Vasopressin Contributes to Hypertension Caused by Nucleus Tractus Solitarius Lesions

ALAN F. SVED, TSUTOMI IMAIZUMI, WILLIAM T. TALMAN, AND DONALD J. REIS

SUMMARY Lesions of the nucleus tractus solitarius (NTS) were studied to determine whether they elevate plasma vasopressin levels and, if so, whether these elevated levels of vasopressin contribute to the hypertension caused by NTS lesions. Bilateral electrolytic lesions of the NTS caused acute, severe hypertension in rats anesthetized with chloralose and in conscious, freely moving rats. After placement of the NTS lesions there was a greater than tenfold elevation in plasma vasopressin levels. Administration of an antagonist of the vasoconstrictor action of vasopressin markedly diminished the hypertension in both conscious and anesthetized rats. Following ganglionic blockade with chlorisondamine, NTS lesions still elicited hypertension, and the magnitude of the hypertension was not different from that observed in rats not treated with chlorisondamine. The hypertension produced by lesions of the NTS in ganglionic-blocked rats was completely abolished by administration of a vasopressin antagonist. These results indicate that (1) NTS lesions elevate plasma vasopressin levels and (2) elevated plasma vasopressin contributes to the hypertension produced by such lesions. (Hypertension 7: 262-267, 1985)

KEY WORDS • sympathetic nervous system • blood pressure • posterior pituitary • brain stem • central nervous system

BILATERAL electrolytic lesions of the nucleus tractus solitarius (NTS) have been shown to elicit arterial hypertension in the rat, cat, and dog.1-4 In the rat, in which the hypertension is fulminant, the increase in arterial pressure (AP) has been attributed solely to an increase in sympathetic discharge. Thus α-adrenergic blockade with phentolamine, chemosympathectomy with 6-hydroxydopamine, or ganglionic blockade will block the development of hypertension after lesion of the NTS.4,5 As the NTS is the site of termination of all arterial and cardiopulmonary baroreceptor afferents,6 the increase of sympathetic activity has been attributed to removal of tonic sympathoinhibitory influences exerted by baroreceptors.

In addition to governing the activity of the autonomic nervous system, information provided by baroreceptors also has been shown to tonically inhibit the release of arginine vasopressin (AVP) from the posterior pituitary.7-9 Therefore, NTS lesions might lead to increased release of this hormone. As baroreceptor denervation markedly potentiates the pressor activity of AVP,10,11 elevated plasma AVP levels may contribute to the hypertension evoked by bilateral NTS lesions. This article describes experiments that support this hypothesis.

Methods

Experiments were performed on male Sprague-Dawley rats weighing approximately 300 g (Taconic Farms, Germantown, NY). Animals were housed in pairs under controlled lighting and temperature conditions, with ad libitum access to food and tap water for at least 1 week before the experiments began.

Rats were anesthetized with halothane (2% in 100% O2) administered by a cone placed over the nose. One cannula (PE-50 tubing filled with heparinized saline) was placed in a femoral artery for recording of arterial pressure (AP) and heart rate (HR) and collecting blood samples for vasopressin assay. Another cannula (0.03 in. Microbore tubing) was placed in a femoral vein for administration of drugs. In experiments requiring in-
travenous infusion of phenylephrine or saline, a cannula also was inserted into the other femoral vein. The animal was placed in a Kopf stereotaxic frame with the incisor bar set 11 mm below the intracranial level. The dorsal surface of the medulla was carefully exposed by limited craniotomy. The calamus scriptorius, which served as stereotaxic zero, was visualized with the aid of a surgical microscope. Chloralose (60 mg/kg i.v.) was administered and the halothane discontinued; the animals continued to breath 100% O₂ throughout the experiment. Experiments were begun 20 minutes later. Additional chloralose was given as needed, generally 20 mg/kg/hour.

Lesions of the NTS were made with electrodes fabricated from Teflon-coated stainless steel wire (outside diameter, 150 μm) with the tip exposed for approximately 150 μm. The coordinates were 0.5 mm rostral to the calamus scriptorius, 0.5 mm lateral from the midline, and 0.5 mm below the dorsal surface of the medulla. Electrolytic lesions were made by passing anodal current (1 mA, 10 sec duration) from a DC constant current source (Grass LMS, Quincy, MA, USA). A clip attached to the neck muscles served as the cathode. In control rats, similar electrodes were lowered into the NTS but no current was passed (sham lesion) or electrolytic lesions of comparable size were placed in the cerebellar cortex.

Blood (0.8 ml) was withdrawn through the arterial cannula 5 minutes before, and 5, 20, and 60 minutes after placement of lesions. The volume of each sample was immediately replaced either with saline (the first sample) or with the red blood cells from the previous sample suspended in saline. Blood samples were immediately centrifuged (Beckman Microfuge, Beckman Instruments, Inc., Palo Alto, CA) for 15 seconds at approximately 10,000 g, and the plasma was removed and frozen (−20°C) until assayed for AVP.

An antagonist of the vasopressor action of AVP, 1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(O-methyl)tyrosine, arginine-vasopressin, d(Ch3)OMe(Tyr)AVP (Bachem, Torrance, CA), was administered intravenously at a dose of 10 μg/kg in saline (1 ml/kg) at the times indicated in individual experiments. At this dose the pressor response to 50 ng of AVP, but not to phenylephrine or angiotensin II, is completely blocked. In some experiments the ganglionic blocking agent chlorisondamine (Ciba Pharmaceutical Co., Summit, NJ) was administered intravenously at a dose of 2 mg/kg. At the time chlorisondamine was injected, an infusion of phenylephrine hydrochloride (1–4 μg/kg/min, Sigma Chemical Co., St. Louis, MO) was started to maintain blood pressure at the pretreatment level. Lesions were made 20 minutes after ganglionic blockade. The effectiveness of ganglionic blockade was confirmed at the end of each experiment by observing that AP fell to less than 60 mm Hg following termination of the phenylephrine infusion and was not further reduced by administration of phentolamine (1 mg/kg i.v.).

In experiments in conscious, freely moving rats, rats were anesthetized with halothane and cannulas were placed in a femoral artery, femoral vein, and trachea. All wounds were closed and treated with procaine (2% solution). The anesthesia was terminated, and the rat was returned to its cage. After 30 to 60 minutes, baseline AP and HR were recorded and a blood sample was taken for AVP determination. The rat was then reanesthetized with halothane, paralyzed with succinylcholine (0.3 mg/kg i.v.), and ventilated with 100% O₂. The dorsal surface of the medulla was exposed, and lesions were placed bilaterally in the NTS. The wound was closed and infiltrated with procaine. The anesthesia was terminated, and the rat was returned to its cage. The AP and HR were recorded for 1 hour. A second blood sample was collected, and was followed by the injection of the AVP antagonist.

At the conclusion of all experiments, rats were anesthetized with pentobarbital (Nembutal) or urethan and were perfused intracardially with saline followed by buffered formalin. The brain stem was removed, sectioned (40 μm) on a cryostat, and stained with cresyl violet. The location and size of each lesion were noted.

After extraction of the hormone from plasma by cation exchange chromatography, AVP was measured by radioimmunoassay. Plasma samples (0.2 ml) were adjusted to pH 4.6 by addition of 0.5 M sodium acetate buffer, pH 4.5. Each sample was then transferred onto a column made from a 1-ml disposable pipette tip plugged at the tip with glass wool. This column contained 0.5 ml of a slurry of 1 g of Amberlite CG-50 resin in 10 ml of water. The resin had previously been washed with 0.1 N acetic acid for several hours followed by repeated washes with water. The Amberlite column was suspended in the mouth of a 12 × 75 mm test tube with a collar made from tubing. This unit (column containing sample suspended in test tube) was then centrifuged at approximately 50 g for 5 minutes to pull the sample through the column. The sample, which collected in the test tube, was reapplied to the column, and the centrifuging process was repeated. The column was then washed with 1 ml of water followed by 1 ml of 50% ethanol and was centrifuged after each wash to pull the liquid through the column. The column was then suspended in a clean test tube, and AVP was eluted from the column with 2 ml of 75% ethanol acidified to pH 1.5 with concentrated hydrochloric acid. The eluate was dried in a Savant Speed Vac sample concentrator (Savant Instruments, Hicksville, NY, USA). The dried extract was reconstituted in 250 μL of radioimmunoassay buffer (50mM NaPO4, 0.9% NaCl, 25mM ethylenediaminetetraacetic acid, 0.5% bovine serum albumin, 0.1% sodium azide, pH 7.5) and centrifuged (3000 g for 10 minutes) to remove any particulate matter. The radioimmunoassay was performed essentially as described by Femstrom and co-workers (antiserum was provided by Dr. J.D. Femstrom, Pittsburgh, PA). Antibody (100 μL of a 1:30,000 dilution) was added to 200 μL of the sample or AVP standard (World Health Organization international arginine-vasopressin standard). Following overnight incubation at 4°C, 100 μL radioimmunoassay buffer containing 3000 cpm I125-AVP (New York, NY) was added, and the mixture was incubated at 4°C for 18 hours. The mixture was then centrifuged at 3000 g to collect the antibody-VASOPRESSIN in NUCLEUS TRACTUS SOLITARIUS HYPERTENSION/Sved et al. 263
England Nuclear Corp., Boston, MA) was added and the tubes were incubated for an additional 2 days. Then 100 μL of normal rabbit serum (1:50 dilution) and 100 μL of goat antirabbit serum (1:30 dilution) were added. Following overnight incubation, the tubes were centrifuged (3000 g for 20 minutes), the supernatants were aspirated, and the tubes were counted in a gamma-scintillation counter.

With these methods recovery of various amounts of AVP added to 200 μL of rat plasma is virtually 100% (e.g., typical assay 95% with a SD of 6; triplicate samples spiked with 2.5, 5, and 10 pg AVP). The sensitivity of the radioimmunoassay is approximately 0.3 pg/tube. The intraassay and interassay coefficients of variation are less than 10%. Baseline AVP levels for rats prepared as described are approximately 10 pg/ml. These levels are elevated over normal basal values (2–3 pg/ml as measured by this assay) because of the operation and anesthesia.

Data were analyzed by analysis of variance followed by the Neuman-Keul’s test. Levels of AVP were subjected to log transformation before statistical analysis.

Results

Effect of NTS Lesions on Plasma Vasopressin Levels

We examined the effect in chloralose-anesthetized rats of bilateral lesions of the NTS on AP, HR, and plasma AVP levels. Following placement of the NTS lesions (n = 6), AP began to increase immediately, and reached maximal elevation approximately 10 minutes after the lesion had been made (Figure 1). The AP remained elevated throughout the postlesion period. Tachycardia also occurred in rats with NTS lesions (Figure 1). In contrast, neither lesion of the cerebellar cortex nor sham-lesion of the NTS elicited changes in AP or HR; no differences were observed between these two groups, so they are combined as a single control group (n = 6) in the results (Figure 1).

Plasma AVP levels also increased rapidly after placement of the NTS lesions and were appreciably elevated 5 minutes postlesion, the earliest time-point sampled (Figure 1a). By 20 minutes postlesion AVP levels reached a maximum — a 20-fold elevation — where they remained for at least 1 hour (at which point the experiment was terminated). Levels of AVP were not elevated in controls.

In the experimental group lesions destroyed most of the caudal and intermediate NTS and usually the dorsal motor nucleus of the vagus (Figure 2). The hypoglossal nucleus and the area postrema typically were spared.

Effect of Vasopressin Antagonist on NTS Hypertension

To examine the contribution of elevated AVP levels to the hypertension caused by destruction of the NTS, rats were treated with a specific antagonist, d(CH₂)₅ OMe(Tyr)AVP, of the vasoconstrictor action of AVP. Ten minutes after bilateral destruction of the NTS or sham lesion, rats received either the AVP antagonist (10 μg/kg) or saline (5 rats per group), and

![Figure 1. Effect of NTS lesion on plasma vasopressin levels, AP and HR. Groups of six chloralose-anesthetized rats received either bilateral lesions of the NTS (open circles) or control lesions (closed circles). The control group consisted of three rats with lesions placed in the cerebellar cortex and three rats with electrodes inserted into the NTS but no current passed. These two control groups were not significantly different from each other. Plasma AVP levels, AP, and HR were measured just before, and 5, 20, and 60 minutes after placement of lesions. • = significant (p < 0.01) difference from control group and baseline value.](http://hyper.ahajournals.org/)

![Figure 2. Photomicrograph of a 40-μm transverse section through the brain stem at the level of the NTS lesion (cresyl violet; × 12.5). This lesion, typical of those in this study, was bilaterally symmetrical and destroyed most of the caudal and intermediate NTS and encroached on the dorsal motor nucleus of the vagus. The hypoglossal nucleus and area postrema were spared.](http://hyper.ahajournals.org/)
the change in AP was monitored. Saline administration had no appreciable effect on AP in either sham- or NTS-lesioned rats. In addition, administration of the AVP antagonist had no effect on AP in sham-lesioned rats. In NTS-lesioned rats the AVP antagonist produced a fall in AP (Table 1; Figure 3) that appeared within 1 minute of its administration and lasted at least 5 minutes (at which point the experiment was terminated).

**Effects of Ganglionic Blockade on NTS Hypertension**

To eliminate any contribution of the sympathetic-adrenal system to the NTS hypertension, rats were treated with the long-acting ganglionic blocking agent chlorisondamine (2 mg/kg i.v.). As ganglionic blockade causes hypotension, which may itself affect AVP levels, phenylephrine (1–4 µg/kg/min) was infused to maintain AP at pretreatment levels.

Despite ganglionic blockade, bilateral lesions of the NTS still caused hypertension \( (n = 4; \text{Table 2; Figure } 4) \). In fact, the magnitude of the increase in AP was not significantly different from that observed in NTS-lesioned rats without drug treatment. In chlorisondamine-treated rats, however, the AVP antagonist completely blocked the hypertension produced by the NTS lesion (Table 2). Also, whereas the AVP antagonist had no effect on AP in sham-lesioned rats, a fall in AP (approximately 10 mm Hg) was observed when the AVP antagonist was administered to sham-lesioned, ganglionic-blocked rats. Ganglionic blockade plus phenylephrine infusion had no effect on plasma AVP levels in either sham-lesioned or NTS-lesioned rats (Table 3).

**NTS Lesions in Conscious, Freely Moving Rats: Effects on Arterial Pressure and Vasopressin Levels**

To eliminate any contribution of anesthesia to the action of AVP observed in chloralose-anesthetized rats, the effects of NTS lesions on AVP and AP were also examined in instrumented, unanesthetized, freely moving rats \( (n = 6) \). While rats were anesthetized with halothane, lesions were placed in the NTS. Following placement of the lesions the anesthesia was terminated and AP was monitored for 1 hour. The AP began to rise approximately 15 minutes after termination of anesthesia, and all rats were markedly hypertensive 60 minutes postlesion (Table 4). As observed in chloralose-anesthetized rats, NTS lesion caused a marked increase in plasma AVP levels (Table 4). Also as observed in chloralose-anesthetized rats, the AVP

### Table 1. Effect of Vasopressin Antagonist on Blood Pressure in NTS Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>Pre-lesion</th>
<th>Post-lesion</th>
<th>Post-drug</th>
<th>Change with drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham lesion</td>
<td>90±4</td>
<td>92±5</td>
<td>92±4</td>
<td>0±2</td>
</tr>
<tr>
<td>NTS lesion</td>
<td>88±4</td>
<td>140±6*</td>
<td>139±6*</td>
<td>-2±2</td>
</tr>
<tr>
<td>VP antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham lesion</td>
<td>87±3</td>
<td>88±2</td>
<td>87±2</td>
<td>-1±2</td>
</tr>
<tr>
<td>NTS lesion</td>
<td>90±4</td>
<td>146±8*</td>
<td>114±6*†</td>
<td>-32±6</td>
</tr>
</tbody>
</table>

Groups of 5 chloralose-anesthetized rats received either sham lesion or NTS lesion and either a vasopressin antagonist \( d(CH_2)_5OMe(Tyr)AVP, (10 \mu g/kg) \) or its vehicle (saline). The antagonist or saline was administered 10 minutes after placement of the lesions.

*Indicates significant change \( (p < 0.05) \) from pre-lesion value.
†Indicates significant change \( (p < 0.05) \) from post-lesion value.

**FIGURE 3. Effect of AVP antagonist on NTS hypertension.** The AP and HR recordings from a typical NTS-lesioned rat and sham-lesioned rat from the experiment presented in Table 1. Administration of the AVP antagonist \( d(CH_2)_5OMe(Tyr)AVP (10 \mu g/kg i.v.) \) is marked by the arrow. Note the decrease in AP elicited by the AVP antagonist in the NTS-lesioned rat but not the sham-lesioned rat.
TABLE 2. Effect of NTS Lesion on Blood Pressure in Ganglion-Blocked Rats

<table>
<thead>
<tr>
<th></th>
<th>Mean arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Saline + sham lesion</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>Saline + NTS lesion</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>Chlorisondamine + sham lesion</td>
<td>88 ± 5</td>
</tr>
<tr>
<td>Chlorisondamine + NTS lesion</td>
<td>90 ± 3</td>
</tr>
</tbody>
</table>

Chloralose-anesthetized rats received either an intravenous injection of the ganglion blocking agent chlorisondamine (2 mg/kg) plus an intravenous infusion of phenylephrine to maintain arterial pressure in the normal range or saline injection plus saline infusion. Twenty minutes after injection, rats received either bilateral NTS lesion or sham lesion. Ten minutes after placement of lesions, all rats were injected with the vasopressin antagonist d(CH2)5OMe(Tyr)AVP (10 μg/kg).

* Indicates significant difference (p < 0.05) from baseline value; n = 4.

TABLE 3. Effect of Chlorisondamine and NTS Lesion on Plasma Vasopressin Levels

<table>
<thead>
<tr>
<th></th>
<th>Plasma vasopressin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Saline + sham lesion</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Saline + NTS lesion</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Chlorisondamine + sham lesion</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Chlorisondamine + NTS lesion</td>
<td>9 ± 2</td>
</tr>
</tbody>
</table>

Chloralose-anesthetized rats received either chlorisondamine (2 mg/kg i.v.), plus an i.v. infusion of phenylephrine to maintain arterial pressure in the normal range or saline plus saline infusion. Twenty minutes after injection, rats received either bilateral NTS lesion or sham lesion. Blood samples for vasopressin measurement were taken before treatment (baseline), 20 minutes after saline or chlorisondamine, and 10 minutes after lesion. The values presented in this table are from the same experiment shown in Table 2.

* Indicates significant difference (p < 0.05) from baseline value; n = 4.

TABLE 4. Effect of NTS Lesion on Arterial Pressure and Plasma Vasopressin in Conscious Rats

<table>
<thead>
<tr>
<th></th>
<th>Before lesion</th>
<th>60 minutes after lesion</th>
<th>Change with AVP antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>107 ± 3</td>
<td>176 ± 2*</td>
<td>-41 ± 14*</td>
</tr>
<tr>
<td>Plasma vasopressin (pg/ml)</td>
<td>6 ± 2</td>
<td>245 ± 39*</td>
<td></td>
</tr>
</tbody>
</table>

Five instrumented, freely moving rats were prepared as described in Methods. Mean arterial pressure and plasma vasopressin levels were determined during a control, baseline period and then again 60 minutes after bilateral lesion of the NTS. Arterial pressure was also determined 5 minutes after administration of a vasopressin antagonist, d(CH2)5Me(Tyr)AVP (10 μg/kg), which was injected 60 minutes after lesion.

* Indicates significant difference (p < 0.05) from preceding value.

Discussion

Results of the present study indicate that bilateral destruction of the NTS elevates the plasma AVP concentration and elevated plasma levels of AVP contribute to the hypertension produced by such lesions. It is not surprising that NTS lesions should evoke the release of AVP; the NTS is the site of termination of antagonist substantially attenuated the hypertension caused by NTS lesion in conscious rats.

FIGURE 4. Effect of NTS lesion and the AVP antagonist on AP and HR in ganglionic-blocked rats. The AP and HR recordings from a typical chlorisondamine-treated, NTS-lesioned rat and chlorisondamine-treated, sham-lesioned rat from the experiment presented in Table 2. Administration of the AVP antagonist d(CH2)5OMe(Tyr)AVP (10 μg/kg i.v.) is marked by the arrow. Note the increase in AP following NTS lesion is completely blocked by the AVP antagonist.
primary afferent fibers from cardiopulmonary and arterial baroreceptors, and these receptors are known to tonically inhibit AVP release. Thus destruction of the NTS should remove this inhibition and lead to release of this hormone. Lesions of the NTS would also interrupt input from chemoreceptors that facilitate AVP release, but the dominant tonic control of vasopressin release exerted from fibers of the ninth and tenth nerves comes from the baroreceptors. It is likely that the release of AVP observed in this study is not secondary to other effects of NTS lesions, such as left ventricular failure and pulmonary congestion, as the increased release of AVP caused by these conditions would be signaled by cardiopulmonary afferents and therefore would be mediated through the NTS, which was destroyed.

It is also not surprising that the elevated levels of AVP observed in NTS-lesioned rats could contribute to the hypertension in these animals. By itself AVP is a potent vasoconstrictor substance; however, baroreceptor input normally antagonizes the effect of AVP on AP.

Our results indicate that AVP contributes to NTS hypertension, but that it is not the only factor involved. Administration of an antagonist to AVP partially, but not completely, reversed NTS hypertension in otherwise untreated rats. In ganglionic-blocked rats, however, the AVP antagonist totally reversed NTS hypertension (Table 2). The larger effect of AVP on AP in the ganglion-blocked rats is expected, as autonomic blockade is known to increase the pressor potency of AVP. These results suggest that AVP and the autonomic nervous system are both involved in NTS hypertension. Indeed, NTS lesions are known to increase sympathoadrenal activity.

These results appear, at first, to contradict previous reports that NTS hypertension is caused solely by increased sympathetic outflow. Such a conclusion was based on the demonstration that destruction of sympathetic nerve terminals by systemic injection of 6-hydroxypaminol plus adrenalectomy or treatment with ganglionic or α-adrenergic blocking agents reversed the hypertension. It is possible that the data do not actually conflict, and that in the absence of AVP, sympathetic blockade would have decreased AP further than observed in the earlier studies.

Conclusion

Our data indicate that a component of the neurogenic hypertension caused by NTS lesion is produced by increased release of AVP into the blood. As we have previously described an AVP component to another form of neurogenic hypertension, that caused by destruction of the caudal ventrolateral medulla, AVP should be considered an important effector mechanism in the central neural control of the circulation.

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