Peripheral Pressor Systems in Hypertension Caused by Nucleus Tractus Solitarius Lesions

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SUMMARY The roles of vasopressin, the sympathoadrenal system, and the renin-angiotensin system in the production of hypertension after bilateral destruction of the nucleus tractus solitarius (NTS) were examined in chloralose-anesthetized rats. Since the activity of the renin-angiotensin system is high in anesthetized rats, additional studies were performed in unanesthetized, freely moving rats to evaluate the role of the renin-angiotensin system in hypertension caused by NTS lesions. Hypertension produced by bilateral electrolytic NTS lesions in rats was accompanied by elevated plasma levels of vasopressin (approximately 7-fold), norepinephrine (> 10-fold), and epinephrine (> 10-fold), but not of plasma renin activity. These results suggest that this form of hypertension is due to increased sympathoadrenal activity and increased vasopressin release into plasma and that the renin-angiotensin system is not involved. In rats with NTS lesions, blockade of vasopressin or the sympathoadrenal system, but not the renin-angiotensin system, elicited an acute decrease in arterial pressure. However, blockade of either vasopressin or the autonomic nervous system before production of the lesions had no effect on the resulting hypertension, indicating that in the absence of either one of these systems bilateral NTS lesions still result in severe hypertension. Although the renin-angiotensin system does not normally contribute to this hypertension, it does appear to contribute to the elevation in blood pressure once the actions of vasopressin have been blocked. In rats pretreated with a vasopressin antagonist, plasma renin activity increased following NTS lesions and the angiotensin converting enzyme inhibitor captopril decreased blood pressure. These results indicate that hypertension induced by bilateral NTS destruction is produced by complex interactions among the sympathoadrenal system, vasopressin, and the renin-angiotensin system. (Hypertension 8: 742–747, 1986)

KEY WORDS • vasopressin • catecholamines • renin-angiotensin system • sympathoadrenal system • brainstem • blood pressure

BILATERAL electrolytic lesions of the nucleus tractus solitarius (NTS), the site of termination in the brainstem of all arterial and cardiopulmonary baroreceptors,1 produce severe hypertension.2,3 Recently, we4 demonstrated that the release of arginine vasopressin (AVP) into the circulation contributes to the fulminating hypertension caused by NTS lesions in the rat. We found that NTS lesions markedly elevated plasma AVP levels and that an AVP antagonist partially reversed the hypertension caused by these lesions. Furthermore, in rats in which the autonomic nervous system had been blocked, NTS lesions still caused hypertension, which was totally reversed by the AVP antagonist. However, the previous studies addressed only whether AVP was involved in NTS hypertension; they did not examine the interactions of AVP with other pressor systems (i.e., sympathoadrenal system and renin-angiotensin system) in producing the hypertension. These interactions are examined in the present study.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats aged approximately 10 weeks and weighing approximately 300 g (Taconic Farms, Germantown, NY, USA). Animals were housed in groups under controlled lighting and temperature conditions, with ad libitum access to food and tap water for at least 1 week before use in experiments.

Most experiments were performed in rats anesthetized with α-chloralose; this preparation has been described previously in detail.5 Briefly, rats were anesthetized with halothane (2% in 100% O₂), and cannulas
(PE-50 tubing) were placed in the right femoral artery and vein. The arterial cannula was connected to a Statham pressure transducer (Oxnard, CA, USA) for recording of arterial pressure and heart rate; this cannula was also used for sampling blood. The venous cannula was used to administer drugs. A second venous cannula was inserted in experiments requiring the infusion of phenylephrine. The animal was placed in a stereotaxic instrument with the incisor bar set 11 mm below the interaural line, and the dorsal surface of the medulla was surgically exposed. Chloralose (60 mg/kg i.v.) was administered, and the halothane was terminated, but the animals continued to breath 100% O₂ throughout the experiment. Experiments were begun 20 minutes after administration of the chloralose. Additional chloralose (20 mg/kg) was given every hour.

The NTS lesions were made using Teflon-insulated stainless steel wire electrodes (150 μm outside diameter) with 150 μm of the tip exposed. Coordinates of the lesion site 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 brainstem. Lesions were made by passing anodal current (1 mA for 10 seconds) from a DC constant current source (LM55, Grass Instruments, Quincy, MA, USA). Control lesions were placed in the spinal trigeminal nucleus (2 mm lateral from the midline, 0.5–1.0 mm below the dorsal surface, at the level of the calamus scriptorius). Arterial pressure and heart rate were measured continually before and after placement of the NTS lesions. Blood samples were collected and drugs administered at times indicated in individual experiments.

Some experiments examined the effects of NTS lesions in conscious, freely moving rats. With the rats under halothane anesthesia, cannulas were placed in the right femoral artery and vein. These cannulas were tunneled subcutaneously and exteriorized and anchored at the back of the neck. The dorsal surface of the medulla was surgically exposed, and lesions were placed in the NTS. The neck muscles were then sutured together, and the wound was infiltrated with 2% lidocaine (Xylocaine) and clipped closed. The rat was placed in a small cage with the cannulas run outside the cage encased in a steel spring. The rats began to awake from the anesthesia within 15 minutes.

In four chloralose-anesthetized rats, both AVP and catecholamine levels were measured in duplicate 50-μL aliquots 5 minutes after the administration of 10 μg/kg of AVP i.v. The AVP antagonist, [1-(β-mercaptop-β,β-cyclopentamethylnepropionic acid), 2(O-methyl)-tyrosine-arginine-epinephrine (d(CH₂)₅OMe(Tyr)AVP (Bachem, Torrance, CA, USA), was injected (10 μg/kg i.v.). At this time, an intravenous infusion of phenylephrine hydrochloride (1 μg/kg/min in 10 μL/min; Sage infusion pump, Cambridge, MA, USA) was initiated and the rat was injected with chlorisondamine (5 mg/kg i.v.; CIBA-Geigy, Summit, NJ, USA). The phenylephrine infusion subsequently was adjusted such that the mean arterial pressure (MAP) was approximately equal to the baseline MAP (1–4 μg/kg/min). After 15 minutes of a stable blood pressure, lesions were placed in the NTS and blood pressure was recorded for an additional hour.

At the conclusion of the experiment, the rat was given an overdose of anesthetic and perfused through the heart with saline followed by 10% buffered formalin. The brainstem was removed and sectioned (40 μm) using a cryostat or Vibratome (Ted Pella Co., Tustin, CA, USA). Brainstem sections were mounted on glass slides and stained with cresyl violet. The location and size of each lesion were noted. Animals without appropriate lesions were excluded from the study. The lesions destroyed most of the intermediate third of the NTS and underlying dorsal motor nucleus of the vagus (see Reference 6). The hypoglossal nuclei and area postrema were typically left intact.

In experiments testing the effects of blockade of the renin-angiotensin system, the angiotensin converting enzyme inhibitor captopril (Squibb, Princeton, NJ, USA), 5 mg/kg, was administered intravenously. In other rats, we have found this dose of captopril to completely inhibit the pressor response to angiotensin I (100 ng/kg i.v.) for at least 45 minutes.

Plasma hormones were measured in blood samples (0.5–1.2 ml depending on the experiment) withdrawn through the arterial cannula at the times indicated for individual studies. The volume of blood removed for each sample was immediately replaced with either saline (the first sample) or the red blood cells from the previous sample suspended in saline. Blood samples were immediately centrifuged (10 seconds in a Microfuge, Allied Scientific, Springfield, NJ, USA), and the plasma was removed and frozen (−70 °C) until assayed for AVP, catecholamines, and renin activity.

The AVP was measured by radioimmunoassay following its extraction from 200-μL aliquots of plasma using microcolumns of Amberlite CG-50 resin (Sigma Chemical, St. Louis, MO, USA). Norepinephrine and epinephrine levels were measured in duplicate 50-μL aliquots of plasma by a radioenzymatic method modified from the procedure of Peuler and Johnson. Renin activity was assayed in 100-μL aliquots of plasma by measuring the amount of angiotensin I generated during a 1- or 3-hour incubation of the sample at 37°C after the sample had been adjusted to pH 6.5 by the addition of 100 μL of a maleate buffer containing phenylmethylsulfonyl fluoride to inhibit the breakdown of angiotensin I. The amount of angiotensin I generated during the incubation was assayed by radioimmunoassay using an antiserum supplied by Dr. Jean Sealey (Cardiovascular Center, Cornell University Medical College, New York, NY, USA), and results are expressed as nanograms of angiotensin I produced per milliliter of plasma per hour.

Data are expressed as means and standard errors. Results were analyzed by analysis of variance, and significant effects were analyzed post hoc by the Bonferroni method. The AVP and catecholamine values were subjected to log transformation before statistical analysis, since the group variance increased as the mean increased.
TABLE 1. Effects of Nucleus Tractus Solitarius Lesions on Plasma Vasopressin, Catecholamine, and Renin Levels in Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minutes after sham lesion (n=6)</th>
<th>Minutes after NTS lesion (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>95 ± 3</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>366 ± 20</td>
<td>382 ± 20</td>
</tr>
<tr>
<td>AVP (pg/ml)</td>
<td>12 ± 2</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>255 ± 25</td>
<td>240 ± 30</td>
</tr>
<tr>
<td>E (pg/ml)</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>8 ± 3</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure; HR = heart rate; AVP = arginine vasopressin; NE = norepinephrine; E = epinephrine; PRA = plasma renin activity; ANG I = angiotensin I.

*p < 0.05, compared with baseline values and values in sham-lesion group.

Results

As previously noted, bilateral lesions of the intermediate portion of the NTS in chloralose-anesthetized rats caused severe hypertension that was accompanied by markedly elevated plasma levels of AVP (Table 1). Plasma norepinephrine and epinephrine levels were also elevated, indicating increased sympathoadrenal activity (see Table 1). Plasma renin activity, however, was not affected significantly by the lesions.

When an AVP antagonist, d(CH₂)₃OMe(Tyr)AVP (10 μg/kg i.v.), was administered 60 minutes after NTS lesions had been placed in chloralose-anesthetized rats, a decrease in MAP of 24 ± 5 mm Hg was elicited (n = 6, p < 0.05). This response was similar to that previously observed when the antagonist was administered 10 minutes after placement of the lesions. In contrast, when the AVP antagonist was given before the NTS lesions were produced, hypertension developed with no difference in time course or magnitude when compared with rats treated with the saline vehicle (Figure 1). Furthermore, there was no difference in the increase in plasma catecholamine levels between NTS-lesioned rats pretreated with the AVP antagonist and rats pretreated with saline (Figure 2). However, following pretreatment with the AVP antagonist, NTS lesions produced a significant increase in plasma renin activity (see Table 1).

These results suggest that in the absence of the contribution of AVP to the NTS lesion-induced hypertension lesions an increase in plasma renin activity occurs. This experiment was subsequently repeated in conscious, freely moving rats because anesthesia artificially elevates plasma renin activity, making the baseline levels very high and making it difficult to assess pharmacologically the contribution of the renin-angiotensin system to the hypertension, since blockade of this system would also have an effect in anesthetized, sham-lesioned rats.

Rats were anesthetized with halothane, and the operation was performed as described in Methods. Rats were injected with either the AVP antagonist or saline 10 minutes before placement of bilateral NTS or sham lesions. The wounds were closed, and each rat was returned to a cage to recover; at 1 hour post lesion, rats appeared totally recovered from the anesthesia. Severe hypertension developed in both groups of NTS-lesioned rats, but plasma renin activity was elevated only in the group that was pretreated with the AVP antagonist (Table 2). Captopril (5 mg/kg i.v.) administered to these rats 70 minutes after placement of the lesions (10 minutes after removal of the blood sample for renin measurement) decreased blood pressure only in the group pretreated with the AVP antagonist (see Table 2).
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We have already reported that inhibition of the autonomic nervous system did not alter the development of NTS hypertension (although the hypertension was totally reversed by the AVP antagonist following autonomic blockade). Since the previous experiment suggested that blockade of AVP did not affect the development of NTS hypertension, we examined the effect of simultaneous blockade of these systems on NTS hypertension. In chloralose-anesthetized rats treated with both the AVP antagonist and the ganglionic blocking agent chlorisondamine (5 mg/kg i.v. with phenylephrine infused to maintain arterial pressure in the normal range), bilateral NTS lesions did not significantly alter arterial pressure (+8 ± 4 mm Hg; maximum increase in MAP following NTS lesion in AVP-blocked plus autonomic-blocked rats; 4 rats were studied for 20 minutes following placement of NTS lesions).

2). Subsequent injection of the AVP antagonist decreased blood pressure in the saline-pretreated group but not in the group pretreated with the AVP antagonist (see Table 2).

Since it appeared that an increase in plasma renin activity could take over for the vasopressin component of the hypertension, we examined 1) whether blood pressure would return to hypertensive levels following the acute decrease in blood pressure that occurs when the AVP antagonist is administered 10 minutes after placement of NTS lesions, and 2) if blood pressure returns to hypertensive levels, whether this is accompanied by an increase in plasma renin activity. Ten minutes after placement of NTS lesions in chloralose-anesthetized rats, rats were injected with either the AVP antagonist (10 μg/kg i.v.) or vehicle. The AVP antagonist produced a prompt decrease in blood pressure, but blood pressure returned to full hypertensive levels within 45 minutes (Figure 3). Although bilateral NTS lesions had no effect on plasma renin activity, plasma renin activity did increase in the rats receiving the AVP antagonist after the NTS lesion (see Figure 3).

Discussion

Bilateral NTS lesions in rats cause severe hypertension. While it was initially suggested that destruction of the NTS produced hypertension solely by increased sympathetic nervous system activity, recent findings indicate that this form of hypertension is produced in part by increased circulating levels of AVP. This conclusion is based primarily on three observations: 1) NTS lesions markedly elevate plasma AVP levels, 2) an antagonist of the vasoconstrictor action of AVP decreased arterial pressure in NTS hypertensive rats but not in control rats, and 3) hypertension still develops following NTS lesions in rats treated with a ganglionic blocking drug, and in these rats, the hypertension was totally reversed by blocking the actions of AVP. Although the hypertension caused by NTS lesions in ganglionic-blocked rats can be accounted for by AVP, AVP clearly is not the only factor involved in NTS hypertension in intact rats. As previously observed and confirmed in these studies, NTS hypertension in intact rats (either conscious or anesthetized with chloralose), although attenuated by injection of an AVP antagonist, was not totally reversed. Furthermore, injection of the AVP antagonist before NTS lesions were placed did not alter the characteristic development of hypertension produced by such lesions (see Figure 1).
TABLE 2.  Contribution of the Renin-Angiotensin System to Nucleus Tractus Solitarius Hypertension in Conscious Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 hour postlesion</th>
<th>Postcaptopril change</th>
<th>Post-AVP antagonist change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>HR (beats/min)</td>
<td>PRA (ng ANG I/ml/hr)</td>
</tr>
<tr>
<td>NTS lesion, saline pretreatment (n = 7)</td>
<td>156 ± 6</td>
<td>352 ± 21</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>NTS lesion, AVP antagonist pretreatment (n = 6)</td>
<td>156 ± 6</td>
<td>340 ± 12</td>
<td>6.5 ± 0.7*</td>
</tr>
<tr>
<td>Trigeminal lesion, no pretreatment (n = 4)</td>
<td>117 ± 5*</td>
<td>335 ± 16</td>
<td>3.6 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. See Table 1 for key to abbreviations. Halothane-anesthetized rats received bilateral NTS lesions. Rats were injected with either an AVP antagonist, d(CH2)5OMe(Tyr)AVP (10 μg/kg i.v.), or its saline vehicle 10 minutes before placement of the lesions. Following lesion placement, the halothane was terminated and rats returned to a cage to recover. One hour later, MAP and HR were monitored, and a 0.5-ml blood sample was taken for measurement of PRA. Ten minutes later, rats were injected with captopril (5 mg/kg i.v.), and MAP and HR were recorded for 15 minutes. The AVP antagonist then was administered to all rats, and MAP and HR were monitored for an additional 10 minutes. A group of animals with bilateral lesions in the spinal trigeminal nucleus is included for comparison.

*p < 0.05, compared with values in the other two groups.

The sympathoadrenal system appears to be the other pressor system involved in producing NTS hypertension. As in an earlier study,3 NTS lesions markedly increased plasma catecholamine levels, indicating increased sympathoadrenal activity (see Table 1). In the same animals, plasma renin activity was not increased, suggesting that the renin-angiotensin system is not involved in this model of hypertension. This finding is in contrast to the earlier work of Zandberg et al.,3 who found a small increase in plasma renin activity in NTS hypertensive rats. The suggestion that NTS hypertension is mediated by increased sympathoadrenal outflow and increased AVP release is supported by the observation that NTS hypertension did not develop in rats treated with a ganglionic blocking agent and an AVP antagonist.

Thus, in normal rats, NTS hypertension appears to have both an AVP component and a sympathoadrenal component. Following the elimination of either system, however, the remaining system can produce the full degree of hypertension. This occurs without greater release of AVP in the case of the sympathoadrenal-blocked rat6 and without greater sympathoadrenal activity (at least as reflected by plasma catecholamine levels) in the AVP-blocked rat (see Figure 2). This finding suggests that the pressor activity of each system is enhanced in the absence of the other system (or, conversely, each system attenuates the response of the other). Indeed, potentiation of the pressor activity of AVP following blockade of the autonomic nervous system has long been known.13

The apparently greater increase in pressure caused by the autonomic nervous system seen in rats pretreated with the AVP antagonist may result from sympathetically stimulated renin release. Plasma renin activity did not change following placement of NTS lesions in otherwise untreated rats (see Tables 1 and 2, Figure 2), but renin activity increased following NTS lesion placement in rats pretreated with the AVP antagonist.
This increase was observed in both chloralose-anesthetized and conscious, freely moving rats. Increased sympathetic activity is known to stimulate renin release, while vasopressin is known to inhibit renin release. It is therefore conceivable that plasma renin activity did not change following NTS lesion placement because the combined effects of increased sympathetic activity and AVP release canceled each other out with respect to renin release. Thus, when NTS lesions are made following pretreatment with the AVP antagonist, the AVP-induced inhibition of renin release does not occur and plasma renin activity increases. Plasma renin activity increased in NTS-lesioned rats pretreated with the AVP antagonist, and this increase appears to contribute to the hypertension, as demonstrated by the ability of captopril to decrease blood pressure in these rats.

Still, it is curious that injection of the AVP antagonist before placement of the NTS lesions should have such a contrasting effect compared with injection of the AVP antagonist after NTS hypertension has developed. Similar findings have been reported with hypertension caused by destruction of the caudal ventrolateral medulla: the hypertension was attenuated by the AVP antagonist when the drug was administered after the hypertension had developed, but not when it was given before the hypertension was produced. The AVP antagonist used in all of these studies appears to be a selective and long-acting antagonist of the vasopressinergic neurons in rabbit brainstem elevates plasma vasopressin, and the renin-angiotensin system.

In summary, bilateral lesions of the NTS elevated plasma levels of AVP, norepinephrine, and epinephrine, but not renin. The hypertension produced by bilateral NTS lesions was caused by increased plasma AVP levels and increased sympathoadrenal activity, although either system alone was capable of causing the hypertension. Although the renin-angiotensin system does not normally contribute to NTS hypertension, following blockade of the AVP component of NTS hypertension, this system did participate in the elevated blood pressure. Taken together, these results indicate that hypertension resulting from bilateral destruction of the NTS in rats is produced by complex interactions among the sympathoadrenal system, vasopressin, and the renin-angiotensin system.

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References
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