Central Nervous System Mechanisms Involved in Inhibition of Renal Sympathetic Nerve Activity Induced by Arginine Vasopressin

Satoshi Suzuki, Akira Takeshita, Tsutomu Imaizumi, Yoshitaka Hirooka, Megumu Yoshida, Shin-ichi Ando, and Motoomi Nakamura

Previous studies have suggested that intravenous infusion of arginine vasopressin (AVP) inhibits sympathetic nerve activity (SNA) in rabbits by its action at the brainstem. This study aimed to determine, first, whether this action of AVP depended on functioning baroreflex mechanisms. The second aim was to determine the role of the nucleus tractus solitarii (NTS) in mediating this action of AVP. Experiments were performed in rabbits anesthetized with chloralose.

Intravenous infusions of AVP (1, 3, and 10 mU/kg/min) resulted in progressive inhibition of renal SNA in rabbits with intact arterial baroreceptors and vagal afferents as well as in rabbits with sinoaortic denervation and bilateral vagotomy. The magnitudes of renal SNA inhibition evoked with AVP were comparable in the two groups. Renal SNA inhibition evoked with AVP at doses of 1 and 3 mU/kg/min in rabbits after sinoaortic denervation and vagotomy was totally blocked by chemical lesions with kainic acid of the bilateral NTS or the area postrema. Lesions of the NTS or the area postrema markedly attenuated (by about 60%) but did not totally block the response evoked with AVP at a dose of 10 mU/kg/min. AVP microinjected directly into the NTS did not evoke changes in renal SNA or arterial pressure, whereas AVP microinjected into the area postrema suppressed renal SNA. These results suggest that intravenous AVP at lower doses inhibited renal SNA by causing excitation of the NTS neurons, and this action of AVP did not depend on functioning baroreflex mechanisms. The second suggestion is that the NTS was unlikely to be the site where AVP directly interacted but instead received a neural connection, presumably from the area postrema, where AVP might directly interact. (Circulation Research 1989;65:1390-1399)

Experimental evidence indicates that arginine vasopressin (AVP) markedly influences control of sympathetic nerve activity (SNA) in rabbits. Investigators have demonstrated that intravenous infusion of AVP caused greater inhibition of renal or lumbar SNA than did phenylephrine for a given increase in arterial pressure, which indicated that AVP facilitated arterial baroreflex inhibition of SNA. It also has been shown that AVP facilitated inhibition of renal SNA mediated through vagal afferents during volume expansion. Furthermore, investigators have shown that the neural effect of AVP resulted largely from the central nervous system mechanism. Undesser et al and Hassel et al have reported that lesions of the area postrema blocked the facilitation of reflex inhibition of renal SNA mediated by arterial baroreceptors and by vagal afferents.

However, the mechanisms involved in the central nervous system by which AVP inhibits SNA are not well understood. Some investigators have suggested that AVP inhibits SNA by facilitation of baroreflex and thus this action of AVP depends on the presence of intact baroreflexes. On the other hand, Bishop et al have recently suggested that AVP causes direct excitations of the central neurons, resulting in a decrease in resting SNA, and this effect was not mediated by facilitation of baroreflex. In fact, Bishop et al have shown that AVP attenuated the parameters describing the arterial baroreflex function, including the maximal gain of the reflex.

Furthermore, the neural pathway involved in the central action of AVP is not known. Block of the...
AVP-induced facilitation of reflex sympathetic inhibition by lesions of the area postrema suggests that circulating AVP interacts with neurons of the area postrema. However, the neural connections relaying excitations from the area postrema to the vasomotor center have not been determined. Based on indirect observations, Undesser et al have suggested that the noradrenergic pathway from the area postrema to the nucleus tractus solitarii (NTS) might play an important role in mediating the effect of AVP.

The aims of this study were, first, to determine whether the inhibitory effect of intravenous AVP on renal SNA in rabbits depends on the presence of intact arterial and/or cardiopulmonary baroreflex. We examined inhibition of renal SNA evoked with progressive infusions of AVP in rabbits with intact arterial baroreceptors and vagi as well as in those with sinoaortic denervation and bilateral vagotomy. The second aim was to examine the role of the NTS neurons in mediating the inhibitory effect of intravenous AVP on renal SNA. We examined the effects of chemical lesions of the NTS or the area postrema on renal SNA inhibition evoked with progressive infusions of AVP in rabbits with intact baroreflex mechanisms as well as in those with sinoaortic denervation and vagotomy. We also examined the arterial pressure and renal SNA responses to AVP microinjected directly in the NTS or area postrema.

**Materials and Methods**

**General Procedures and Recording of Nerve Activity**

Male New Zealand white rabbits (2.2–2.8 kg) were anesthetized with α-chloralose (60 mg/kg i.v.) after induction with thiamylal sodium (25 mg/kg i.v.) and mechanically ventilated with room air supplemented with oxygen through an endotracheal tubing. Supplemental doses of α-chloralose (20 mg/kg) were given hourly, and rabbits were immobilized with 0.5 mg/kg d-tubocurarine. Blood gas was monitored, and ventilation was adjusted to maintain Po2 greater than 100 mm Hg, PCO2 at 35–45 mm Hg, and pH at 7.35–7.45. Body temperature was maintained at 37–40°C by a heating pad and a heating lamp.

A femoral artery and a vein were cannulated with PE 90 tubing for arterial pressure recording and drug administration, respectively. The arterial cannula was connected to a pressure transducer (TM1, Toyo Baldwin, Tokyo, Japan), and arterial pressure and heart rate were continuously monitored and recorded.

With the use of a retroperitoneal approach, the left renal nerve was isolated. A branch of the nerves was separated from surrounding connective tissues, cut distally, desheathed, and covered with mineral oil for subsequent recordings of action potentials from the central cut end of the nerves. The nerves were placed on silver/silver chloride bipolar electrodes for subsequent recording of nerve traffic activity. The technique for amplification and quantification of nerve traffic activity has been previously described in detail. In brief, the action potentials of nerves were amplified and fed into a nerve traffic analyzer (DPA 1000, DIA Medical System, Tokyo, Japan) and converted to spikes by a window discriminator. The raw electroneurogram and the integrated output from the spike counter were displayed on a recorder.

**Sinoaortic Denervation and Vagotomy**

Sinoaortic denervation and bilateral vagotomy were performed by bilateral section of the aortic depressor nerves, sympathetic nerves, and vagi and by interrupting all nerves between the internal and external carotid arteries, stripping the adjacent adventitia, and painting the region of the carotid sinus with 10% phenol. Completeness of sinoaortic denervation was ensured in each rabbit by the absence of changes in renal SNA with an increase in arterial pressure of 20–30 mm Hg caused by an intravenous injection of phenylephrine.

**Chemical Lesions of the NTS and the Area Postrema**

The rabbits were placed in a stereotaxic frame with the head inclined downward by 45°. An incision was made between the ears, and muscles were dissected to expose the cisterna magna. The atlantooccipital membrane was cut and removed. The dura was incised, and the obex was visualized.

Chemical lesions of the NTS were made with kainic acid (Sigma Chemical, St. Louis, Missouri), a substance that has been shown to selectively damage nerve cell bodies with minimal or no damage to nerve terminals or to axons passing through the lesioned area. A glass micropipette (50 μm o.d.) was filled with kainic acid (200 ng/μl) dissolved in artificial cerebrospinal fluid containing (mM) NaCl 123, CaCl2 0.86, KCl 3.0, MgCl2 0.89, NaHCO3 25, NaH2PO4 0.5, and Na2HPO4 0.25 and gassed with 5% CO2-95% O2 mixture. The pipette was placed in a micromanipulator and positioned in the injection sites. The NTS lesions were made by injecting kainic acid in the four sites at each side of the NTS as shown in Figure 1. The sites of injection were defined according to an atlas in the rabbit, with reference to the midline, the dorsal surface of the medulla, and the rostral border of the area postrema (Figure 1). Kainic acid in a volume of 0.2 μl (40 ng) was injected at each site over 1 minute, and the pipette was left in situ for another minute. The total volumes and doses of kainic acid injected in each side of the NTS were 0.8 μl and 160 ng, respectively. Lesions of the bilateral NTS were confirmed as follows: First, reflex inhibition of renal SNA with an increase in arterial pressure caused by phenylephrine was lost after the NTS lesions in rabbits with intact arterial baroreceptors (reflex renal SNA inhibition with phenylephrine was pres-
ent in these rabbits before the NTS lesions. Second, each injection of kainic acid into the NTS caused immediate but transient decreases in renal SNA and arterial pressure through its initial neuroexcitatory effect. However, the completion of injections into the four sites in each side of the NTS resulted in marked increases in renal SNA and arterial pressure, which suggests effective bilateral destruction of the NTS. Third, the sites of injection were marked by methylene blue added to the injectates and confirmed at postmortem in each rabbit as described below. The dye added to the injectate did not alter the responses to kainic acid.

Chemical lesion of the area postrema was made by a single injection of kainic acid (0.2 μl, 40 ng). The site of injection in the area postrema is also presented in Figure 1. The site of injection into the area postrema was confirmed by the dye at postmortem in each rabbit.

**Microinjection of AVP Into the NTS or Area Postrema**

Injections of AVP into the NTS or area postrema region were made in the same way as injections of kainic acid. AVP (16 mU) dissolved in artificial cerebrospinal fluid (0.2 μl) was injected into the unilateral NTS in rabbits with intact baroreflexes. AVP (4 mU, 0.2 μl) was injected into the unilateral NTS or area postrema in rabbits with sinoaortic denervation and bilateral vagotomy. At the end of experiments, the micropipette containing AVP was removed. The micropipette containing methylene blue was then positioned at the exact site of the AVP injection, and methylene blue (0.2 μl) was injected to examine the site of injection at postmortem.

**Histological Examination**

After completion of the experiments, the brain was perfused with 0.9% saline followed by 10% formalin solution through the heart. The brainstem was removed, and frozen sections (50 μm) were cut serially. The locations of methylene blue staining were identified with a microscope. The animals were excluded from the study if injections were not at the proper sites.
TABLE 1. Responses to Intravenous Arginine Vasopressin in Rabbits With Intact Arterial Baroreceptors and Vagi

<table>
<thead>
<tr>
<th></th>
<th>AVP 1</th>
<th>AVP 3</th>
<th>AVP 10 (mU/kg/min)</th>
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<tr>
<td></td>
<td>Control</td>
<td>During infusion</td>
<td>Control</td>
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<tr>
<td>Before lesions of NTS (n=6)</td>
<td>Renal SNA (spikes/sec)</td>
<td>137±13</td>
<td>132±10</td>
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<td></td>
<td>Mean AP (mm Hg)</td>
<td>97±5</td>
<td>95±5</td>
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<td></td>
<td>Heart rate (beats/min)</td>
<td>293±12</td>
<td>293±12</td>
</tr>
<tr>
<td>After lesions of NTS</td>
<td>Renal SNA (spikes/sec)</td>
<td>173±18</td>
<td>162±18</td>
</tr>
<tr>
<td>1 Hour after lesions (n=6)</td>
<td>Mean AP (mm Hg)</td>
<td>125±11</td>
<td>122±11</td>
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<tr>
<td></td>
<td>Heart rate (beats/min)</td>
<td>275±7</td>
<td>273±5</td>
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<tr>
<td>3-4 Hours after lesions (n=4)</td>
<td>Renal SNA (spikes/sec)</td>
<td>141±22</td>
<td>141±19</td>
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AVP, arginine vasopressin; NTS, nucleus tractus solitarii; SNA, sympathetic nerve activity; AP, arterial pressure.

*p<0.01, †p<0.05 vs. control value (paired t test). There were no differences among control values before each dose of AVP in rabbits before the NTS lesions or after the lesions. Control values before infusions of AVP did not significantly differ before and after the NTS lesions.

Experiment 3. In this experiment, we determined the effect of an injection of AVP into the NTS in rabbits with intact arterial baroreceptors and vagal nerves. In five rabbits, we continuously recorded arterial pressure, heart rate, and renal SNA for 5 minutes after an injection of AVP (16 mU, 0.2 μl) into the unilateral NTS. In another two rabbits with sinoaortic denervation and bilateral vagotomy, we also observed the effect of an injection of AVP (4 mU, 0.2 μl) into the unilateral NTS or area postrema.

Statistical Analysis

In experiments 1 and 2, comparisons between values at control and those with each dose of AVP were made by paired t test. The magnitudes of changes in renal SNA evoked with AVP before lesions were compared with those after lesions of the NTS or the area postrema by two-way analysis of variance and Tukey's multiple comparison test. In experiment 3, one-way analysis of variance was used to examine significance of changes after injection of AVP into the NTS. All values are expressed as mean±SEM. Significance was accepted for values of p<0.05.

Results

Responses to Intravenous AVP in Rabbits With Intact Arterial Baroreceptors and Vagi (Table 1)

Before lesions of the NTS, intravenous infusions of AVP decreased renal SNA in a dose-dependent fashion (p<0.01 at each dose) (changes in renal SNA evoked with each dose of AVP are shown in Figure 2). Mean arterial pressure increased significantly only at the highest dose (10 mU/kg/min) of AVP (p<0.05). Heart rate decreased at doses of 3 and 10 mU/kg/min (p<0.01). After the NTS lesions, baseline arterial pressure and renal SNA at the time of the experiments with infusions of AVP tended to be higher than values before the NTS lesions, but differences were not significant. After the NTS...
Arterial Baroreceptors Denervated and Vagi Responses to Intravenous AVP in Rabbits With sinoaortic baroreceptors denervated and vagi sectioned are shown in Figure 3. Intravenous infusions of AVP decreased renal SNA and increased mean arterial pressure in a dose-dependent fashion (p<0.01 for each dose). After lesion of area postrema, AVP at doses of 1 and 3 mU/kg/min did not decrease renal SNA. AVP at the dose of 10 mU/kg/min decreased renal SNA (p<0.05), but the magnitude of the decrease in renal SNA was markedly reduced (p<0.01) compared with that before the area postrema lesion (Figure 4). After the area postrema lesion, AVP at doses of 3 and 10 mU/kg/min increased arterial pressure (p<0.05 and p<0.01, respectively) but did not alter heart rate.

Responses to Microinjection of AVP Into the Unilateral NTS or Area Postrema

Microinjection of AVP (16 mU) into the unilateral NTS did not elicit significant changes in renal SNA, mean arterial pressure, or heart rate during 5 min-

<table>
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<th>TABLE 2. Responses to Intravenous Arginine Vasopressin in Rabbits After Sinoaortic Denervation and Bilateral Vagotomy</th>
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<tr>
<td>Before lesions (n=8)</td>
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<td></td>
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<tr>
<td>Renal SNA (spikes/sec)</td>
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<tr>
<td>Mean AP (mm Hg)</td>
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<td>Heart rate (beats/min)</td>
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After lesions of NTS (n=4)

| Renal SNA (spikes/sec) | 149±12 | 146±11 | 154±10 | 148±9 | 158±7 | 139±8† |
| Mean AP (mm Hg) | 137±8 | 142±7 | 138±6 | 150±5† | 135±6 | 152±5* |
| Heart rate (beats/min) | 255±20 | 261±22 | 261±21 | 256±20 | 266±22 | 258±22 |

After lesion of area postrema (n=5)

| Renal SNA (spikes/sec) | 126±15 | 127±20 | 125±15 | 120±18 | 125±15 | 108±18† |
| Mean AP (mm Hg) | 85±3 | 89±3 | 85±3 | 97±4* | 84±5 | 102±5† |
| Heart rate (beats/min) | 249±14 | 248±13 | 254±13 | 242±13 | 245±12 | 231±10* |

AVP, arginine vasopressin; SNA, sympathetic nerve activity; AP, arterial pressure.
FIGURE 3. Representative recordings of arterial pressure and renal sympathetic nerve activity (SNA) with infusions of arginine vasopressin (AVP) in rabbits with sinoaortic baroreceptors denervated and vagi sectioned. Left panel: Arterial baroreflex-mediated inhibition of renal SNA with a bolus injection of phenylephrine (PE) was abolished. Right panel: Intravenous infusions of AVP decreased renal SNA in a dose-dependent fashion. ENG, electroneurogram.

Discussion

The major findings in this study are as follows: First, intravenous infusions of progressive doses of AVP resulted in progressive decreases in renal SNA in rabbits with and without functioning arterial and cardiopulmonary baroreflex. Second, decreases in renal SNA evoked with lower doses of intravenous AVP were totally abolished and those with the highest dose of AVP were markedly attenuated by chemical lesions of the bilateral NTS or the area postrema. Third, microinjections of AVP into the NTS region did not alter arterial pressure or renal SNA, whereas microinjections of AVP into the area postrema suppressed renal SNA. These findings allow two conclusions. First, the inhibitory effect of intravenous AVP on renal SNA resulted from the direct influence on brainstem neurons and did not depend on functioning arterial and cardiopulmonary baroreflex. Second, the NTS neurons were involved in the neural pathway mediating this effect of AVP but were unlikely to be the site where AVP directly interacted. In the paragraphs that follow, we will discuss each of these points.

Mechanisms of AVP-Induced Inhibition of Renal Sympathetic Nerve Activity

Previous studies have suggested that intravenous infusion of AVP facilitated arterial and cardiopulmonary baroreflex control of lumbar and renal SNA in rabbits.1-6 It also has been shown that AVP might interact at several sites to cause this effect, which included the central nervous system,1-2,4,6 ganglionic transmission,8 and baroreceptor afferents.4 However, major interaction appears to occur at the central nervous system.1-2,4,6 Undesser et al1 and Hassner et al6 have established most definitively that AVP inhibits renal SNA through a central action with the demonstration that lesions of the area postrema blocked this effect. Our results are consistent with this suggestion. We also observed that chemical lesions of the NTS or the area postrema blocked the decrease in renal SNA evoked with lower doses of AVP and markedly attenuated the decrease in renal SNA with the high dose of AVP.
An important finding in this study is that intravenous infusions of AVP resulted in decreases in renal SNA in a dose-dependent fashion in rabbits after sinoaortic denervation and bilateral vagotomy. The magnitudes of decreases in renal SNA were comparable between rabbits with intact baroreceptors and those with baroreceptors denervated. Furthermore, lesions of the NTS or the area postrema blocked the responses evoked with AVP at doses of 1 and 3 mU/kg/min and markedly attenuated the response with AVP at a dose of 10 mU/kg/min.

The conclusion that renal sympathetic inhibition with intravenous AVP resulted from a direct action on brainstem neurons differs from that of other investigators. Other investigators have suggested that intravenous AVP inhibited lumbar or renal SNA by facilitating arterial and/or cardiopulmonary baroreflex and this effect of AVP was dependent on functioning baroreflexes.\(^1,^3,^5\) Their suggestion was based on the finding that sympathetic inhibition evoked with AVP was no longer observed after acute denervation of arterial baroreceptors and/or bilateral vagotomy.\(^1,^3,^5\)

The reason for the difference between our results and those of others is not clear. Rabbits were anesthetized with \(\alpha\)-chloralose in our study as well as in studies by Sharabi et al\(^3\) and Gupta et al\(^5\) so that anesthetics did not account for the differences in the results. However, the designs of the experiments differed between this and their studies. Gupta et al have reported that AVP did not inhibit renal SNA after acute vagotomy in rabbits with arterial baroreceptors previously denervated. In their study, the responses to AVP were examined 10 minutes after vagotomy. In contrast, in our study, the examinations were begun at least 2 hours after sinoaortic denervation and vagotomy. It is possible that acute denervation might have increased the plasma level of endogenous vasopressin\(^16-18\) so that the effect of exogenous AVP might have been masked. In a preliminary study, we have observed that intravenous infusion of AVP (3 mU/kg/min) did not significantly inhibit renal SNA (Δ renal SNA: \(-8\pm4\%\), NS vs. control) at 10 minutes after bilateral vagotomy in rabbits with previous sinoaortic denervation (n=3) but inhibited it at 2 hours after vagotomy (Δ renal SNA: \(-28\pm3\%, \ p<0.01\) vs. control). Similar mechanisms might have caused the discordant results as to the effect of sinoaortic denervation on AVP-induced SNA inhibition in rabbits with intact vagi.\(^3,^5\) Sharabi et al\(^3\) have reported that acute sinoaortic denervation in rabbits with intact vagi blocked AVP-induced lumbar SNA inhibition. On the other hand, Gupta et al\(^5\) have shown that AVP decreased renal SNA before vagotomy in rabbits with sinoaortic baroreceptors previously denervated. Undesser et al\(^1\) also have shown that AVP decreased renal SNA before vagotomy in conscious rabbits with sinoaortic baroreceptors denervated. They reported that acute vagotomy blocked the response evoked with AVP in these rabbits. However, the studies were apparently done acutely after vagotomy in only two rabbits and they did not report the detail of the procedures or the results.

### Table 3. Responses to Arginine Vasopressin Microinjected Into NTS

<table>
<thead>
<tr>
<th>Changes (%) over time (min)</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
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<tr>
<td>Renal SNA (spikes/sec)</td>
<td>130±10</td>
<td>±4</td>
<td>±2</td>
<td>±4</td>
<td>±6</td>
<td>±4</td>
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<tr>
<td>Mean AP (mm Hg)</td>
<td>77±3</td>
<td>±2</td>
<td>±1</td>
<td>±3</td>
<td>±3</td>
<td>±1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>268±21</td>
<td>±2</td>
<td>±2</td>
<td>±2</td>
<td>±2</td>
<td>±1</td>
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NTS, nucleus tractus solitarii; SNA, sympathetic nerve activity; AP, arterial pressure. Changes in renal SNA, AP, and heart rate were not significant.
FIGURE 5. The representative recordings of the effects of arginine vasopressin (AVP) microinjected into the unilateral nucleus tractus solitarii (NTS) (Panel A) or the area postrema (AP) (Panel B) on arterial pressure and renal sympathetic nerve activity (SNA) in rabbits with sinoaortic baroreceptors denervated and vagi sectioned. Microinjection of AVP (AVP inj.) into the NTS did not alter renal SNA, whereas microinjection of AVP into the area postrema decreased renal SNA. The injection site of AVP is indicated as a hatched area in the drawing of the section of the brainstem. ENG, electroneurogram; Sm, medial solitary nucleus; T, solitary tract; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus. The distance from the rostral border of the AP is given on the lower right side of each drawing.

Imaizumi and Thames, like us, observed significant decreases in renal SNA evoked with intravenous AVP in rabbits after sinoaortic denervation and vagotomy. They attributed this effect to an action of AVP on ganglionic transmission since AVP at a large dose (40 mU) inhibited renal SNA even after spinal cord transection. However, they did not examine the effects of smaller doses of AVP on renal SNA after spinal cord transection. Our results indicate that lesions of the NTS or the area postrema totally blocked the renal sympathetic responses evoked with AVP at doses of 1 and 3 mU/kg/min in rabbits after sinoaortic denervation and vagotomy. These results indicate that decreases in renal SNA with these doses of AVP resulted from the central action but not from the effect on ganglionic transmission. However, our results with AVP at the dose of 10 mU/kg/min were compatible with the suggestion by Imaizumi and Thames since the response evoked with this dose of AVP was markedly attenuated but not totally blocked by lesions of the NTS or the area postrema.

Bishop et al have recently shown that infusions of AVP at doses of 1.5 and 3 mU/kg/min in conscious rabbits decreased resting renal SNA and resulted in progressive decreases in the parameters describing the arterial baroreflex function, including the maximal gain of the reflex. These observations have challenged the view that AVP inhibits SNA by facilitating arterial baroreflex. Bishop et al have suggested that AVP at doses of 1.5 and 3 mU/kg/min caused direct excitation of brainstem neurons leading to a reduction in resting renal SNA and a blunting of the arterial baroreflex. Our results are consistent with this conclusion of Bishop et al and strongly suggest that renal sympathetic inhibition evoked with intravenous AVP resulted from its direct effect on brainstem neurons and did not depend on functioning arterial and cardiopulmonary baroreflex.
Intravenous infusion of AVP also decreased heart rate in a dose-dependent fashion in rabbits after sinoaortic denervation and vagotomy. Lesions of the NTS blocked or attenuated the responses of heart rate with AVP. These results indicate that decreases in heart rate evoked with AVP also were mediated by its direct central action.

**Role of the NTS Neurons in Mediating the Central Effect of AVP**

As discussed previously, Undesser et al. and Hasser et al. have shown that the area postrema was the most likely site where plasma AVP interacted with the central neurons in rabbits with the demonstration that electrical lesion of the area postrema blocked the neural effect of AVP. This notion was further supported by the finding that transection at the midcollicular level did not significantly alter the responses of renal SNA to intravenous AVP. Our results are consistent with this notion because chemical lesion of the area postrema with kainic acid abolished renal nerve responses to lower doses of AVP and microinjection of AVP into area postrema suppressed renal SNA. We need to consider the possibility that block of the response evoked with AVP by lesion of the area postrema might have resulted from erroneous lesions of the NTS since lesions of the NTS also blocked the response with AVP. However, this possibility is unlikely because subsequent electrical stimulation of the NTS or subsequent injections of kainic acid into the NTS in rabbits subjected to chemical lesion of the area postrema evoked transient decreases in renal SNA and arterial pressure (Δ renal SNA: −81±9%; Δ mean arterial pressure: −37±3% at peak response). These results indicate that the NTS neuron remained functionally intact in rabbits with lesion of the area postrema. Thus, we consider that block of the responses evoked with AVP in these rabbits indeed resulted from lesion of the area postrema. However, it should be noted that the area postrema is not the only site where intravenous AVP interacts with central neurons. Patel and Schmid have suggested that the central effect of AVP on control of heart rate involved different neural pathways from that on control of SNA. They showed that microinjections of lidocaine into the median preoptic nucleus blocked reflex bradycardia induced by AVP but did not alter reflex inhibition of SNA in rabbits.

The central neural pathway in the brainstem mediating the inhibitory effect of AVP on SNA has not been determined. Undesser et al. have shown that 6-hydroxydopamine administered into the fourth cerebral ventricle blocked the facilitation of reflex inhibition of renal SNA induced by AVP without altering reflex inhibition of renal SNA induced by phenylephrine. They found significant depletion of catecholamine content in the NTS and the spinal cord in rabbits treated with 6-hydroxydopamine. Based on these findings, Undesser et al. have suggested that the noradrenergic pathway from the area postrema to the NTS might be important in eliciting the neural effect of AVP. Our study established more definitively that the neural connections from the area postrema to the NTS were involved in the central pathway mediating the effect of AVP. Chemical lesions of the NTS neurons abolished the effect of lower doses of AVP and markedly attenuated that of the high dose of AVP.

We should consider the possibility that kainic acid injected into the NTS region might have lesioned the area postrema in addition to the NTS and that lesions of the area postrema but not of the NTS were responsible for block of the responses evoked with AVP. However, this possibility appears unlikely because injections of AVP into the NTS region in other rabbits in the same quantity as kainic acid did not alter arterial pressure or renal SNA, whereas injections of AVP into the area postrema resulted in decreases in renal SNA (Figure 5). We also should consider the possibility that increased resting renal SNA after the NTS lesion might have prevented AVP to decrease renal SNA. Thus, we examined responses to intravenous AVP when resting renal SNA after lesions of the NTS returned to the level comparable to that before the NTS lesion in some rabbits. The neural response evoked with AVP at lower doses were not observed in these rabbits. Therefore, increased resting sympathetic activity itself did not account for block of the effect of AVP after the NTS lesion.

Our results suggest that the NTS neurons were not directly excited by AVP but received the neural connection from the area postrema since injections of AVP into the NTS region did not alter arterial pressure or renal SNA. We should consider the possibility that the latter findings might have resulted from the damage to the NTS or an insufficient dose of AVP. However, we observed in a few rabbits that increases or decreases in arterial pressure induced by intravenous phenylephrine or nitroglycerin, respectively, caused the expected reflex changes in renal SNA after the injection of AVP into the bilateral NTS. Such results suggest that the NTS neurons remained functionally intact after microinjection of AVP. Further, failure for AVP to inhibit SNA as injected into the NTS was not due to an insufficient dose because the dose of AVP microinjected into the NTS (16 mU) was larger than the dose of AVP microinjected into the area postrema (4 mU), which inhibited renal SNA.

The finding that AVP microinjected into the NTS region did not alter arterial pressure differs from the finding in rats reported by Matsuguchi et al. They have reported that AVP injected into the NTS in rats caused hypertension and tachycardia. The reason for this difference is not clear but species difference may account for these discordant results. It has been suggested that the neural effect of AVP markedly differs between rabbits and rats.
Our results suggest that the NTS neurons are involved in the neural connections mediating AVP-induced inhibition of SNA as well as baroreflex control of SNA. However, this study does not permit us to determine if these two mechanisms involve the same NTS neurons. Further studies are needed to delineate the precise neural pathways within or beyond the NTS region that mediate AVP-induced inhibition of SNA.

In summary, intravenous infusions of AVP caused progressive inhibition of renal SNA in rabbits with and without functioning cardiac and arterial baroreflex. Lesions of the bilateral NTS or the area postrema abolished or markedly attenuated the responses evoked with AVP. These findings suggest that intravenous AVP inhibited renal SNA by causing excitation of the NTS neurons and this action of AVP did not depend on functioning baroreflex mechanisms. Because AVP microinjected directly into the NTS did not inhibit renal SNA, the NTS was unlikely to be the site where AVP directly interacted but received a neural connection, presumably from the area postrema where AVP directly interacted.

Acknowledgments

We thank Ms. Junko Yasumoto for her technical assistance and Ms. Mieko Itoyama for secretarial assistance.

References


Key Words • arginine vasopressin • central nervous system • nucleus tractus solitarii • renal sympathetic nerve • area postrema • rabbits
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Circ Res. 1989;65:1390-1399
doi: 10.1161/01.RES.65.5.1390

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