Interactions of Vasopressin with the Area Postrema in Arterial Baroreflex Function in Conscious Rabbits

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SUMMARY. This study compares the effect of arginine-vasopressin with phenylephrine on arterial pressure, heart rate, and renal sympathetic nerve activity in conscious rabbits with and without functional arterial baroreflexes and in rabbits with lesions of the area postrema. In intact rabbits, progressive infusions of arginine-vasopressin result in large decreases in renal sympathetic nerve activity and heart rate for a given increase in blood pressure as compared to progressive infusions of phenylephrine. In sinoaortic-denervated rabbits, the responses of arterial pressure on heart rate and renal sympathetic nerve activity to both arginine-vasopressin and phenylephrine are markedly attenuated, indicating the necessity for afferent baroreceptor activity in this response. This observation indicates that arginine-vasopressin is acting centrally to enhance the baroreflex. A central site of action of circulating vasopressin may be the area postrema, since it is the only circumventricular organ in the hindbrain. Lesioning the region of the area postrema resulted in a normalization of the responses evoked with arginine-vasopressin and phenylephrine. There was no difference in the phenylephrine responses of arterial pressure on renal sympathetic nerve activity or heart rate in area postrema-lesioned animals, compared to control rabbits. Therefore, we conclude that the area postrema or its surrounding tissue is either a site of action of circulating arginine-vasopressin or contains fibers of passage from another site where arginine-vasopressin acts to enhance baroreflex activity. (Circ Res 56: 410-417, 1985)

ALTHOUGH arginine-vasopressin (AVP) has been shown to be a potent vasoconstrictor agent (Altura and Altura, 1977; Cowley et al., 1974), increases in mean arterial pressure (MAP) have often been shown to be less than those observed with other vasoconstrictor agents when administered to animals with intact reflexes. A number of studies (Cowley et al., 1974; Montani et al., 1980; Liard et al., 1981; Guo et al., 1982) have indicated that AVP interacts in the central nervous system to modify arterial baroreflexes. It has been postulated that the central action of AVP results in increases in the gain of the arterial baroreflexes (Cowley et al., 1974; Montani et al., 1980). The resulting enhanced inhibition would result in a greater fall in sympathetic activity for any given increase in MAP. On the other hand, microinjections of AVP into the nucleus tractus solitarius results in increases in arterial pressure and heart rate (Matsuguchi et al., 1982), while administration of AVP into the 4th cerebral ventricle causes bradycardia (Varma et al., 1969). Although the effects of AVP appear to be dependent upon the site or route of administration, data from these studies suggest that AVP can act centrally to alter the control of the circulation.

Liard et al. (1981) demonstrated that intravertebral infusions of AVP, in concentrations which do not exert a peripheral effect, result in a decrease in cardiac output and heart rate, suggesting that the hindbrain is involved in the AVP interaction with the baroreflex. The area postrema may be such a site, since it is the only area in the hindbrain devoid of a blood-brain barrier. The area postrema has also been implicated in modifying baroreflex control to increase sympathetic outflow for another circulating neuropeptide, angiotensin II (Ferrario et al., 1972).

The purpose of this study was to examine reflex changes in renal sympathetic nerve activity and heart rate in response to the two pressor agents, AVP and phenylephrine. In addition, this study identifies a site which normalizes the differences seen in response to AVP and phenylephrine.

Methods

Experiments were performed on male New Zealand white rabbits weighing between 2 and 3 kg. Rabbits were anesthetized with sodium-pentobarbital (25 mg/kg). Through a midcervical incision, two catheters were inserted into the right jugular vein for administration of drugs, one for phenylephrine and one for arginine-vasopressin. A catheter was also inserted into the right femoral artery for monitoring arterial pressure. The left kidney was exposed retroperitoneally. Renal sympathetic nerves were isolated and two stainless steel electrodes were placed around the nerve. Electrodes for measuring renal sympathetic nerve activity were fabricated from seven
Intact Animals

In nine rabbits, control curves were produced by progressive infusions of either phenylephrine (0.5–12 μg/kg per min) or arginine-vasopressin (2–50 mU/kg per min). Infusions were maintained for 3-minute periods to allow RSNA, HR, and MAP to stabilize before values were recorded. Flow rates ranged from 0.02–0.5 ml/min. Rabbits were allowed to recover a minimum of 30 minutes after an infusion before the other drug was administered. Recovery was considered to be complete once MAP, HR, and RSNA had returned to levels comparable to preinfusion values. The two drugs were administered randomly, and the order of administration had no effect on the HR, MAP, and RSNA responses.

Sinoaortic Denervation

Animals were anesthetized with sodium-pentobarbital (25 mg/kg). The aortic depressor and carotid sinus nerves were exposed through a midcervical incision. Aortic depressor nerves were identified both electrophysiologically and anatomically. The aortic depressor nerves were cut at the level of the nodose ganglion.

The bifurcation of the external and internal carotid artery was isolated and stripped of all nerve fibers in this region. The internal carotid artery was ligated and cut. The region of the carotid sinus was stained with cresyl violet or Weil stain. Microscopic examinations of the sections were performed to localize and determine the extent of each lesion.

Histology

After experimentation, the animals were anesthetized with sodium-pentobarbital, and, in each, the chest was opened via a midsternal incision so as to expose the heart. The descending aorta was ligated at the level of the heart and the ascending aorta was ligated just as it leaves the heart. An 18-gauge needle was inserted into the ascending aorta for administration of the perfusion fluids. An incision was made in the right atrium to allow the perfusion fluids to leave the system.

The brain then was perfused with 100 ml of 27°C saline, followed by a 10% buffered formalin (37–40% formalin, 100 ml; distilled water, 900 ml; sodium monophosphate, 4 g; sodium dihydrogen phosphate, 6.5 g). The brains were removed and stored in buffered formalin. Paraffin (15-μm) sections were cut serially, mounted, and stained by the Klüver-Barrera procedure (Klüver and Barrera, 1953). In some cases, alternate paraffin sections were stained either with cresyl violet or Weil stain. Microscopic examinations of the sections were performed to localize and determine the extent of each lesion.

Statistical Analysis

The arterial pressure, heart rate, and renal nerve activity responses to progressive infusions of drugs were analyzed by one-way analysis of variance with repeated measures (Snedecor et al., 1967). All values are shown with SEM. Dose–response curves were analyzed by two-way analysis of variance with repeated measures (Winer, 1971) or linear regression analysis. Differences in slopes were determined by unpaired t-test. Significant differences revealed by analysis of variance were identified using the Newman-Keuls multiple range test (Winer, 1971). Differences between responses produced by either AVP or phenylephrine were analyzed by Students’ paired t-test (Snedecor et al., 1967). Statistical significance was considered to be a probability level of less than 0.05.

Results

Intact Rabbits

No significant differences in the heart rate and renal sympathetic nerve activity responses to increases in arterial pressure produced with either AVP or phenylephrine were noted between intact and sham-lesioned rabbits (n = 5). Therefore, sham-
lesioned rabbits will be referred to as intact rabbits. An analog recording comparing the effects of AVP and phenylephrine on arterial pressure and RSNA is seen in Figure 1. At equal pressor doses, vasopressin completely abolished nerve activity, whereas phenylephrine reduced nerve activity approximately 25%. Elimination of nerve activity with phenylephrine required that arterial pressure be raised to a substantially higher level.

The effects of increasing MAP with progressive infusions of AVP and phenylephrine on the percent change in RSNA and HR in nine rabbits are shown in Figure 2. A marked difference is seen between the effects of AVP and phenylephrine when the RSNA and HR responses to changes in MAP are compared. With phenylephrine infusion, increases in MAP of 10 mm Hg reduced RSNA by 58 ± 11%. In all rabbits, when MAP was increased by 30 mm Hg with phenylephrine, RSNA approached zero. With AVP infusions, RSNA was reduced 58% before detectable increases in MAP were observed; RSNA was abolished when MAP had increased by 7 mm Hg. Thus, for any given increase in MAP, there was a greater inhibition of RSNA with AVP, compared to phenylephrine. A similar response is seen when comparing ΔMAP vs. ΔHR. With AVP, there is a much greater fall in HR for any given increase in MAP, compared to the response produced with phenylephrine (Fig. 2).

Sinoaortic-Denervated Rabbits

In six sinoaortic-denervated rabbits (SAD), increases in MAP with either phenylephrine or AVP produced similar effects on RSNA (Fig. 3). Resting values for MAP and HR are shown in Table 1. No
TABLE 1

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<th>Control</th>
<th>SAD</th>
<th>AP lesion</th>
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<tr>
<td>MAP (mmHg)</td>
<td>83 ± 4</td>
<td>83 ± 4</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>289 ± 11</td>
<td>339 ± 10</td>
<td>306 ± 7</td>
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* In control, sinoaortic-denervated (SAD), and area postrema-lesioned (AP lesion) rabbits. In the SAD rabbits, the resting heart rate is significantly higher than in the other groups. † Significantly different from control and AP-lesioned groups (P < 0.05).

Significant difference was seen between decreases in RSNA to increases in MAP with either AVP or phenylephrine. Significant decreases in RSNA were seen when MAP was increased 20 mm Hg with AVP, or 40 mm Hg with phenylephrine. In two SAD rabbits, vagotomy prevented the decrease in RSNA during phenylephrine and AVP infusions. Significant decreases in heart rate from control were seen only when MAP was increased by 40 mm Hg with AVP, and no decrease was seen with phenylephrine. At all values, the decrease in heart rate produced with AVP was greater than that produced with phenylephrine.

SAD rabbits exhibited no significant decreases in heart rate when MAP was increased with phenylephrine. However, as pressure was increased with AVP, there was a significant decrease in HR, suggesting a differential effect on HR and RSNA with phenylephrine but not with AVP.
Area Postrema-Lesioned Rabbits

In six rabbits, lesion of the area postrema had no effect on resting MAP or HR (Table 1). However, lesion of the area postrema abolished the differential response between phenylephrine and AVP (Fig. 4). After ablation of the area postrema, the ΔMAP vs. %RSNA relationship obtained with AVP infusion was shifted to approximate the relationship obtained with phenylephrine infusions in either intact or sham-lesioned animals. There was no significant difference in the ΔMAP vs. %RSNA or MAP-HR relationship during phenylephrine infusions between intact, sham-lesioned, or area postrema-lesioned animals. Also, as shown in Figure 4, after lesion of the area postrema, the ΔMAP-AHR responses produced with AVP were similar to those observed with phenylephrine in intact, sham lesion, or lesion rabbits.

Figures 5 and 6 summarize the HR and RSNA responses to increases in MAP produced with phenylephrine and AVP infusions. During AVP infusion, lesions of the area postrema shifted RSNA and HR responses to the right, during increases in MAP, but

**FIGURE 7.** Dose-response curves of increases in arterial pressure produced with progressive doses of phenylephrine in control, sinoaortic-denervated (SAD) or area postrema-lesioned (AP lesion) rabbits. Arterial pressure (ΔMAP) increases significantly (P < 0.05) with increasing doses of phenylephrine in control, lesioned, and sinoaortic-denervated rabbits. No difference is seen between control and lesioned rabbits, however, sinoaortic-denervated rabbits are different (P < 0.05) from both control and lesioned rabbits. Renal sympathetic nerve activity (RSNA) decreases significantly (P < 0.05) with increasing doses of phenylephrine in control and area postrema-lesioned rabbits. The slopes of the control and area postrema-lesioned animals are not different from one another and they are both different from sinoaortic-denervated rabbits (P < 0.05).

**FIGURE 8.** Dose-response curves of increases in arterial pressure produced with progressive doses of AVP in control, sinoaortic-denervated (SAD), or area postrema-lesioned (AP lesion) rabbits. Arterial pressure (ΔMAP) increases significantly (P < 0.05) with increasing doses of AVP in lesioned and sinoaortic-denervated but not control rabbits. Lesioned rabbits are different (P < 0.05) from control and sinoaortic-denervated rabbits. Sinoaortic-denervated rabbits are also different (P < 0.05) from control rabbits. Renal sympathetic nerve activity (RSNA) decreases significantly (P < 0.05) with increasing doses of AVP in control and area postrema-lesioned but not sinoaortic-denervated rabbits. The slopes of the control and area postrema-lesioned rabbits are not different from one another, but both are different from sinoaortic-denervated rabbits (P < 0.05). At the higher doses of AVP, RSNA is significantly different between the control and area postrema-lesioned rabbit (P < 0.05).
did not alter these responses during phenylephrine infusions. Sinoaortic denervation essentially abolished the RSNA and HR responses to increases in MAP during phenylephrine infusions. However, some residual effects were noted at higher MAP during AVP infusion.

**Dose-Response Curves**

MAP and RSNA dose-response curves for AVP and phenylephrine in intact, sinoaortic-denervated, and area postrema-lesioned rabbits are shown in Figures 7 and 8. Increasing doses of either pressor agent caused a dose-related increase and decrease in MAP and RSNA, respectively. Because, with either agent, the maximum dose tested was the dose that resulted in reducing RSNA to zero in the intact animal, the dose-response curves do not reach an asymptote. The MAP dose-response curve during phenylephrine infusion was unaltered after lesion of the area postrema. However, sinoaortic denervation resulted in a significant shift of the dose-response relationship to the left.

In intact rabbits, AVP infusion resulted in only small increases in MAP. However, lesions of the area postrema shifted the dose-response curve to the left, resulting in a greater increase in MAP for any given dose, compared to intact rabbits. Sinoaortic denervation also resulted in a large shift in the MAP dose-response curve to AVP. However, area postrema lesions shifted the AVP dose-response substantially to the left, resulting in a difference between the SAD and area postrema AVP dose-response curve which was similar to that observed between the SAD and control phenylephrine dose-response curve.

RSNA decreases comparable amounts to increasing doses of phenylephrine in both area postrema and control rabbits. In SAD rabbits, the decrease in RSNA to increasing doses of phenylephrine is abolished by vagotomy. The slopes of the AVP dose- 

%RSNA relationship for control and area postrema-lesioned rabbits are not different from one another, and both are different from those of SAD rabbits. The decrease in RSNA seen in SAD rabbits is eliminated by vagotomy. At the higher dose of AVP, the amount of RSNA remaining is greater in the area postrema-lesioned rabbits, than in control.

**Verification of Lesions**

Lesions that normalized the differences between responses produced with phenylephrine and AVP destroyed the area postrema and variably damaged tissue lateral and ventral to the site of maximum damage (Fig. 9). Lesions ranged from minimal damage of the area postrema to removal of the entire area postrema and surrounding tissue, including portions of the NTS and dorsal motor nucleus. In one rabbit, the medial portion of the nucleus tractus solitarius (NTS) was damaged; however, in this animal, the reflex response produced with phenylephrine was not altered from that of control rabbits. In three rabbits that had incomplete lesions of the area postrema, the responses produced with phenylephrine and AVP were not normalized. These three rabbits were not included in our analysis. Baroreflex responses to phenylephrine were not altered in any animals subjected to lesions as compared to control.

**Discussion**

The purposes of this study were to evaluate the effects of AVP and phenylephrine on renal sympathetic efferent nerve activity and to localize a site of action of AVP in the central nervous system. Three conclusions are drawn from this study. (1) The reflex response to increases in MAP produced by progressive infusions of AVP was greater than that produced with phenylephrine. For any given increase in MAP, there was a greater decrease in RSNA or HR with AVP, compared to phenylephrine; (2) Si-
noaortic denervation abolished the response to phenylephrine, whereas the response to AVP was markedly attenuated, but not completely abolished. (3) The difference seen between AVP and phenylephrine infusions was normalized by lesioning of tissue of the area postrema and its surrounding tissue.

Our observation that increases in mean arterial pressure with infusion of AVP produced greater inhibition of RSNA than similar increments in mean arterial pressure with phenylephrine is consistent with the results of previous studies. Results from separate laboratories have shown that AVP interacts with the arterial baroreflexes to increase the inhibitory influence of the reflex (Cowley et al., 1974; Montani et al., 1980; Guo et al., 1983). Cowley et al. (1974) noted that, in SAD dogs, pressor responses to norepinephrine were increased only 5-fold, whereas pressor responses to AVP increased 100-fold. They concluded that AVP potentiates the arterial baroreflexes. More recently, using anesthetized rabbits, Guo et al. (1982) observed a substantially greater inhibition of lumbar sympathetic nerve activity for a given increase in arterial pressure with AVP, compared to phenylephrine. In the above studies, functional arterial baroreflexes were necessary for the effects of AVP. However, in our study, sinoaortic deafferentation greatly attenuated but did not completely abolish the effects of AVP. In the conscious rabbit, following sinoaortic deafferentation, the mean arterial pressure dose-response curve to phenylephrine was shifted significantly to the left, and the reflex heart rate and RSNA nerve responses to increases in pressure of 30 mm Hg were abolished. Compared to the effects on the phenylephrine dose-response curve, sinoaortic denervation caused a greater shift in the arterial pressure/dose-response curve to AVP. However, sinoaortic deafferentation did not completely abolish the HR and RSNA reflex responses to increases in arterial pressure. Vagotomy after sinoaortic deafferentation abolished the reflex responses to large increases in pressure (40 mm Hg) during phenylephrine infusion, as well as those during AVP infusion. The decrease in RSNA and HR with AVP during large increases in MAP in SAD rabbits may be due to several causes. First, AVP has been shown to interact with the cardiopulmonary reflexes to inhibit sympathetic outflow (Hasser et al., 1984). Thus, the resulting activation of cardiopulmonary afferents with distension of the heart during exposure to elevations in MAP may lead to a greater inhibition of sympathetic outflow when MAP is increased with AVP, compared to that obtained with phenylephrine. Second, it is possible that AVP may have a greater effect on venous compliance than phenylephrine. For a given increase in MAP, AVP may cause a greater increase in filling pressure, resulting in a greater activation of cardiopulmonary afferents as compared to phenylephrine.

Various investigators have suggested that AVP acts at a central site to increase baroreflex sensitivity (Cowley et al., 1974; Montani et al., 1980; Hasser et al., 1983). Additional support for a central site of action was provided by Liard et al. (1981). AVP infused via the vertebral artery, as opposed to intravenous infusion, produced a greater inhibitory effect, suggesting that the central site for the action of AVP was in the region perfused by the vertebral arteries. Since the area postrema, which is the only circumventricular organ in the hindbrain, has been implicated as the site of action of angiotensin II to increase sympathetic outflow (Ferrario et al., 1972), we reasoned that the area postrema may also be involved in the central action of AVP.

In rabbits with lesions of the area postrema, the effects of AVP to increase the inhibitory influence of the arterial baroreflex on RSNA and heart rate were abolished. Since the arterial baroreflex responses to pressor challenges induced with phenylephrine were unaltered, lesions of the area postrema normalized the difference between the reflex responses to AVP and phenylephrine. Although the extent of the lesions varied among rabbits, the area postrema was the single area that was consistently destroyed in the rabbits exhibiting a normalized arterial baroreflex response between phenylephrine and AVP. In the majority of these rabbits, tissue surrounding the area postrema was also damaged. The largest lesion included a portion of the NTS and the dorsal motor nucleus. However, none of the lesioned rabbits displayed an altered arterial baroreflex control of heart rate or RSNA to increases in pressure with phenylephrine. Furthermore, lesions of the area postrema did not alter the magnitude or lability of arterial pressure (Nathan and Reis, 1977; Buchholz and Nathan, 1984). Thus, the damage produced by the lesions to other structures did not interfere with the normal arterial baroreflexes.

The relative difference between the AVP dose-response curve in area postrema-lesioned rabbits and sinoaortic-deafferentated rabbits was similar to the difference observed between the phenylephrine dose-response curves determined in intact rabbits and sinoaortic-deafferentated rabbits. Thus, the area postrema lesion, by abolishing the effects of AVP on the arterial baroreflex, normalized the AVP-arterial pressure dose-response curve.

In the intact rabbit, infusions of AVP decreased RSNA by 80% and heart rate by 40 beats/min before increases in MAP were detected. Even though intact baroreflexes were necessary to demonstrate this effect, the data suggest that the apparent buffering effect of AVP is related to dose, as well as to changes in MAP. After lesion of the area postrema and surrounding tissue, decreases in RSNA and HR during AVP infusions were similar to that observed with phenylephrine (Fig. 4). In this situation, the stimulus for the decrease in RSNA and HR appeared to be dependent upon MAP. Analysis of the AVP dose-response curves for RSNA shows that there is little difference between the intact and lesion groups (Fig. 8). However, in intact animals, the data indicate that, at the doses of AVP administered, the decrease...
in sympathetic outflow exactly counterbalanced the vasoconstrictor effects of AVP, suggesting an extremely fine regulation of MAP.

Previous studies in the dog have indicated that the area postrema is the site for the central actions of angiotensin II to increase sympathetic outflow. Our data indicate that it is also important in mediating the central effects of AVP to increase the apparent gain of the arterial baroreflex in conscious rabbits. Thus, these two observations suggest that the area postrema plays an important role in the modulation of the sympathetic outflow.

In summary, our results show that AVP acts via the area postrema, or surrounding tissue, to increase the arterial baroreflex inhibition of RSNA and heart rate in conscious rabbits.

We gratefully acknowledge the help of LuAnn Laubach and R. Allen Buchholz in preparing and interpreting the histology; we also thank Linda Stahl and Judith Smith for their expert technical assistance, and Janet Wall for secretarial assistance in preparing the manuscript.

Supported by National Institutes of Health Grant HL-12415.

Dr. Johnson was a Visiting Professor from the Department of Psychology and the Cardiovacular Center, University of Iowa, Iowa City, Iowa, during the time this work was carried out.

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Received April 2, 1984; accepted for publication December 17, 1984.

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INDEX TERMS: Sinoaortic denervation • Renal sympathetic nerves • Nerve recording • Phenylephrine • Dose response
Interactions of vasopressin with the area postrema in arterial baroreflex function in conscious rabbits.
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Circ Res. 1985;56:410-417
doi: 10.1161/01.RES.56.3.410

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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