Reflex Effect of Vasopressin After Blockade of V₁ Receptors in the Area Postrema

Eileen M. Hasser and Vernon S. Bishop

This study investigated the effect of micropressure injection of the V₁ arginine vasopressin (AVP) receptor antagonist into the area postrema on the ability of circulating AVP to augment baroreflex inhibition of renal sympathetic nerve activity (RSNA) in urethane-anesthetized rabbits. In addition, the effects of micropressure injections of AVP into the area postrema on RSNA, arterial pressure, heart rate, and baroreflex control of RSNA were evaluated. Injection of 100 ng (in a 10-nl volume) of AVP antagonist into the area postrema abolished the ability of AVP to enhance baroreflex inhibition of RSNA compared with phentolamine (-8.84±0.89 before antagonist versus -4.83±0.44 %RSNA/mm Hg after antagonist). Normal baroreflex inhibition to phentolamine (-3.95±0.26 versus -4.10±0.33 %RSNA/mm Hg) was unaltered. This dose of AVP antagonist given intravenously or into the adjacent medial nucleus tractus solitarius was without effect. Micropressure injection of AVP directly into the area postrema produced a dose-dependent decrease in RSNA without significant effects on arterial pressure or heart rate. Local injection of 4±0.6 ng (in a 4-nl volume) of AVP produced an average 27±3% decrease in resting RSNA. Continuous injection of AVP into the area postrema using short-duration, low-frequency pressure pulses significantly augmented the baroreflex inhibition of RSNA during phentolamine infusion (during AVP injection, -7.12±1.60 %RSNA/mm Hg; control, -3.38±0.55 %RSNA/mm Hg). These data support the hypothesis that circulating AVP acts at the area postrema to augment baroreflex inhibition of RSNA by a V₁ receptor mechanism. (Circulation Research 1990;67:265–271)

Circulating arginine vasopressin (AVP) interacts with arterial and cardiopulmonary baroreflexes to modulate reflex inhibitory effects on the sympathetic nervous system.¹⁻⁴ When arterial pressure is elevated by infusions of AVP, the reflex inhibition of renal or lumbar sympathetic nerve activity is markedly greater than when pressure is raised by phentolamine.⁵⁻⁶ In addition, elevations in plasma AVP concentration that exert minimal effects on resting arterial pressure, heart rate, or renal sympathetic nerve activity (RSNA) enhance the ability of the arterial and cardiopulmonary baroreflexes to inhibit RSNA in response to another pressor stimulus or in response to volume expansion.⁶⁻⁷

The ability of AVP to augment reflex inhibitory effects is eliminated by lesion of the area postrema, suggesting that the area postrema mediates the interaction of AVP with baroreflexes.⁴⁻⁶ Recent work also indicates that low-level electrical stimulation of the area postrema causes a current- and frequency-dependent inhibition of RSNA and significantly augments the baroreflex-mediated inhibition of RSNA in response to phentolamine.⁸ Inhibition of sympathetic nerve activity can also be elicited by microinjection of glutamate directly into the area postrema.⁸

Previous work has also reported that reflexly or osmotically released AVP can modulate baroreflex control of the sympathetic nervous system.²⁻⁷⁻⁹ This interaction can be eliminated either by lesion of the area postrema or by intravenous administration of the specific V₁ vasopressin receptor antagonist. Therefore, the present study was designed to evaluate the effects of microinjection of small amounts of the V₁ receptor antagonist directly into the area postrema on the ability of circulating AVP to augment baroreflex inhibition of RSNA. In addition, the effects of microinjection of AVP directly into the area postrema on resting hemodynamic parameters and RSNA, and on baroreflex control of RSNA were examined.

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Materials and Methods

Preparation
Male New Zealand White rabbits (1.5–2.5 kg) were anesthetized with urethane (1 g/kg i.v.), prepared with an endotracheal tube, and mechanically ventilated. Arterial and venous catheters were inserted into the femoral artery and jugular vein for the measurement of arterial pressure and drug administration, respectively. By using a retroperitoneal approach, the left renal artery and vein were exposed and a branch of the left renal nerve was isolated. Two stainless steel electrodes were placed around the nerve, the nerve and electrodes were covered with a silicone gel, and the gel was allowed to harden. Details of the procedure for implantation of renal recording electrodes are described elsewhere.4

RSNA was amplified (model P511H, Grass Instrument Co., Quincy, Mass.), and whole nerve activity was obtained by rectifying and integrating the action potentials with a root mean square integrator with a time constant of 28 msec. This signal was then filtered at 0.08 Hz for quantitation. Background noise level was determined when nerve activity was eliminated by elevating arterial pressure with phenylephrine (8–10 μg/kg) or after ganglionic blockade with hexamethonium bromide (15 mg/kg i.v.) and atropine (0.05 mg/kg i.v.).

Area Postrema Microinjection
Vasopressin antagonist. After instrumentation, rabbits (n = 8) were placed in a stereotaxic head holder, a cervical incision was made, and the neck muscles were dissected to expose the cisternum magnum. The atlantooccipital membrane was cut and removed to expose the underlying brain stem. The arachnoid membrane was then severed and peeled from the brain stem surface. Under microscopic guidance, a glass micropipette (tip diameter, 5–15 μm) filled with the AVP V1 receptor antagonist (1β-mercapto-β,β-cyclopentamethylene propionic acid 2-[O-(methyl)tyrosine] arginine vasopressin (d[CH2][Tyr(Me)]AVP) was inserted into the area postrema. The electrode tip was positioned on the midline and inserted just below the surface of the area postrema (approximately 100–200 μm) midway between the rostral-caudal extent of the structure.

Arterial pressure was elevated with increasing doses of intravenously administered phenylephrine (0.5–12 μg/kg/min) for 1-minute periods, and baroreflex curves were constructed relating mean arterial pressure and RSNA. After a recovery period, increasing doses of AVP (5–125 ng/kg/min) were infused intravenously, and baroreflex responses were observed again. After a second recovery period, the AVP antagonist (100 ng) was injected into the area postrema in a volume of 10 nl by using a custom micropressure injection system with 100-pl resolution.10 Ejectate volumes were measured directly by monitoring the movement of the fluid meniscus in a pipette barrel with the aid of a ×150 compound microscope equipped with a fine reticule. Baroreflex responses to progressive infusions of phenylephrine and AVP were observed again.

To determine that the effect of the AVP antagonist was specific to the area postrema, baroreflex inhibitory effects on RSNA in response to intravenous phenylephrine and AVP were compared under control conditions and after administration of 100 ng of the AVP antagonist intravenously (n = 3) or microinjection into the medial nucleus tractus solitarius (NTS) 750 μm lateral to the area postrema site and at a depth of 200–300 μm (n = 2).

Vasopressin. The brain stem was exposed as in the previous experiments, and a glass micropipette filled with AVP was inserted into the area postrema. Vasopressin was then injected into the area postrema in volumes ranging from 2 to 20 nl (2 to 20 ng), and effects on resting arterial pressure, heart rate, and RSNA were observed. Volumes of AVP were administered as single pulses or slowly injected by a series of short-duration pressure pulses. In three animals, the AVP receptor antagonist (15 μg/kg) was administered intravenously, and microinjection of AVP into the area postrema was repeated. As a control for nonspecific pressure effects, equivalent volumes of artificial cerebrospinal fluid were injected into the area postrema.

In six rabbits, increasing doses of phenylephrine (0.5–12 μg/kg/min) were infused intravenously for 1-minute periods, and baroreflex curves were constructed. After a recovery period, vasopressin was continuously pulsed into the area postrema by short-duration (100–500-msec) pressure (0.1–3.0 atm) pulses at a rate that produced an average sustained reduction in resting RSNA of 7±3%. After RSNA stabilized at this level, infusions of phenylephrine were repeated while the injection of AVP was continued. Baroreflex responses to phenylephrine under control conditions and during AVP microinjection were then compared. The order of infusion under control conditions or during AVP microinjection was randomized.

Data Analysis
Effects of microinjection of the AVP antagonist or of AVP into the area postrema on resting arterial pressure, heart rate, and RSNA were evaluated by paired t test. Baroreflex function relating increases in arterial pressure with phenylephrine or AVP to RSNA was analyzed with linear regression analysis. Slopes of baroreflex curves for phenylephrine and AVP under control conditions or after intravenous administration of AVP antagonist into the NTS or area postrema were compared with two-way analysis of variance. The Student-Newman-Keuls multiple range test was used to determine significance when indicated by analysis of variance. Slopes of baroreflex curves for phenylephrine under control conditions and during administration of AVP into the area postrema were compared by paired t test. A probability level of p<0.05 was considered to be statistically significant.
Results

Microinjection of Arginine Vasopressin Antagonist Into the Area Postrema

Average resting mean arterial pressure and heart rate before any experimental manipulations were 83±5 mm Hg and 231±12 beats/min, respectively. Baroreflex curves relating increases in mean arterial pressure to inhibition of RSNA during intravenous infusions of sequentially increasing doses of phenylephrine and AVP under control conditions are presented in Figure 1. Progressive increases in mean arterial pressure with phenylephrine resulted in a linearly related reflex decrease in RSNA, with a slope of −3.95±0.26 %RSNA/mm Hg and r=−0.97±0.01 (Table 1). When arterial pressure was raised with systemic administration of AVP, the reflex inhibition of RSNA in response to any given increase in arterial pressure was greater than observed with phenylephrine. This is reflected in a significantly greater slope of the baroreflex relation (−8.84±0.89 %RSNA/mm Hg, r=−0.96±0.01) for AVP as compared with phenylephrine. Baroreflex inhibition of hear rate exhibited a similar pattern. The slope of the mean arterial pressure–heart rate relation for AVP was −3.00±0.81 beats/min/mm Hg compared with −1.21±0.07 beats/min/mm Hg for phenylephrine.

Microinjection of AVP antagonist (100 ng in 10 nl) into the area postrema resulted in no significant change in resting mean arterial pressure (83±5 versus 83±5 mm Hg), heart rate (230±13 versus 231±12 beats/min), or RSNA (+7±4%). Baroreflex responses to increases in arterial pressure caused by infusion of phenylephrine or AVP after microinjection of AVP antagonist into the area postrema are depicted in Figure 2. Reflex inhibition of RSNA in response to increases in arterial pressure with phenylephrine was similar to that observed under control conditions (Figure 2, Table 1), indicating that normal baroreflex function was not altered by local injection of the AVP antagonist into the area postrema. However, baroreflex responses to intravenous AVP were now similar to those observed for phenylephrine. The slope of the baroreflex relation for intravenous AVP after local injection of AVP antagonist into the area postrema was significantly reduced compared with control conditions or after intravenous (Table 1) or NTS injection of 100 ng of the AVP antagonist. In addition, the slopes for baroreflex responses to AVP and phenylephrine were no longer significantly different (−4.83±0.44 versus −4.10±0.33 %RSNA/mm Hg). The mean arterial pressure–heart rate relation for AVP (−1.09±0.24 beats/min/mm Hg) was also similar to that for phenylephrine (−0.91±0.14 beats/min/mm Hg) after injection of the AVP antagonist into the area postrema.

Intravenous administration of 100 ng of the V1 receptor antagonist (n=3) had no significant effect on resting arterial pressure, heart rate, or RSNA. In addition, the slopes of the baroreflex curves for phenylephrine and AVP were not altered by intra-

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TABLE 1. Slopes of Baroreflex Responses to Phenylephrine and Arginine Vasopressin Before and After Administration of Vasopressin Antagonist (100 ng i.v.) or Into the Area Postrema

<table>
<thead>
<tr>
<th></th>
<th>PE</th>
<th>AVP</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>−3.95±0.26</td>
<td>−8.84±0.89*</td>
</tr>
<tr>
<td>(n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVP antagonist (i.v.)</td>
<td>−3.94±0.51</td>
<td>−9.47±2.16*</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
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<tr>
<td>AVP antagonist (area postrema)</td>
<td>−4.10±0.33</td>
<td>−4.83±0.44†</td>
</tr>
<tr>
<td>(n=8)</td>
<td></td>
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Values are mean±SEM. PE, phenylephrine; AVP, arginine vasopressin.
*Significantly different from phenylephrine.
†Significantly different from control and intravenous administration of vasopressin antagonist.
venous application of AVP antagonist (Figure 3, Table 1). In two animals, NTS injection of AVP receptor antagonist also had no effect on baroreflex curves for AVP (4.6 %RSNA/mm Hg before versus 4.0 %RSNA/mm Hg after) or phenylephrine (2.7 %RSNA/mm Hg before versus 2.6 %RSNA/mm Hg after). The ability of AVP to augment baroreflex inhibition of RSNA in response to increases in arterial pressure was also not altered.

Microinjection of Arginine Vasopressin Into the Area Postrema

Micropressure injection of AVP into the area postrema resulted in an inhibition of RSNA and bradycardia. Figure 4 (top panel) shows the effects of injections of 1 ng (5 nl over 25 seconds), 2.4 ng (12 nl over 45 seconds), and 4.2 ng (21 nl over 60 seconds) of AVP into the area postrema on resting arterial pressure, heart rate, and RSNA in a single rabbit. Microinjection of AVP into the area postrema produced a dose-related inhibition of RSNA, with minimal effects on arterial pressure. Responses to microinjection of AVP into the area postrema were somewhat variable, probably because of the dense vasculature in the area postrema, which might be expected to result in rapid clearing of AVP from this region. However, local injection of 4±0.6 ng of AVP (1 ng/nl) in eight animals resulted in a significant inhibition (~27±3 %RSNA) (Figure 4, bottom panel). Equivalent or larger volumes of artificial cerebrospinal fluid injected directly into the area postrema were without effect. In three animals, the response to AVP microinjection into the area postrema was eliminated by intravenous injection of AVP receptor antagonist (15 μg/kg).

Baroreflex inhibition of RSNA in response to increases in arterial pressure with phenylephrine under control conditions and during local administration of AVP into the area postrema is illustrated in Figure 5 (n=6). During continuous administration of AVP into the area postrema at a rate that produced an average reduction in RSNA of 7±3%, baroreflex inhibition of RSNA in response to increases in arterial pressure was greater for any given pressure than observed under control conditions. Because the baseline level of RSNA was altered slightly by microinjection of AVP, the slope of the baroreflex relation was calculated both from the level of RSNA after stabilization of the response to AVP (Figure 5) and from the original control value before administration of AVP. When the level of RSNA attained during local administration of AVP into the area postrema was used as the baseline value (100%) of RSNA, the slope of the mean arterial pressure–RSNA relation was significantly augmented during local application of AVP (~7.12±1.60 versus ~3.38±0.55 %RSNA/mm Hg). Baroreflex inhibition of RSNA was also significantly enhanced (slope,
The circulating peptide hormone AVP augments the inhibitory effects of arterial baroreflexes. Baroreflex inhibition of renal or lumbar sympathetic nerve activity and heart rate is significantly greater during elevations in arterial pressure produced by infusions of AVP compared with equivalent pressure elevations caused by phenylephrine. This sympathoinhibitory action is dependent on an intact area postrema. Recent studies indicate that the response to low-intensity electrical or chemical (L-glutamate) stimulation of the area postrema resembles the actions of circulating AVP on baroreflex function. The effects of directly activating the area postrema include inhibition of resting RSNA as well as augmentation of the inhibitory effects of the baroreflex on RSNA. In view of these previous studies, it was postulated that AVP acts at the area postrema to augment the inhibitory effects of baroreceptors.

Previous studies have suggested that the actions of vasopressin to augment baroreflex inhibition of sympathetic nerve activity are related to sensitization of the baroreceptors or to an inhibition of sympathetic ganglionic transmission. In contrast, the findings of the present study provide strong support for a central rather than peripheral site of action of AVP. Perhaps the most pertinent result is that microinjection of the V₁ receptor antagonist (100 ng in 10 nl) directly into the area postrema completely abolished the augmentation of baroreflex responses to intravenous infusions of AVP. This same amount of AVP antagonist was without effect when delivered peripherally or into the NTS. If the mechanism of action of AVP were at the ganglionic level or at the level of peripheral baroreceptors, then central administration of AVP antagonist would not be expected to completely eliminate baroreflex augmentation by circulating vasopressin. These peripheral mechanisms may be operative, but their relative contribution to augmentation of the baroreflex is minor compared with the central effect. The importance of these peripheral mechanisms may, however, become more significant in conjunction with the central action of circulating AVP.

It is important to note that microinjection of the V₁ antagonist into the area postrema specifically abolished the enhancement of baroreflex inhibition of RSNA in response to AVP without altering normal baroreflex function. After injection of the antagonist into the area postrema, baroreflex effects of phenylephrine infusions were not modified, and increases in pressure during AVP infusions elicited reflex responses similar to those obtained with phenylephrine. Thus, the area postrema appears to specifically mediate the effects of AVP to modulate baroreflex function through a V₁ receptor mechanism.

The importance of the area postrema in mediating the interaction of circulating AVP with baroreflex function is further supported by the fact that microinjection of AVP directly into the area postrema produced a significant inhibition of RSNA at rest and a significant augmentation of baroreflex inhibition of RSNA in response to increases in pressure with phenylephrine. The effects of injection of AVP could be eliminated by intravenous administration of the V₁ antagonist. It is possible that some of the AVP that was injected into the area postrema leaked into the circulation through the dense vasculature in this region, accounting for some of the variability in response to AVP injection. Thus, some of the effects observed may have been due to an action of circulating AVP at a site other than the area postrema.

For example, the median preoptic nucleus has been suggested as a site that may be involved in the effects of AVP on heart rate. However, the fact that area postrema lesions, and in particular that microinjection of the AVP antagonist into the area postrema, blocks the effects of circulating AVP on baroreflex function supports the concept that the major site of action for the effects of AVP on the baroreflex is at the area postrema. These data also support previous work that indicates that the central interaction of reflexly or osmotically released AVP with arterial or cardiopulmonary baroreflexes could be eliminated by either intravenous administration of the V₁ antagonist or by lesion of the area postrema.

The changes in RSNA and baroreflex control of RSNA caused by injections of AVP into the area postrema are likely to result from changes in the

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Mean regression lines relating the reflex decrease in renal sympathetic nerve activity (RSNA) to increases in mean arterial pressure (MAP) with phenylephrine under control conditions (PE only) or during injection of vasopressin (AVP) into the area postrema [(AVP → AP) + PE] at a level that reduced resting RSNA by an average of 7±3% (n=6). Open circles indicate the mean values during PE infusion alone. Closed triangles indicate the mean values during PE infusion when AVP was injected into the area postrema.

% RSNA

**Discussion**

-8.02±2.21 versus -3.38±0.5 %RSNA/mm Hg) when the original control value used was 100% of RSNA. Baroreflex inhibition of heart rate was also augmented by local administration of AVP into the area postrema. The slope of the mean arterial pressure–heart rate relation for phenylephrine alone was 1.08±0.18 beats/min/mm Hg compared with -2.51±0.90 beats/min/mm Hg during injection of AVP.
discharge of neurons with AVP receptors on their somatodendritic membranes, but not on fibers of passage, in the immediate vicinity of the electrode tip. A mechanism of action via AVP receptor activation is supported by the ability of a selective AVP receptor blocking agent to antagonize the responses of AVP. Nonspecific effects caused by artifacts of pressure injection are ruled out by the absence of effects of equivalent or larger volumes of artificial cerebrospinal fluid or the antagonist. Pressure artifacts are also unlikely since the time course of action of AVP or its antagonist greatly outlasted the time of drug injection.

The effect of AVP on single neurons in the central nervous system has not been extensively investigated, although some results are known. Both excitation and inhibition of spontaneous discharge to iontophoretic application of AVP have been observed. Detailed studies of AVP effects in nonmammalian preparations indicate that AVP has an excitatory or augmenting effect on neuronal tissues and can induce bursting activity. Carpenter et al. have reported that AVP produces excitation of area postrema neurons, although this observation was based on a small number of cells. An excitatory action of AVP on neurons in the area postrema seems most likely since microinjection of AVP directly into this structure mimics the effects of electrical stimulation or L-glutamate microinjection. The effective spread of AVP or its antagonist is difficult to gauge without direct measurement. Several factors, such as available extracellular space, the number of receptors on regional neurons, and pharmacodynamics, would all affect the drug spread and response. The possibility that the observed responses are due to the spread of AVP from the area postrema into surrounding structures, particularly the NTS, is unlikely. Theoretical calculations predict that injectate concentrations would fall very rapidly within small distances (<300 μm) from the injection site when administered in similar volumes and concentrations as those used in this study. AVP inactivation mechanisms such as hydrolysis or active uptake by surrounding neurons or glia would further reduce the effective spread. The short latency onset of inhibition of RSNA after AVP injection is also not consistent with the idea of drug spread into the surrounding tissue. These observations strongly support the area postrema as the site of action of AVP, rather than adjacent structures.

The actions of AVP need not be confined to neurons intrinsic to the area postrema. Golgi studies of this region in cats indicate that some NTS neurons, particularly those with somas bordering the area postrema, send dendritic arborizations into the area postrema. Thus, it is possible that AVP could activate these neuronal processes. Lesions of the area postrema, or microinjections of drugs, could not effectively discriminate between activation of dendrites of NTS neurons and neurons residing entirely within the area postrema. However, the rather limited extent of arborization of these dendrites and their confinement to the deep portions of the area postrema diminishes their possible importance in mediating AVP responses. A more likely possibility involves catecholaminergic neurons intrinsic to the area postrema. These cells reside in close proximity to blood vessels within the area postrema and form numerous axodendritic synapses within the adjacent dorsolateral and medial NTS. Recent work from this laboratory indicates that intracisternal 6-hydroxydopamine treatment eliminates the augmentation of the baroreflex by intravenous AVP. Depletion of catecholamine terminals in the adjacent NTS appears to be related to the effects of 6-hydroxydopamine.

The lack of major effect of the AVP antagonist on resting RSNA, blood pressure, or heart rate suggests several possibilities concerning AVP’s actions on the neurons producing the reflex inhibitory responses. AVP may excite neurons with somatodendritic membranes in the vicinity of the injection pipette, but these neurons may not fire spontaneously or may discharge with a low spontaneous rate in our experimental preparation. Therefore, AVP receptor blockade would have no substantial effect. The lack of effect of AVP antagonist on baroreflex function also suggests that endogenous levels of AVP in anesthetized rabbits may not be sufficiently high to influence neurons in the area postrema or that these neurons have adapted to the given level of circulating AVP.

In summary, microinjection of the specific V1 receptor antagonist into the area postrema eliminated the ability of intravenous AVP to augment arterial baroreflex inhibition of RSNA, without altering normal baroreflex responses to phenylephrine. In addition, microinjection of AVP into the area postrema inhibited resting RSNA and augmented the inhibitory effects of arterial baroreflexes. These data suggest that the area postrema mediates the modulation of baroreflex function by circulating AVP via a V1 receptor mechanism.

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References


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