Role of Central Catecholamines on the Potentiation of the Baroreflex Produced with Vasopressin

A Study Using 6-Hydroxydopamine

Karl P. Undesser, Angelo J. Trapani, William W. Morgan, and Vernon S. Bishop
From the Department of Pharmacology, Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas

SUMMARY. This study compares the effect of arginine-vasopressin and phenylephrine on renal sympathetic nerve activity, arterial pressure, and heart rate in vehicle- and intraventricular 6-hydroxydopamine-treated conscious rabbits. In addition, this study examines the involvement of forebrain structures on these variables. In vehicle-treated rabbits, compared to phenylephrine, vasopressin produces a greater decrease in renal sympathetic nerve activity and heart rate for a given increase in pressure. After administration of intraventricular 6-hydroxydopamine, the responses produced with vasopressin are similar to those produced with phenylephrine. Analysis of catecholamine content in the nucleus of the solitary tract, parabrachial nucleus, area postrema, and spinal cord indicates that the only regions of significant catecholamine depletion are in the nucleus of the solitary tract and the spinal cord. Transection at the mid-collucular level does not alter significantly the responses of vasopressin or phenylephrine on renal sympathetic nerve activity or heart rate. This indicates that forebrain structures are not involved in mediating the enhanced buffering effect produced with vasopressin. (Circ Res 58: 882-889, 1986)

WE recently have demonstrated that the reflex decrease in renal sympathetic nerve activity and heart rate for a given increase in arterial pressure is greater when pressure is elevated with vasopressin than when elevated with phenylephrine (Undesser et al., 1985a). The augmented baroreflex response to vasopressin is eliminated by lesions of the area postrema. Although this enhanced buffering ability of vasopressin is abolished by the lesion, these studies did not indicate whether vasopressin acts at the area postrema or whether the area postrema contains fibers of passage that are involved in the response. Studies by Pardridge (1981) and Wang et al. (1981) indicate that peptides such as vasopressin do not cross the blood-brain barrier, and thus, the central site of action for vasopressin must involve a circumventricular organ. An involvement of the hindbrain in the central action of vasopressin is supported by the studies of Liard et al. (1981) in which they demonstrated that vasopressin causes a greater reduction in cardiac output when infused into the vertebral artery, as opposed to systemic administration.

The area postrema has been shown to send projections to nuclei involved in cardiovascular function. Specifically, the area postrema projects to the nucleus of the solitary tract (NTS) and the parabrachial nucleus in the rostral pons (Morest, 1967; Vigier et al., 1979; Van der Koog et al., 1983). The area postrema has also been shown to contain noradrenergic cell bodies (Fuxe and Owman, 1965; Torack et al., 1973), some of which may project to the parabrachial nucleus and NTS (Morest, 1967; Armstrong et al., 1981). The purpose of our study was to determine whether central catecholaminergic projections participate in the enhanced buffering ability of vasopressin. In addition, decerebration was utilized to determine whether the integrity of neural connections between hindbrain and forebrain cardiovascular control centers is required for vasopressin to exert its central effect on baroreflex function.

Methods

General Protocol

Experiments were performed on male New Zealand white rabbits (1.5-2.5 kg). Rabbits were anesthetized with pentobarbital sodium (25 mg/kg) administered intravenously (iv) into the marginal ear vein. Catheters were inserted into the femoral artery for monitoring arterial pressure and into the jugular vein for administration of drugs. The left kidney was exposed retroperitoneally. Renal nerves were isolated, and two stainless steel electrodes were placed around the nerves. The electrodes were fabricated from seven strands of wire, 0.002 inch in diameter over quad Teflon 0.009 inch (Medwire Corp.). The nerves and electrodes were covered with silicone gel.
Systemic arterial pressure and heart rate were displayed electrically shielded holding unit. Arterial pressure was monitored by means of the arterial catheter connected to a Gould Statham (P23Db) pressure gauge transducer. Heart rate (HR) was obtained from a Beckman cardiometer (9857B) triggered by the arterial pressure pulse. Renal sympathetic nerve activity (RSNA) was amplified by a Grass P511H preamplifier and filtered to exclude frequencies outside the range of 100–3000 Hz. A recording of RSNA from a rabbit instrumented chronically is shown in Figure 1. This recording was obtained 3 days after electrode implantation. Whole nerve activity was determined by rectifying and integrating the action potentials with a root mean square integrator (RMS). The RMS had a 28-msec time constant. The integrated signal was filtered at 0.08 Hz (time constant = 198 sec) for quantification. The baseline level of RSNA was normalized to 100% in all animals. Background noise was determined when nerve activity was completely suppressed by increasing arterial pressure. Before any experimental intervention, rabbits were allowed at least 60 minutes to adjust to the laboratory environment. Only rabbits that exhibited a stable level of RSNA over the 60-minute period were used in these studies. Baroreflex curves were generated by progressive 3-minute infusions of either phenylephrine (0.5–12 μg/kg per min, Winthrop Lab) or arginine vasopressin (2–50 μU/kg per min, Bachem Inc.), administered in random order. Curves produced in this manner gave reproducible results (Undesser et al., 1985b). All experiments were performed in conscious rabbits.

**Central Catecholamine Depletion**

Rabbits were treated with either 6-hydroxydopamine hydrobromide (600 μg/kg) or vehicle injected into the 4th cerebral ventricle. Animals were anesthetized with pentobarbital sodium (25 mg/kg) and placed in a stereotaxic head holder. The muscles at the back of the neck were dissected to expose the atlanto-occipital membrane. A 26-gauge needle attached to a 1-cc syringe was inserted into the 4th ventricle, and 250 μl of cerebrospinal fluid were withdrawn. This volume was replaced with either 6-hydroxydopamine (6-OHDA) in 1% ascorbic acid or vehicle. Vehicle or 6-OHDA was gassed with nitrogen for 2 minutes prior to injection. Vehicle contained only 1% ascorbic acid in saline. A minimum of 7 days was allowed for catecholamine depletion before electrodes and catheters were implanted. This method has been demonstrated to be adequate to deplete central nerve terminals of catecholamines (Chalmers and Reid, 1972).

**Quantification of Tissue Catecholamine Levels**

Rabbits were killed with an injection of pentobarbital sodium (iv) following the experimental protocol. The brain, spinal cord, and heart of each animal were removed, frozen on dry ice, and placed in a −80°C freezer for storage until the time for analysis of tissue catecholamine levels. On the day of analysis, the tissues were removed from the freezer and mounted on a microtome (American Optical). Frozen coronal brain sections were cut (600 μm) and placed on a glass plate which was cooled with dry ice. Under a dissecting microscope, tissue punches in the area postrema, nucleus of the solitary tract, and the parabrachial nucleus were made with a 22-gauge needle. A section of the spinal cord halfway between the cervical and lumbar enlargement and a portion of the right atrium of the heart also were taken. The tissue punches were stored in containers and kept frozen on dry ice.

Norepinephrine and dopamine values were quantified by liquid chromatography with electrochemistry. The methodology used was similar to that described by Proll et al. (1982). Briefly, tissue was sonified in 0.1 N HClO4 (200 μl) that contained 2 ng of 3,4-dihydroxybenzylamine as an internal standard and 10 mm sodium metabsulphite. Sonified samples were centrifuged for 4 minutes in a microfuge (Eppendorf Centrifuge 3200). The supernatant was added to 15 mg of acid washed alumina, and the pellet was saved for protein analysis by the method of Lowry et al. (1951) adapted for the Technicon Autoanalyzer. Catecholamines were allowed to adsorb on the alumina and then were eluted with acid. Samples of the eluate were injected into the Beckman liquid chromatograph. All values are presented as ng/mg protein. Content was determined by comparing the peak height of the unknown sample to that of the internal standard.

**Decerebration**

Rabbits were instrumented with electrodes and catheters, and control arterial baroreflex curves were obtained with infusion of phenylephrine (PE) and vasopressin (AVP) in conscious animals as previously described. After control curves had been obtained, the rabbits were anesthetized with halothane, intubated, and placed in a stereotaxic head holder. The cranium was removed at a level that exposed the inferior and superior colliculi. A mid-collricular transection was performed with a spatula. After wound closure and the removal of halothane, ani-
mals were given at least 60 minutes to recuperate before the experimental protocol was performed. Vasopressin and phenylephrine curves were repeated.

### Statistical Analysis

The slopes of the MAP-RSNA and MAP-HR curves were determined using a least-squares best-fit linear regression analysis (Snedecor and Cochran, 1967). The significance of the linearity of each curve was tested by means of analysis of variance. In all cases, there was a significant linear relationship over and above any curvilinear relationship. The mean correlation coefficient for all curves was 0.9870 ± 0.012 (range = 0.4489–0.9935). The statistical significance of differences in the slopes and in tissue catecholamine concentrations was determined by paired and unpaired Student’s t-tests, where appropriate (Snedecor and Cochran, 1967). Statistical analysis of the dose-response relationships was performed by analysis of variance with repeated measures. The criterion for significance was $P < 0.05$. All values are expressed as mean ± SEM.

### Results

#### Central Catecholamine Depletion

Intraventricular treatment with 6-OHDA did not alter body weight. There were no significant differences in body weight between pre- (2.02 ± 0.07 kg), post- (2.06 ± 0.2 kg), and vehicle-treated (1.90 ± 0.18 kg) animals. In addition, 6-OHDA treatment did not significantly alter resting MAP (84 ± 8 vs. 93 ± 3) or HR (290 ± 13 vs. 284 ± 22) in sham vs. 6-OHDA-treated rabbits, respectively.

In five vehicle-treated rabbits, progressive infusions of PE resulted in a continuous decrease in both RSNA and HR (Fig. 2). RSNA was reduced to 50% and 0% of control when MAP had increased 6 ± 2 mm Hg and 17 ± 2 mm Hg, respectively. HR was decreased by 60 beats/min when MAP had increased 10 ± 3 mm Hg. In vehicle-treated animals, RSNA was reduced to zero after small increases in MAP (2 mm Hg) produced by AVP infusions. HR was also decreased to a much greater extent for a given increase in MAP with AVP, compared to that with PE. The slopes of the MAP-RSNA and MAP-HR relationships generated with AVP were significantly ($P < 0.05$) greater than those produced with PE (Table 1). The results seen in Figure 2 are similar to those obtained with untreated rabbits, indicating that vehicle alone does not effect normal reflex responses.

Intracisternal administration of 6-OHDA altered the slope of the relationship of decreases in RSNA and HR for increases in MAP produced with AVP (Fig. 3). After treatment with 6-OHDA, the arterial baroreflex response to AVP was similar to that produced with PE. However, 6-OHDA did not affect the RSNA and HR response produced with PE, indicating normal baroreflex function (Table 1). Thus, central administration of 6-OHDA caused the baroreflex control of RSNA and HR during AVP infusions to be similar to that produced with PE.

#### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>6-OHDA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Phenylinephrine</td>
<td>Vasopressin</td>
</tr>
<tr>
<td>MAP-RSNA</td>
<td>6 22 ± 0 51†</td>
<td>26 16 ± 2 62‡</td>
</tr>
<tr>
<td>MAP-HR</td>
<td>4 85 ± 0 86†</td>
<td>28 69 ± 3 78‡</td>
</tr>
<tr>
<td>$n$</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

* Treated with intracisternal vehicle or 6-OHDA injections produced with phenylephrine and vasopressin
† Significantly different at $P < 0.05$ between phenylephrine and vasopressin within the same group
‡ Significantly different at $P < 0.05$ for same drug between groups
Decreases in Renal Sympathetic Nerve Activity and Heart Rate in 6-OHDA Treated Animals

**Figure 3.** Responses of renal sympathetic nerve activity (RSNA) and heart rate (HR) to increases in arterial pressure (MAP) produced with phenylephrine (PE) or vasopressin (AVP) in 6-OHDA-treated conscious rabbits. The decreases in RSNA and HR to increases in MAP produced with PE and AVP are similar. RSNA is represented in arbitrary units where 100 is the amount of activity present in the resting animal.

**Quantification of Catecholamines**

The levels of tissue norepinephrine found in the area postrema, heart, NTS, parabrachial nucleus, and spinal cord are shown in Table 2. There was no significant difference in norepinephrine levels between 6-OHDA- and vehicle-treated rabbits in the area postrema, heart, or parabrachial nucleus. 6-OHDA treatment significantly decreased norepinephrine levels in the NTS (−49%) and the spinal cord (−64%). 6-OHDA treatment did not alter dopamine levels in any of these brain regions or the heart.

**Dose-Response Curves**

Dose-response curves for MAP and RSNA were generated for AVP and PE in 6-OHDA- and vehicle-treated rabbits. Increasing doses of PE produced

**Figure 4.** Phenylephrine dose-response curves for increases in arterial pressure (MAP) and decreases in renal sympathetic nerve activity (RSNA) for 6-OHDA- and vehicle-treated rabbits. 6-OHDA does not alter the dose-response relationship from vehicle-treated rabbits.

Increases in arterial pressure and decreases in RSNA for both groups of rabbits (Fig. 4). Comparison showed no significant differences between 6-OHDA- and vehicle-treated groups.

Increasing doses of vasopressin had no effect on arterial pressure until the highest dose in vehicle-treated rabbits (Fig. 5). After 6-OHDA treatment, increasing doses of AVP resulted in progressive increases in arterial pressure. There were no differences in the decreases in RSNA produced by increasing doses of AVP when 6-OHDA- and vehicle-treated rabbits were compared.

**Table 2**

<table>
<thead>
<tr>
<th>Area postrema</th>
<th>Heart</th>
<th>NTS</th>
<th>Parabrachial nucleus</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>6-OHDA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

All values are expressed as ng/mg protein

* P ≤ 0.05
Decerebration
In five conscious rabbits, MAP, HR, and RSNA were measured in response to progressive infusions of AVP and PE before and after mid-colicular decerebration (Figs. 6 and 7). We ran an internal control in these experiments by always comparing the reflex curves produced with AVP with those produced with PE. In addition, in three rabbits, sham decerebrations were performed in which the animals were run through the same protocol as the decerebrate rabbits; however, after the cranium was opened, the brain was not transected. In these rabbits, no difference was seen in the PE and AVP curves, compared to the pretransected group of rabbits. There was no difference in the baseline level of MAP before (81 ± 7 mm Hg) and after (81 ± 4 mm Hg) decerebration. The transection resulted in a significant decrease in resting HR from 289 ± 13 to 184 ± 10 beats/min. The slopes of the MAP-RSNA and MAP-HR relationships produced with AVP and PE were significantly different before decerebration (Table 3). After the transection, the slopes of MAP-RSNA relationship produced with PE and AVP remained significantly different from each other (Fig. 7), and neither curve was different from the corresponding curve obtained before decerebration. The slopes of the MAP-HR relationship produced with AVP and PE were not significantly different from one another in decerebrate animals. This may have been due to the decrease in resting heart rate and large variability observed in the MAP-HR relationship following decerebration. However, it should be noted that, in every rabbit, the MAP-HR relationship produced with AVP had a greater slope than that produced with PE.

Discussion
The major findings of this study are that intravenicularly applied 6-OHDA abolishes the enhancing effects of AVP on arterial baroreflex control of RSNA, without altering normal baroreflex control during pressure increases with PE. This effect is correlated with a reduction in catecholamine content in the NTS and spinal cord. In addition, the experimentation in decerebrate animals demonstrates that the integrity of neural connections between forebrain regions and more caudal cardiovascular structures is not required for the expression of the central actions of vasopressin.
FIGURE 7. Responses of renal sympathetic nerve activity (RSNA) and heart rate (HR) to increases in arterial pressure (MAP) produced with phenylephrine or vasopressin in mid-collicular transected conscious rabbits. RSNA and HR are decreased to a greater extent for a given increase in MAP produced with AVP as compared to PE. RSNA is represented in arbitrary units where 100 is the amount of activity present in the resting animal. For simplicity, only two points were drawn for the HR response to AVP on this curve. The points included are the mean value of HR where MAP begins to increase and the final value at the highest dose of AVP administered. Statistical analysis was performed using at least four data points following the increase in MAP.

In the present study, micropunches of various brain regions were performed to determine the extent of 6-OHDA catecholamine depletion. No change was seen in heart tissue, indicating that 6-OHDA administered into the 4th ventricle at 600 µg/kg did not affect peripheral nerve terminals. Administration of 6-OHDA did not affect norepinephrine content in the parabrachial nucleus or the area postrema. A lack of depletion in the parabrachial nucleus is not surprising, since it is an area that lies deeper within the brainstem than the NTS or area postrema. 6-OHDA administered intraventricularly may not have reached this area. Although norepinephrine content in the area postrema was not significantly decreased, it had a tendency to be depleted. However, the area postrema probably is not a site where 6-OHDA is acting to alter the effect of AVP, since the selective microinjection of 6-OHDA into this area did not alter the response (unpublished observation).

The regions where significant norepinephrine depletion was seen were the NTS and spinal cord. Decreased norepinephrine content in the spinal cord agrees with the studies of Chalmers and Reid (1972). The spinal cord also was the area of maximum depletion in their study. They noted a 52% depletion of norepinephrine in the medulla-pons. This value is comparable to the 49% depletion we noted in the NTS in the present study.

The NTS is richly innervated by catecholaminergic neurons. The area postrema contains noradrenergic projections to the NTS (Vigier and Portalier, 1979). Bilateral injections of 6-OHDA into the NTS have shown various results. Snyder et al. (1978) demonstrated that arterial pressure became labile and HR was unaffected following chronic microinjections of 6-OHDA into the NTS. Baroreflex gain was reduced but not abolished. Other studies by Healy et al. (1981) demonstrated a persistent bradycardia following 6-OHDA microinjections into the A2-cell group of the NTS. Mean arterial pressure was unchanged. In the present study, 6-OHDA had no effect on the basal arterial pressure or heart rate. Furthermore, baroreflex control of renal sympathetic nerve activity and heart rate also were not affected. The differences seen in those studies, compared to ours in the present investigation, may be due to the varying degree of destruction produced by 6-OHDA in these brainstem regions. In the study by Korner et al. (1978), rabbits that received 6-OHDA exhibited a significant decrease in body weight which may have contributed to the observed changes in resting MAP and HR. In our studies, the effect of 6-OHDA on normal baroreflex function is relatively subtle and difficult to detect with the degree of residual noradrenaline seen in these experiments. However, 6-OHDA did prevent the facilitation of baroreflexes.

### TABLE 3
Slopes of the Relationship between Arterial Pressure, Renal Sympathetic Nerve Activity, and Heart Rate*

<table>
<thead>
<tr>
<th></th>
<th>Before Phenylephrine</th>
<th>After Phenylephrine</th>
<th>Before Vasopressin</th>
<th>After Vasopressin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP-RSNA</td>
<td>4.4 ± 0.7 f</td>
<td>4.1 ± 2.1 f</td>
<td>17.8 ± 6.6</td>
<td>15.4 ± 5.7</td>
</tr>
<tr>
<td>MAP-HR</td>
<td>5.1 ± 0.9 f</td>
<td>2.3 ± 0.3 f</td>
<td>15.2 ± 3.0</td>
<td>10.9 ± 4.1</td>
</tr>
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* Before and after brain transection produced with phenylephrine and vasopressin
f Significantly different at P ≤ 0.05 for phenylephrine and vasopressin within same group
† Significantly different at P ≤ 0.05 for same drug between groups
by vasopressin. Previous studies performed in our laboratory have indicated that the area postrema is involved in mediating the effect of AVP. The noradrenergic pathway from the area postrema to the NTS may be an important pathway in eliciting this response.

Intracisternal 6-OHDA normalized the MAP-RSNA and MAP-HR relationship for AVP to that of PE. The MAP dose-response curve was shifted to the left so that the baroreflex-buffering ability of AVP was not as great following 6-OHDA administration. The PE dose-response curves were unaffected by 6-OHDA. Similar results were seen with lesions of the area postrema (Undesser et al., 1985a). In control rabbits, decreases in RSNA and HR produced by AVP are dose-dependent but not pressure-dependent. Following lesions of the area postrema or intracisternal 6-OHDA, the decreases in RSNA and HR are both dose- and pressure-dependent. In the latter case, decreases in RSNA and HR are produced by increases in arterial pressure, as they would be with phenylephrine. There appears to be a fine regulation of vasopressin on the baroreflex in studies performed in area postrema-lesioned rabbits, as well as those treated with intraventricular 6-OHDA. These studies indicate the involvement of both the area postrema and central catecholaminergic projections in mediating the enhanced buffering ability of circulating AVP.

The results of studies examining the involvement of supramedullary structures on the baroreflex function are equivocal. In the present study, no significant changes were observed in the baroreflex curves generated with AVP or PE in decerebrate rabbits. However, the variability seen in the reflex curves generated in the transected animals was much greater than in intact rabbits. In three of the five rabbits, the increase in pressure necessary to eliminate RSNA in the decerebrate state was higher than in the intact state. In the remaining two rabbits, no change was seen. Katz et al. (1967) demonstrated that decerebration in the cat does not eliminate the response to carotid occlusion. In addition, Chai et al. (1963) showed that mid-collicular decerebration does not alter the vasomotor response or the cardiac response elicited by dorsal medulla or sciatic nerve stimulation, or by bilateral carotid occlusion. On the other hand, Reis and Cuenod (1965) demonstrated that the pressor response to carotid occlusion is reduced while the depressor response to sinus stretch is augmented in decerebrate cats.

Transmission of the brain at the mid-collicular level interrupts the neural connections between all circumventricular organs, except the area postrema and cardiovascular centers caudal to the midbrain. Since peptides probably do not readily cross the blood-brain barrier (Pardridge et al., 1981), the area postrema is a prime candidate as the central site of action of AVP. Baroreflex control of RSNA and HR in decerebrate rabbits was not significantly different from that of intact rabbits, in the present study. Therefore, the neural mechanisms that underlie the enhanced buffering effect of AVP appear to be located in the hindbrain or spinal cord.

In conclusion, these studies demonstrate that the enhanced buffering ability of AVP as compared to PE involves central catecholaminergic pathways which do not affect normal baroreflex function. Furthermore, brain regions rostral to the midbrain are not involved in the interaction of AVP with the arterial baroreflex.

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Dr. Morgan is affiliated with the Department of Cellular and Structural Biology.

Address for reprints: Vernon S. Bishop, Department of Pharmacology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7704.

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