Intravenous 6-hydroxydopamine attenuates vasopressin and oxytocin secretion stimulated by hemorrhage and hypotension but not hyperosmolality in rats

**Stocker, Sean D., Melinda E. Wilson, Christopher J. Madden, Usman Lone, and Alan F. Sved.** Intravenous 6-hydroxydopamine attenuates vasopressin and oxytocin secretion stimulated by hemorrhage and hypotension but not hyperosmolality in rats. *Am J Physiol Regul Integr Comp Physiol* 291: R59–R67, 2006. First published February 23, 2006; doi:10.1152/ajpregu.00772.2005.—The present study sought to determine whether chemical destruction of peripheral catecholaminergic fibers with 6-hydroxydopamine (6OHDA) attenuates vasopressin (VP) and oxytocin (OT) secretion stimulated by hemorrhage, hypotension, and hyperosmolality. Rats received 6OHDA (100 mg/kg iv) or vehicle (1 ml/kg iv) on days 1, 2, and 7, and experiments were performed on day 8. Serial hemorrhage (4 samples of 2 ml per 300 g body wt at 10-min intervals) increased plasma VP and OT levels in both groups; however, the increase in plasma VP and OT levels was significantly attenuated in 6OHDA-treated vs. control rats despite a significantly lower mean arterial blood pressure. Similarly, the increase in plasma VP and OT levels in response to hypotension produced by the selective arteriolar vasodilator diazoxide was significantly attenuated in 6OHDA-treated rats. In marked contrast to hemorrhage and hypotension, hyperosmolality produced by an infusion of 1 M NaCl (2 ml/h iv) increased plasma VP and OT levels that were not different between 6OHDA-treated and control rats. In a parallel set of experiments, intravenous 6OHDA treatment reduced dopamine-β-hydroxylase immunoreactivity in the posterior pituitary but had no substantial effect in the hypothalamic paraventricular and supraoptic nuclei. In each experiment, the pressor response to tyramine (250 μg/kg iv) was significantly attenuated in 6OHDA-treated rats, thereby confirming that 6OHDA treatment destroyed sympathetic catecholaminergic fibers. Collectively, these findings suggest that catecholaminergic fibers located outside the blood-brain barrier contribute to VP and OT secretion during hemorrhage and arterial hypotension.

**MAGNOCELLULAR NEUROSECRETORY** neurons of the hypothalamic supraoptic and paraventricular nuclei synthesize vasopressin (VP) and oxytocin (OT) and release these hormones directly into the circulation from axon terminals located in the posterior pituitary. VP promotes water reabsorption in the kidney by altering membrane permeability of the distal tubules and collecting ducts and, at higher levels, produces vasoconstriction (6). OT, in addition to its well-known roles in lactation and parturition, stimulates natriuresis in rats (33) and modulates hypotension-evoked renin secretion (13). Together, these hormones act in concert with other physiological responses (e.g., ingestion of water and salt, changes in autonomic nervous system activity) to regulate body fluid homeostasis under a variety of circumstances, including alterations in blood volume, arterial blood pressure, or plasma osmolality (Posmol).

The secretion of VP and OT is influenced by a number of neuroactive substances acting within the supraoptic and paraventricular nuclei (25). For example, noradrenergic fibers originating from the central nervous system innervate both VP and OT magnocellular neurons (32). Functional studies suggest that these noradrenergic inputs relay visceral information from the hindbrain to affect the excitability of putative VP and OT neurons and subsequent release of VP and OT (4, 11, 25, 26). On the other hand, VP and OT secretion may also be modulated by substances acting directly at the level of magnocellular nerve terminals in the posterior pituitary. For example, the posterior pituitary is innervated by catecholaminergic fibers that likely arise from both central and peripheral origins (3, 10). With regard to the latter, removal of the superior cervical ganglion decreases posterior pituitary norepinephrine (1, 19), and electrical stimulation of the superior cervical ganglion increases VP and OT secretion (16, 17). Recently, we reported that intravenous infusion of the α1-adrenergic agonist phenylephrine at doses that do not cross the blood-brain barrier potentiate plasma VP and OT levels in hyperosmotic rats (29).

Collectively, these observations suggest that peripheral catecholaminergic inputs to the posterior pituitary enhance VP and OT secretion.

In the present study, we sought to determine whether the catecholaminergic fibers located outside the blood-brain barrier contributed to VP and OT secretion during various physiological challenges. Peripheral catecholaminergic fibers were destroyed by intravenous administration of the neurotoxin 6-hydroxydopamine (6OHDA), and VP and OT secretion was stimulated by hemorrhage, hypotension, or hyperosmolality.

**METHODS**

**Animals**

Adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 300–400 g were housed individually in a temper-
Chemical Sympathectomy

Chemical sympathectomy was performed in rats with intravenous administration of 6OHDA (100 mg/kg) on days 1 and 7, as described previously (7, 18). On day 1, rats were anesthetized with halothane (2–3% in 100% O2), and 6OHDA followed by 0.3 ml isotonic saline was given through a Silastic catheter inserted into the right jugular vein. The catheter was removed, the jugular vein tied off, and the incision closed with suture. Rats received an injection of antibiotic (Dual-cillin, 30,000 U im) and were returned to home cages. On day 7, catheters were implanted in the left femoral artery (Silastic or Microcath tubing, 0.012 in. ID and 0.025 in. OD, Braintree Scientific, Braintree, MA) and vein (Silastic, 0.025 in. ID and 0.037 in. OD, Fisher Scientific, Pittsburgh, PA) while rats were anesthetized with halothane (2–3% in 100% O2). These catheters were tunneled subcutaneously to exit between the scapulae and were filled with heparinized saline (arterial, 1,000 U/ml; venous, 40 U/ml). Rats were fitted with an infusion harness (Harvard Apparatus) that allowed the catheters to pass outside the cage while protected by a steel spring. Then, rats were injected with 6OHDA through the femoral venous catheter. On both days, control rats received 0.1 M ascorbic acid vehicle (1 ml/kg iv). Rats received an injection of antibiotic (Dual-cillin, 30,000 U im), and experiments began the next day.

Effect of 6OHDA Treatment on VP and OT Secretion

At least 1 h before experiments began, rats were weighed and returned to the home cage without food and water. Arterial blood pressure was recorded by connecting the arterial line to a Statham pressure transducer (Grass Instruments, Quincy, MA) and a polygraph chart recorder (model 7G, Grass Instruments). The pulsatile arterial blood pressure signal was filtered electronically to obtain mean arterial blood pressure (MAP). Heart rate (HR) was obtained through a tachograph (Grass Instruments, Model 7P44) triggered by the pulsatile arterial blood pressure signal.

Tyramine test. The completeness of the chemical sympathectomy was assessed by the magnitude of the pressor response to tyramine (250 μg/kg iv) in both 6OHDA-treated and control rats. Tyramine stimulates vasoconstriction and increases blood pressure by promoting endogenously stored norepinephrine release from sympathetic nerve terminals (7, 12). Each rat was tested three times separated by 5 min, and the peak changes in MAP were averaged. Hemorrhage, hypotension, or hyperosmolality protocols began 1 h after the last tyramine injection.

Hemorrhage. Rats were hemorrhaged serially as described previously (23, 24). After a 20-min recording of baseline MAP and HR, four successive blood samples (2 ml/300 g body wt over 1 min) at 10-min intervals were collected from the arterial line into microcentrifuge tubes containing heparin (20 U/1.5 ml blood). Samples were centrifuged immediately (10,000 g, 1 min), and the plasma was stored at −80°C until VP and OT levels were determined by radioimmunoassay, as described below. Plasma VP and OT levels were determined by radioimmunoassay in the posterior pituitary, hypotalamic nuclei, and forebrain circumventricular organs.

Hypotension. A second group of 6OHDA-treated and control rats received an injection of the arteriolar vasodilator diazoxide (DZX; 15 mg/kg iv) to decrease MAP. Because destruction of sympathetic nerves would be expected to exaggerate the hypertensive effect of DZX, an additional group of control rats received a larger dose of DZX (25 mg/kg iv) to produce similar degrees of hypotension as those observed in 6OHDA-treated rats receiving DZX (15 mg/kg iv). The dose of DZX will be referred to as DZX plus the respective dose (i.e., DZX15 or DZX25). Blood samples (2.0 ml) were collected from the arterial line into a microcentrifuge, as described above, at 5 min before initiation of the 1 M NaCl infusion, and 10 and 30 min after the start of the infusion. Plasma VP and OT levels were determined as described in the experiments in Hemorrhage.

Effect of 6OHDA Treatment on Catecholaminergic Innervation of the Posterior Pituitary, Hypothalamic Nuclei, and Forebrain Circumventricular Organs

To determine whether 6OHDA treatment destroyed catecholaminergic innervation of the posterior pituitary without affecting the innervation of structures inside the blood-brain barrier, we examined dopamine-β-hydroxylase (DBH) immunoreactivity in the posterior pituitary and hypothalamic structures in 6OHDA and vehicle-treated rats. A separate group of rats were treated with 6OHDA (n = 3) or vehicle (n = 3) on day 1 and 7, and the pressor response to tyramine (250 μg/kg iv) was examined as described above. Then, rats were deeply anesthetized with Inactin (100 mg/kg iv) and perfused transcardially with 100 ml saline followed by 250 ml of 4% formaldehyde in 0.1 M PBS (4°C). Brains and pituitaries were removed, postfixed overnight in 4% paraformaldehyde in 0.1 M PBS (4°C), and immersed in 30% sucrose for 2–3 days.

Pituitaries were sectioned at 20 μm using a cryostat, mounted on slides in a series of 1 in 8, and stored at −20°C. Tissue sections were then rinsed with 0.1 M PBS and 0.1% Triton X-100, incubated with 0.3% Triton X-100 in 100% methanol for 1 h, and quenched endogenous peroxidase activity, and blocked with 0.3% Triton X-100, and 10% normal donkey serum (blocking solution) for 30 min. Sections were incubated overnight at 4°C with monoclonal mouse anti-DBH antibody (1:500, MAB308; Chemicon, Temecula, CA) in 4% paraformaldehyde in 0.1 M PBS (4°C) and rinsed with PBS. Finally, sections were incubated with a biotinylated secondary antibody for 1 h at room temperature. Then, peroxidase activity was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. Axon terminal-like structures were confirmed by immunoreactivity in the posterior pituitary.
blocking solution followed by a 1-h incubation at room temperature with a Cy3 donkey anti-mouse IgG (1:100, Jackson ImmunoResearch Laboratories, West Grove, PA). Forebrains were sectioned at 30 μm using a cryostat and collected into 0.1 M PBS at 4°C in a one in three series. Then, free-floating sections were rinsed with 0.1 M PBS, and incubated with a monoclonal anti-DβH antibody (1:500 MAB308; Chemicon) at 4°C for 48 h followed by an overnight incubation in a Cy3 donkey anti-mouse IgG (1:100, Jackson Immunoresearch Laboratories). Sections were mounted on slides and coverslipped with Cytoseal 60 (Fisher Scientific).

**Statistical Analysis**

All data are expressed as means ± SE. MAP and HR were analyzed by a two-way ANOVA with repeated measures (Systat 10.2, Systat Software, Point Richmond, CA). When significant F values were obtained for the group factor, independent t-tests corrected with a layered Bonferroni analysis were performed. When significant F values were obtained for the time factor, an ANOVA with repeated measures was performed followed by paired t-tests corrected with a layered Bonferroni analysis. Plasma VP and OT concentrations were log transformed and analyzed as described for MAP and HR. Posm_σ were analyzed as described for MAP and HR. For hypotension experiments, a linear regression analysis was performed between plasma VP and OT levels and the level of MAP (Sigma Plot 2000, SPSS). A P value of <0.05 was significant in all statistical tests.

DβH immunoreactivity was ranked by two experimenters blind to the treatment group using the following scale: 0, no labeling; 1, sparse; 2, light moderate; 3, heavy moderate; and 4, dense. Scores were averaged and analyzed by a Mann-Whitney U-test. Scores for a section did not vary more than one unit on the scale. Tissue sections were sampled from several brain regions, including the subfornical organ, dorsal and ventral median preoptic nucleus, organum vasculosum of the lamina terminalis, the supraoptic nucleus, hypothalamic paraventricular nucleus, and the posterior pituitary. One section per animal was scored for each area except for the posterior pituitary that was scored from two sections per animal. In addition, the hypothalamic paraventricular nucleus was sampled from three rostrocaudal levels, as described previously (27, 28, 30): level 1 was the most rostral and consisted of a ventrally located magnocellular division; level 2 displayed a prominent and laterally positioned posterior magnocellular division and both dorsal and ventrolateral parvocellular divisions; level 3 was the most caudal and consisted of the lateral and medial parvocellular divisions. Digital images were taken of each area using a Nikon Eclipse TE2000-E microscope connected to a Spot camera (Spot RT Slider, Diagnostic Instruments, Sterling Heights, MI) using Spot Imaging Software (version 3.5).

**RESULTS**

**Effect of 6OHDA Treatment on Tyramine-Evoked Pressor Response**

To assess whether 6OHDA treatment destroyed sympathetic catecholaminergic fibers, the pressor response to tyramine (250 μg/kg iv) was measured in 6OHDA-treated and control rats. Tyramine stimulates vasoconstriction and increases blood pressure by promoting endogenous norepinephrine release from sympathetic nerve terminals (7, 12). Thus a diminished pressor response would reflect a depletion of norepinephrine from nerve terminals and/or destruction of catecholaminergic nerve terminals. As expected, the tyramine-evoked increase in MAP was markedly attenuated in 6OHDA-treated vs. control rats in all experiments (5 ± 1 mmHg vs. 47 ± 3 mmHg, respectively; P < 0.001).

**Effect of 6OHDA Treatment on Hemorrhage-Evoked Increase in Plasma VP and OT Levels**

Serial hemorrhage significantly increased plasma VP and OT levels from baseline levels at 20 and 30 min in both 6OHDA-treated and control rats (Figs. 1, A and B). However,
the hemorrhage-evoked increase in plasma VP and OT levels was significantly attenuated in 6OHDAtreated rats compared with control rats at both times (Fig. 1, A and B). Baseline plasma VP and OT levels did not differ between groups. The attenuated increase in plasma VP and OT levels of 6OHDAtreated rats occurred despite a significantly lower MAP compared with control rats between 2 and 15 min (Fig. 1C). In fact, MAP of 6OHDAtreated rats fell significantly below baseline values immediately after the first blood withdrawal, whereas MAP of control rats did not significantly drop until the second blood withdrawal. In both 6OHDAtreated and control rats, hemorrhage produced a biphasic response in HR with an initial tachycardia followed by a significant bradycardia. Although baseline HR were not different between groups (6OHDA: 383 ± 5 bpm; control: 386 ± 11 bpm), the magnitude of the tachycardic response was significantly attenuated in 6OHDAtreated vs. control rats (6 min values: 34 ± 8 vs. 70 ± 14 bpm, respectively; \( P < 0.05 \)); the magnitude of the bradycardia was not different between groups (−91 ± 19 bpm vs. −115 ± 33 bpm). \( P_{\text{osmol}} \) was not significantly different between the two groups at any time (Table 1).

**Effect of 6OHDATreatment on Hypotension-Evoked Increase in Plasma VP and OT Levels**

Administration of DZX15 significantly decreased MAP in both 6OHDAtreated and control rats (Fig. 2C). However, 6OHDAtreated rats displayed a significantly lower MAP than control rats receiving DZX15, which would provide a greater stimulus for VP and OT secretion. Therefore, to produce similar levels of MAP between 6OHDAtreated and control rats, a larger dose of DZX was administered to a separate group of control rats. MAP of 6OHDAtreated rats given DZX15 was not significantly different from control rats given DZX25 (Fig. 2C). Despite similar degrees of hypotension, plasma VP and OT levels were significantly lower in 6OHDAtreated rats vs. control rats given DZX25 (10 and 30 min; Figs. 2, A and B). A linear regression analysis between plasma VP and OT levels vs. MAP in control rats receiving DZX15 or DZX25 revealed a significant correlation at 10 min (Fig. 3) and 30 min (plot not shown). Interestingly, every 6OHDAtreated rat receiving DZX15 fell outside the 95% confidence intervals of this regression line. That is, 6OHDAtreated rats had significantly lower plasma VP and OT levels for a similar drop in MAP.

Table 1. **Plasma osmolality of 6OHDAtreated and control rats that were hemorrhaged serially or received an injection of DZX**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Baseline</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>289±2</td>
<td>287±3</td>
<td>287±3</td>
<td>291±2</td>
</tr>
<tr>
<td>6OHDA</td>
<td>9</td>
<td>290±3</td>
<td>289±3</td>
<td>292±3</td>
<td>293±3</td>
</tr>
<tr>
<td>DZX-Hypotension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DZX25)</td>
<td>6</td>
<td>292±3</td>
<td>292±3</td>
<td>293±2</td>
<td></td>
</tr>
<tr>
<td>Control (DZX15)</td>
<td>7</td>
<td>290±3</td>
<td>290±3</td>
<td>288±1</td>
<td></td>
</tr>
<tr>
<td>6OHDA (DZX15)</td>
<td>7</td>
<td>289±2</td>
<td>286±2</td>
<td>292±3</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. No differences were observed in Plasma osmolality (\( P_{\text{osmol}} \)) between 6-hydroxydopamine (6OHDA)-treated and control rats at any time that were hemorrhaged or received an injection of diazoxide (DZX). Dashes indicate no data available. DZX25, 25 mg/kg iv DZX; DZX15, 15 mg/kg iv DZX.
response was significantly attenuated in 6OHDA-treated rats given DZX15 vs. control rats receiving DZX25 (43 ± 8 bpm vs. 100 ± 13 bpm, respectively; P < 0.01).

Effect of 6OHDA Treatment on Hyperosmotic-Evoked Increase in Plasma VP and OT Levels

The infusion of HS significantly increased plasma VP and OT levels and $P_{\text{osmol}}$ in both control and 6OHDA-treated rats (Fig. 4). In marked contrast to hemorrhage and hypotension, the increase in plasma VP and OT levels during the infusion of HS was not different between control and 6OHDA-treated rats at 60 and 120 min (Figs. 4, A and B). In addition, $P_{\text{osmol}}$ was not different between groups at any time (Fig. 4C). The infusion of HS did not alter MAP (Fig. 4D) or HR from baseline values in 6OHDA-treated vs. control rats (baseline HR: 389 ± 7 vs. 406 ± 11 bpm, respectively).

Effect of 6OHDA Treatment on DBH Immunoreactivity in the Posterior Pituitary and Hypothalamic Nuclei

To determine whether 6OHDA treatment reduced catecholaminergic innervation of the posterior pituitary without affecting those structures inside the blood-brain barrier, we examined DBH immunoreactivity in a separate group of 6OHDA and vehicle-treated rats. Again, the tyramine-evoked pressor response was markedly blunted in 6OHDA vs. vehicle-treated rats (6 ± 1 mmHg vs. 52 ± 2 mmHg, respectively; P < 0.001). Summary data for DBH immunoreactivity in the structures examined are summarized in Table 2.

The posterior pituitary of vehicle-treated rats contained numerous DBH immunoreactive axons and terminals (Fig. 5A). In contrast, only sparse DBH immunoreactivity was present in the posterior pituitaries of 6OHDA-treated rats (Fig. 5B). The anterior pituitary contained few, if any, DBH-positive fibers in either group and therefore was not scored (data not shown). The ability of 6OHDA treatment to reduce DBH immunoreactivity in the posterior pituitary was in stark contrast to the effect of 6OHDA treatment on the hypothalamic paraventricular and supraoptic nuclei (Fig. 5, C–F). As expected (32), the hypothalamic paraventricular and supraoptic nuclei contained a dense level of DBH-immunoreactivity in vehicle-treated rats (Fig. 5, C and E). In the paraventricular nucleus, DBH-positive fibers were present in both parvocellular and magnocellular divisions throughout its rostral-caudal extent. In marked contrast to its effect on the posterior pituitary, 6OHDA did not alter the level of DBH immunoreactivity in either the paraventricular or supraoptic nuclei (Fig. 5, D and F; Table 2).

The forebrain laminar terminals of vehicle-treated rats also contained numerous DBH-immunoreactive axons and terminals (Fig. 6). We observed several DBH-positive fibers in the subfornical organ, the dorsal and ventral median preoptic nucleus, and the organum vasculosum of the lamina terminalis. With regard to the latter, numerous axons and terminalis were located in the lateral and dorsal boundaries with only sparse labeling in the central core (Fig. 6G). 6OHDA treatment did not affect the level of DBH immunoreactivity in these structures except for the subfornical organ (Fig. 6; Table 2).

DISCUSSION

Peripheral catecholaminergic inputs to the posterior pituitary may influence VP and OT release from neurohypophysial
nerve terminals (1, 16, 17, 19, 29), but their role in VP and OT secretion during physiological challenges has received little attention. In the present study, destruction of catecholaminergic nerve terminals located outside the blood-brain barrier with 6OHDA significantly attenuated the increase in plasma VP and OT levels stimulated by hemorrhage and hypotension but not hyperosmolality. These findings suggest that peripheral cat-
elaminergic fibers contribute to VP and OT secretion during conditions associated with sympathoadrenal activation.

Intravenous administration of the neurotoxin 6OHDA destroys sympathetic catecholaminergic fibers (15, 18). Because 6OHDA cannot cross the blood-brain barrier, catecholaminergic neurons and fibers within the central nervous system and inside the blood-brain barrier should remain largely unaffected in adult rats (15, 20). The present findings provide strong support for this notion as 6OHDA treatment did not reduce DβH immunoreactivity in the hypothalamic paraventricular and supraoptic nuclei. Here, the effectiveness of the 6OHDA treatment to destroy peripheral catecholaminergic fibers was assessed by the magnitude of the tyramine-evoked pressor response, and this pressor response was virtually abolished in 6OHDA-treated rats. Because the ability of tyramine to increase arterial blood pressure depends upon the integrity of presynaptic terminals and norepinephrine concentrations (7, 12), a diminished pressor response would reflect a depletion of terminal noradrenergic stores or destruction of sympathetic nerve terminals. Given the mechanism of action of 6OHDA, it seems likely that the 6OHDA treatment regimen used in the present study destroyed the majority of sympathetic catecholaminergic terminals, consistent with previous observations (7, 18).

Table 2. Quantification of DβH immunoreactivity in the posterior pituitary, hypothalamic nuclei and forebrain circumventricular organs of 6OHDA and vehicle-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 3)</th>
<th>6OHDA (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior pituitary</td>
<td>1.9±0.1</td>
<td>0.9±0.1*</td>
</tr>
<tr>
<td>Paraventricular nucleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>3.0±0.0</td>
<td>2.8±0.2</td>
</tr>
<tr>
<td>Level 2</td>
<td>3.1±0.1</td>
<td>3.0±0.0</td>
</tr>
<tr>
<td>Level 3</td>
<td>3.1±0.1</td>
<td>3.0±0.0</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>3.3±0.2</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>Subfornical organ</td>
<td>1.5±0.0</td>
<td>0.7±0.3*</td>
</tr>
<tr>
<td>Dorsal median preoptic nucleus</td>
<td>2.2±0.2</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>Ventral median preoptic nucleus</td>
<td>2.5±0.0</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>Organum vasculosum of the lamina terminalis</td>
<td>2.3±0.2</td>
<td>2.3±0.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Dopamine-β-hydroxylase (DβH) immunoreactivity was qualitatively scored by two experimenters blind to the treatment group using the following scale: 0, none; 1, sparse; 2, light moderate; 3, heavy moderate; 4, dense. Each score was based on one section per animal except for the posterior pituitary, which was ranked from two sections per animal. *Significant difference from vehicle-treated rats (P < 0.05).
Fig. 5. Dopamine-β-hydroxylase (DBH) immunoreactivity in the posterior pituitary, hypothalamic paraventricular nucleus, and supraoptic nucleus of 6OHDA and vehicle-treated rats. 6OHDA treatment reduced DBH immunoreactivity in the posterior pituitary (A and B). In marked contrast, 6OHDA treatment did not reduce DBH immunoreactivity in any rostral-caudal level of the paraventricular nucleus, including level 2 with a prominent posterior magnocellular cell group (C and D) or the supraoptic nucleus (E and F). 3V, third ventricle; OC, optic chiasm. Scale bars = 100 μm.

Fig. 6. DBH immunoreactivity in the forebrain lamina terminalis of 6OHDA and vehicle-treated rats. 6OHDA treatment reduced DBH immunoreactivity in the subfornical organ (A and B) but had no effect in the dorsal median preoptic nucleus (C and D), ventral median preoptic nucleus (E and F), and organum vasculosum of the lamina terminalis (G and H). Scale bar A–F = 100 μm, G–H = 200 μm.
creases VP and OT secretion (16, 17). However, some catecholaminergic input to the posterior pituitary may originate from the central nervous system. For example, lesion of the ventral noradrenergic tract has been reported to reduce norepinephrine levels of the neural lobe (2), and Garten et al. (10) reported that a small number of A2 neurons were labeled after injection of a retrograde tracer targeted at the posterior pituitary. On the other hand, several reports suggest that the superior cervical ganglion, not central catecholaminergic neurons/tracts, provides the vast majority of the catecholaminergic innervation of the posterior pituitary (1, 3, 19). In the present study, 6OHDA treatment markedly reduced DBH immunoreactivity in the posterior pituitary, and this could be attributed to the actions of 6OHDA on catecholaminergic fibers of sympathetic or central origin but located outside the blood-brain barrier. Although 6OHDA treatment did not destroy DBH-positive fibers within the hypothalamic paraventricular and supraoptic nuclei, we did observe a decrease in DBH immunoreactivity within the subfornical organ. This effect is likely explained by the lack of a complete blood-brain barrier at this circumventricular organ; however, noradrenergic innervation of the organum vasculosum of the lamina terminalis was unaffected. The origin of noradrenergic input to the subfornical organ arises from the A1 and A2 cell groups (5, 14, 36), but there is currently no available evidence to suggest that noradrenergic inputs to the subfornical organ contribute to VP and OT secretion. Therefore, it seems likely that the effects of 6OHDA treatment on VP and OT release can be largely attributed to the destruction of peripheral catecholaminergic innervation of the posterior pituitary. The destruction of sympathetic fibers likely contribute to this response, but a contribution from the small number of brainstem neurons that project outside the blood-brain barrier cannot be excluded.

Hemorrhage and arterial hypotension stimulate the secretion of VP and OT. With both stimuli, the increase in plasma VP and OT levels was blunted in 6OHDA-treated rats vs. control rats. The attenuation of VP and OT levels still occurred despite a significantly lower MAP of 6OHDA-treated rats in some instances, which would be expected to produce an even greater stimulus for VP and OT secretion. Although ascending noradrenergic inputs facilitate putative VP cell responses and associated VP secretion in response to hemorrhage/hypotension (4, 11, 25, 26), these inputs are largely unaffected by peripheral 6OHDA administration in adult rats (15, 20). Indeed, the present findings provide strong support for this notion as DBH immunoreactivity was not different in the hypothalamic paraventricular and supraoptic nuclei between 6OHDA and vehicle-treated rats. Therefore, it appears that peripheral catecholaminergic inputs to the posterior pituitary contribute to VP and OT secretion during hemorrhage and arterial hypotension.

The inability of intravenous 6OHDA treatment to affect VP and OT secretion during hyperosmolality is in stark contrast to its ability to blunt plasma VP and OT levels to hemorrhage and hypotension. Hemorrhage and hypotension produce robust increases in sympathetic nerve activity (8, 21, 34), whereas hyperosmolality produces substantially weaker and nonuniform changes in sympathetic outflow (35). For example, Weiss et al. (35) reported that systemic hyperosmolality produced by intravenous infusion of hypertonic saline moderately increases lumbar sympathetic nerve activity but decreases or does not change renal and splanchnic sympathetic nerve activity. In light of the present findings, it is interesting to speculate that the ability of 6OHDA treatment to attenuate VP and OT secretion stimulated by hemorrhage and hypotension, but not hyperosmolality, is related to the effect of each stimulus on sympathetic nerve activity. That is, an increase in sympathetic outflow at the level of the posterior pituitary during hemorrhage and hypotension enhances VP and OT secretion. The lack of an effect of 6OHDA treatment on plasma VP and OT levels in hyperosmotic rats may be due to the osmotic stimulus producing minimal changes in the relevant sympathetic nerves. Future experiments that record activity of sympathetic nerves innervating the posterior pituitary under these various physiological conditions are needed to fully explore this possibility.

In summary, the present findings suggest that peripheral catecholaminergic fibers enhance VP and OT secretion under certain physiological conditions, as systemic administration of the neurotoxin 6OHDA blunted VP and OT levels stimulated by hemorrhage and hypotension, but not hyperosmolality. Because the former two stimuli are associated with pronounced increases in sympathetic outflow, this mechanism might permit the enhancement of VP and OT secretion under conditions in which pronounced activation of multiple pressor mechanisms is needed to maintain arterial blood pressure. The present findings also highlight potential concerns for the use of peripheral 6OHDA administration to elucidate the role of different pressor mechanisms in physiological responses. The results of studies using peripheral 6OHDA treatment to distinguish between the contribution of different pressor systems (sympathetic vs. VP) should be interpreted cautiously, as the present findings clearly demonstrate that systemic 6OHDA treatment eliminates peripheral sympathetic fibers but also blunts VP secretion under conditions of sympathoadrenal activation.

ACKNOWLEDGMENTS

The authors thank Kimberly Alfred for technical assistance and Dr. John Fernstrom for his generous gift of the VP and OT antibodies.

GRANTS

This research was supported by National Institutes of Health Grants HL-55687 (to A. F. Sved), HL-073661 (to S. D. Stocker), HL-073693 (to M. E. Wilson), COBRE Grant P20 RR-015592 (to M. E. Wilson), and a Scientist Development Grant from the American Heart Association (S. D. Stocker). C. J. Madden was supported by a Predoctoral Fellowship from the American Heart Association.

Present address for Christopher J. Madden: Oregon Health & Science University, Neurological Science Institute, 505 NW 185th Ave., West Campus, Beaverton, OR 97006 (e-mail: maddench@ohsu.edu).

REFERENCES


