

Effect of Central Catecholamine Depletion on the Osmotic and Nonosmotic Stimulation of Vasopressin (Antidiuretic Hormone) in the Rat

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ABSTRACT The central nervous system (CNS) mechanism(s) for the release of antidiuretic hormone (ADH) by various stimuli is unknown. In this study, the role of CNS catecholamines in effecting ADH release was examined in conscious rats 10–14 d after the cerebroventricular injection of 6-hydroxydopamine (6-OHDA). This dose of 6-OHDA caused a 67% depletion of brain tissue norepinephrine and only 3% depletion of heart norepinephrine, as compared with controls, which were injected with the vehicle buffer alone. Either intravenous 3% saline (osmotic stimulus) or intraperitoneal hyperoncotic dextran (nonosmotic stimulus) was administered to water-diuresing rats through indwelling catheters. Neither of these maneuvers changed arterial pressure, pulse, or inulin clearance in control or 6-OHDA rats. The 3% saline caused similar increases in plasma osmolality (15 mosmol/kg H₂O) in control and 6-OHDA rats. The control rats, however, increased urinary osmolality (U_{osm}) to 586 mosmol/kg H₂O, whereas 6-OHDA rats increased U_{osm} only to 335 mosmol/kg H₂O ($P < 0.005$). These changes in U_{osm} were accompanied by an increase in plasma ADH to 7.6 μ IU/ml in control animals vs. 2.9 μ IU/ml in 6-OHDA rats ($P < 0.005$). All water-diuresing animals had undetectable plasma ADH levels. Dextran-induced hypovolemia caused similar decrements (–10%) in blood volume in both control and 6-OHDA animals, neither of which had significant changes in plasma osmolality. This nonosmotic hypovolemic stimulus caused an increase in U_{osm} to 753

mosmol/kg H₂O in control rats, whereas U_{osm} in 6-OHDA rats increased to only 358 mosmol/kg H₂O ($P < 0.001$). At the same time, ADH levels also were significantly greater in Cont rats (2.4 μ IU/ml) than in the 6-OHDA animals (0.69 μ IU/ml; $P < 0.05$). These results therefore suggest that CNS catecholamines may play an important role in mediating ADH release in response to both osmotic and nonosmotic (hypovolemic) stimuli.

INTRODUCTION

During the 40 yr since the original studies of Rydin and Verney (1) there has been considerable interest in the role of the sympathetic nervous system in renal water excretion. In these early studies of Verney (2) the antidiuresis associated with electrical stimulation of dog's flanks ("emotional stress") was enhanced by sympathetic denervation procedures and impaired by either intravenous epinephrine or tyramine administration. Because neither of these latter sympathomimetic agents altered the antidiuretic response to the administration of posterior pituitary extract, Verney suggested (2) that sympathetic stimulation may inhibit a sequence of chemical events in the central nervous system which leads to the release of antidiuretic hormone (ADH).¹

The possibility, however, that catecholamines may interfere with the effect of ADH at the level of the renal tubule was proposed later on the basis of *in vitro* studies in anuran membranes (3), canine renal medullary tissue (4), and intact man (5). Recent studies in ADH deficiency in dog (6, 7), rat (8), and man (9), however, have demonstrated that the primary *in vivo* effect of catecholamines on water excretion is modulated by altering

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¹*Abbreviations used in this paper:* ADH, antidiuretic hormone; BV, blood volume; Hct, hematocrit; 6-OHDA, 6-hydroxydopamine; Posm, plasma osmolality; U_{osm}, urinary osmolality.

endogenous ADH release. These latter results thus support the original hypothesis of Verney that suggested a primary central effect of catecholamines on ADH release. However, in contrast to the proposal of a direct central effect of catecholamines on ADH release, the effects of both alpha (10) and beta adrenergic stimulation (11) on ADH release, are mediated by extracerebral arterial baroreceptors, pathways that now appear to modulate many nonosmotic stimuli for ADH release (12).

The possibility remains, however, that intracerebral catecholamines may be important as central neurotransmitters in either the osmotic or nonosmotic release of ADH, or both (13, 14). Some *in vivo* evidence for such a role of catecholamines has been obtained by the demonstration that the intracerebral injection of norepinephrine stimulates ADH release, an effect which can be blocked by alpha adrenergic blockade (15–17). Although this antidiuretic effect of central norepinephrine administration (15) is opposite from the water diuresis induced by intravenous norepinephrine (10), it must be remembered that the central and peripheral effects of alpha adrenergic stimulation on blood pressure also are in the opposite direction (18). In this regard it is important to note that systemic drug administration may bring into play a variety of efferent reflex mechanisms as a result of the effects of the drug on systemic hemodynamics. Likewise, some caution should be exercised in interpreting the results of studies with the acute intracerebral injection of supra-physiological doses of various drugs. In this regard, it seems rather clear that the *in vivo* interruption of parasympathetic afferent pathways to the brain stimulates ADH release (19), whereas activation of the efferent limb of the autonomic nervous system by intracerebral injections of acetylcholine and carbachol also has been found to stimulate ADH release (15, 17).

In this study another approach has been used in conscious rats to evaluate the potential importance of brain catecholamines on ADH release. Brain catecholamines were depleted chemically by the use of intraventricular 6-hydroxydopamine (6-OHDA), a procedure which does not alter catecholamine stores in extracerebral tissues. As compared with control animals, rats with a 67% depletion of brain catecholamines demonstrate a significant impairment in the release of ADH in response to both osmotic and nonosmotic stimuli despite comparable systemic and renal hemodynamics. These results, therefore, support a central role of catecholamines in modulating the release of ADH.

METHODS

The studies were performed in male Sprague-Dawley rats that weighed 150–200 g. Throughout the 10- to 14 d interval between the administration of 6-OHDA and the time of the

acute experiments, the animals were maintained on standard rat chow (Ralston Purina Co., St. Louis, Mo.) and *ad lib.* water intake.

Injection of 6-OHDA (experimental group) or vehicle buffer (control group). Administration of 6-OHDA into the lateral ventricle was based upon the technique of Uretsky and Iverson (20, 21). The rats were lightly anesthetized with ether and a midline incision 2-cm long was made through the skin to the skull. The intersection of the coronal and sagittal sutures was identified, and a 0.5-mm burr hole was made 1.5-mm lateral and 2.5-mm caudal to this intersection. The experimental animals received 250 μ g of 6-OHDA (Regis Chemical Co., Morton Grove, Ill.) in a volume of 20 μ l of vehicle buffer (ascorbic acid 1 mg/ml in distilled water, corrected to pH 5.0). The control animals received 20 μ l of the vehicle buffer alone. Administration was by a 0.05-ml Hamilton syringe (Hamilton Co., Reno, Nev.) equipped with a 26-gauge needle that had a thick polyethylene sleeve covering all but the last 3.5 mm.

After the initial injection, wound clips were placed and the animal recovered for 24–48 h. The animals then were again anesthetized and given a second injection of 6-OHDA through the same burr hole.

Balance studies. Six control-injected and six 6-OHDA-injected rats were placed in individual metabolic balance cages 24 h after their second intraventricular injection. The rats were allowed *ad lib.* food (standard rat chow) and water intake. Daily food and water intake, as well as urine volumes, were recorded. The rats were weighed every other day. Systolic blood pressures were measured in conscious animals by the indirect tail cuff method on days 1, 5, and 11 after injection.

Hypertonic saline protocol. Acute experiments were performed in a separate group of rats. The animals were prepared as above with intraventricular injections of either 6-OHDA or vehicle buffer and were allowed to recover for 10–14 d. On the day of the acute experiments, the animals were weighed and anesthetized with methoxyflurane. This agent was used because of the profound sensitivity of 6-OHDA-treated rats to ether and to barbiturates (22), and because Sprague-Dawley rats are not prone to nephrogenic diabetes insipidus after methoxyflurane anesthesia (23). This latter fact was confirmed by administering exogenous ADH (500 μ U Pitressin, Parke, Davis & Co., Detroit, Mich.) to three water-diuresing, 6-OHDA-injected and three control-injected animals and observing an equivalent antidiuresis in both groups (mean urinary osmolality [Uosm] 685 and 710 mosmol/kg H₂O, NS, respectively). Although maximal Uosm cannot be attained after exogenous ADH administration in water-diuresing animals, because of washout of medullary tonicity, we have previously used this method of exogenous ADH administration successfully to distinguish differences in ADH responsiveness (24).

The anesthetized rats then underwent placement of external jugular and carotid artery catheters (PE 50, Clay-Adams, Div., Beckton, Dickinson & Co., Parsippany, N. J.) as well as a suprapubic polyethylene catheter (PE 250, Clay-Adams). The animals were then placed in a restrainer (Narco Bio-Systems, Inc., Houston, Tex.) and allowed to awaken. At that time a base-line blood sample (0.2 ml) was taken for determination of hematocrit (Hct) (model Mb, International Equipment Co., Div. Damon Corp., Needham Heights, Mass.) and osmolality by vapor pressure osmometer (Wescor Inc., Logan, Utah), and a urine sample was taken for determination of osmolality (Uosm) by freezing-point depression (model 3L, Advanced Instruments, Inc., Needham Heights, Mass.). The vapor pressure osmometer correlates within 1% of the value recorded by both freezing-point depression and calculated osmolality (25). In our laboratory

the osmolality of five samples of rat and dog plasma were determined in quintuplicate by this method. The greatest variation of any single measurement from the mean was 1.3% (4 mosmol).

Next, an infusion of 0.33% saline with 1,200 mg inulin/100 ml was started at a rate of 9 ml/h (model 355, Sage Instruments Div., Orion Research Inc., Cambridge, Mass.). In most cases, a volume equaling ~3% of body weight was administered as Uosm fell to below 150 mosmol/kg. Once a stable water diuresis was achieved, three 5-min urine collections were made for Uosm, volume, and inulin concentration (Auto-Analyzer, Technicon Instruments Corp., Tarrytown, N. Y.). Simultaneous 0.2-ml blood samples were taken for Hct, plasma osmolality (Posm), and inulin. Blood pressure and pulse were continuously monitored by a strain gauge (model 267AC, model 7702B, Hewlett-Packard Co., Palo Alto Calif.) connected to the arterial line.

After the control period an infusion of 3% saline, 2 ml/100 g body wt, was administered over a period of 4–6 min. Blood pressure, pulse, and respirations were monitored continuously throughout this infusion. After completing the infusion, the experimental periods were started and urine was collected at 5-min intervals and blood samples, 0.2 ml, were taken every 10 min until a postcontrol water diuresis occurred (Uosm < 150 mosmol/kg). At this time the hypotonic infusion was discontinued and the animals were sacrificed. Tissue was removed for assay of catecholamine concentrations as described below.

Another group of 6-OHDA-injected and control-injected animals received the hypertonic saline infusion and had blood ADH levels measured. Because the maximum urinary concentration after hypertonic saline was found in earlier experiments to occur between 18 and 23 min, all animals in this group had 2 ml of blood for ADH removed rapidly over 10 s through the arterial catheter exactly 20 min after the start of the experimental period. As a control to establish that blood removal over 10 s did not stimulate ADH release rapidly enough to effect the ADH level in the withdrawn blood specimen, a group of control-injected and 6-OHDA-injected animals had ADH levels drawn over a 10-s period while undergoing a water diuresis.

Hyperoncotic dextran protocol. As a nonosmotic, hypovolemic stimulus to ADH release, the intraperitoneal administration of 6% dextran-70 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) was used. Rats were prepared in the same manner as described above for the hypertonic saline protocol, with the exception of placement of an indwelling peritoneal catheter (PE 250, Clay-Adams) at the time of surgery. After establishing a water diuresis as described above, collection of appropriate control blood and urine samples were obtained. The animals then received intraperitoneal dextran (1.8 ml/100 g body wt) at a rate of 1 ml/min. The estimated oncotic pressure of this solution (van't Hoff equation) was 800 mm H₂O. Blood pressure, pulse, and respiration were monitored throughout the infusion. Urine was then collected at 5-min intervals and 0.2 ml of blood was taken every 10 min for a period of 60 min after dextran administration (experimental period). Blood removed was replaced with equivalent volumes of isotonic saline. The hypotonic infusion was then stopped and the animals sacrificed. Tissue was then obtained and prepared for the catecholamine assay. Calculation of estimated changes in blood volume (BV) was done by the formula $[\Delta BV = 1 - (Hct\ 2/Hct\ 1)]$. This method probably underestimates ΔBV because it does not account for erythrocyte volume removed by blood sampling.

As in the hypertonic saline protocol a separate set of animals was then studied for ADH levels. Because the time of peak Uosm after dextran varied from 30 to 60 min, a summed

sampling technique was used for blood ADH samples. With this technique, 0.1 ml of blood was drawn at 30 min, and then similar samples were drawn every 3 min for the next 30 min. Blood ADH was then measured on the pooled specimens. Blood pressure and pulse were monitored throughout the experiments. Samples of blood (Hct, Posm, inulin) and urine (volume, Uosm, inulin) were also taken. Animals were then sacrificed, and tissue was obtained and prepared for catecholamine assay.

ADH assay. All blood samples for ADH were drawn into syringes that contained EDTA, then transferred to clean tubes on ice. These samples were centrifuged at 4°C for 10 min at 2,000 rpm (model PR-J, International Equipment Co.); the plasma was removed, measured for volume, and frozen at -20°C. Within 5 d the sample was thawed, 1.0 ml was removed and acidified with 0.2 ml 1 N hydrochloric acid, and then 1.0 ml of Bentonite (3.0 mg/cm³, Fisher Scientific Co., Pittsburgh, Pa.) was added. The tubes were then vortexed and centrifuged. The supernate was discarded and the Bentonite resuspended in a solution of 1.0 ml of 80% acetone in 1 N hydrochloric acid. After centrifugation and removal of the supernate, the precipitate was washed with 1.0 ml of ether. The ether layer was then aspirated and discarded. The aqueous layer was blown to dryness under room air. The dry residue was stored at -20°C until it was assayed for ADH. The assay for ADH was performed by radioimmunoassay as previously described in our laboratory (26).

The vasopressin antiserum used in this radioimmunoassay cross-reacts by <0.1% with vasotocin and oxytocin and 10% with lysine vasopressin. The recovery of immunoassayable arginine vasopressin is 79% over a range of 0.8–5.0 μ IU/ml. The intra-assay coefficient of variation ranges from 6 to 14% and the interassay coefficient of variation ranges from 14 to 29%. The threshold sensitivity of the assay is 0.25 μ IU/ml, which is significantly different from the blank plasma.

Assay of pituitary ADH content. On day 12 after the intraventricular injections, the six animals in each group (control and 6-OHDA) that were included in the balance studies were guillotined and their pituitaries were rapidly removed for determination of ADH content. Simultaneous truncal blood was obtained for determination of serum osmolality by freezing-point depression. The pituitary glands were removed rapidly, placed individually in 0.5 ml of 0.1 M HCl, and frozen immediately at -80°C. At the time of assay, the samples were thawed quickly in a 30°C water bath and homogenized. Two 0.1-ml aliquots of the tissue suspension then were removed. The first aliquot was centrifuged at 3,000 rpm for 30 min. 10 μ l of the supernate was removed and dried under air, then reconstituted to 1 ml and diluted 1:100 with assay buffer (26). The assay for ADH was then performed with 50- and 100- μ l aliquots of this sample.

A second 0.1-ml aliquot of tissue suspension underwent the following extraction procedure before ADH assay. As above, 0.5 ml of 0.1 M HCl was added to the 0.1-ml tissue suspension aliquot. 2 ml of acetone was then added to the suspension, which was then vortexed and centrifuged at 3,000 rpm for 10 min. The supernate was then decanted and washed twice with 2 ml of ether. The ether layer was aspirated after each wash. The aqueous layer was then reconstituted to 1 ml and diluted 1:1,000 with assay buffer. The ADH assay was then performed with 50- and 100- μ l aliquots of this sample. Recovery of a known amount of ¹²⁵I-labeled arginine vasopressin added before the extraction procedure to the tissue suspension averaged 75.2% for four determinations.

Samples of both Sprague-Dawley cortical brain tissue as well as pituitary tissue from a homozygous diabetes insipidus rat (Brattleboro strain) were measured concomitantly with the experimental samples by both methods. ADH content in these

cortical and Brattleboro pituitary samples was not significantly different from zero with either the extracted or unextracted tissues.

Assay of tissue catecholamines. Brain and heart tissue from 6-OHDA-injected and control-injected animals were prepared for catecholamine (norepinephrine) assay. The heart was used as a reference to establish whether or not the catecholamine-depleting action of 6-OHDA had crossed the blood-brain barrier. The catecholamine assay used was the radioenzymatic technique of Henry et al. (27), as modified by Arnold et al. (28).

Immediately after sacrifice the whole brain was removed and weighed. The tissues were homogenized at 4°C (Polytron, Brinkman Instruments, Inc., Westbury, N. Y.) in saline, and then the samples were made up to 0.1 N perchloric acid. The samples were frozen until the assay was performed. The assay was performed with only slight variation in the originally reported reaction mixture (28).

Statistics. For each experimental animal, changes in mean arterial pressure, pulse, inulin clearance, Posm, and Uosm were compared by paired Student's *t* test. Comparisons of the above parameters as well as ADH levels among the control-injected and 6-OHDA-injected animals were made by unpaired *t* test. In both cases, a *P* value <0.05 was considered significant.

RESULTS

Balance studies. The percentage of weight gain during the 12-d study in the control and 6-OHDA animals was not significantly different (30.5 vs. 26.7%) nor was the food intake (24.6 vs. 26.8 g). The water intake was slightly less in the 6-OHDA rats (43.2 vs. 39.3 ml/d) but this difference did not reach a level of statistical significance. This modest decrease in water intake was associated with a significantly lower daily urine volume (13.7 vs. 10.6 ml/d, *P* < 0.05). As expected, however, this adequate fluid intake was sufficient to avoid fluid dehydration as documented by the absence of either a weight loss or a rise in serum osmolality (see below).

Measurement of pituitary ADH content. Results of pituitary ADH content on day 12 after intraventricular injection for six control-injected and six 6-OHDA-injected rats are shown in Table I. Pituitary ADH content was not different in 6-OHDA-injected rats from control-injected rats when measured either in unextracted

(702.3 vs. 650.6 mU/pituitary, NS) or extracted (412.1 vs. 401.7 mU/pituitary, NS) pituitary tissue. The results in control rats correlate well with results previously published for unextracted pituitary tissue (29). Serum osmolalities in the two groups were no different (295 mosmol/kg H₂O in 6-OHDA-injected vs. 300 mosmol/kg H₂O in control-injected rats, NS).

Catecholamine depletion. The results of tissue norepinephrine levels for all experimental animals are shown in Table II. It should be pointed out that individual assay runs contained tissue samples from brains and hearts of both control-injected and 6-OHDA-injected animals. The values given in Table II are for the animals that underwent either hypertonic saline or hyperoncotic dextran stimulation.

It can be seen that the brains of animals that received intraventricular 6-OHDA had a 67% depletion of assayed norepinephrine. This agrees with literature values for animals treated in a similar fashion (20, 22). Furthermore, other authors have shown that such animals also have a 60–75% depletion in assayed brain dopamine (20, 22).

The values for heart norepinephrine revealed that there was no significant depletion in the 6-OHDA rats as compared with control-injected rats. This finding indicates that the catecholamine-depleting effect of intraventricular 6-OHDA is restricted to the brain. Similarly treated animals also have been shown by other investigators (22) to have no depletion in assayed heart dopamine.

Hypertonic saline stimulation. The responses of Posm and Uosm to hypertonic saline administration are shown in Fig. 1. During base-line water diuresis there were no significant differences in the Posm and Uosm between the control-injected and 6-OHDA-injected rats. There was an equivalent rise in Posm of ~15 mosmol/kg H₂O in both animal groups when measured 10 min after the hypertonic saline infusion. However, the peak Uosm after hypertonic saline in the control-injected and 6-OHDA-injected rats was significantly different (586 vs. 335 mosmol/kg H₂O, *P* < 0.001).

TABLE I
Serum Osmolality and Pituitary ADH Content 12 d after Intraventricular Injection in Control and 6-OHDA-injected Rats

| | Control* | Experimental* | <i>P</i> value |
|--|------------|---------------|----------------|
| Serum osmolality, mosmol/kg H ₂ O | 300.2±5.9 | 295.1±3.9 | NS |
| Pituitary ADH content, mU/pituitary | | | |
| Unextracted | 702.3±38.5 | 650.6±32.8 | NS |
| Extracted | 412.1±16.1 | 401.7±18.0 | NS |

* *n* = 6.

TABLE II
Effect of 6-OHDA on Norepinephrine Concentration in Brain and Heart Tissue

| | Values |
|----------------------------|--------|
| ng norepinephrine/g tissue | |
| Brain tissue | |
| Control (<i>n</i> = 15) | 169.6 |
| 6-OHDA (<i>n</i> = 13) | 55.5 |
| <i>P</i> value | <0.005 |
| Heart tissue | |
| Control (<i>n</i> = 16) | 200.6 |
| 6-OHDA (<i>n</i> = 13) | 194.2 |
| <i>P</i> value | NS |

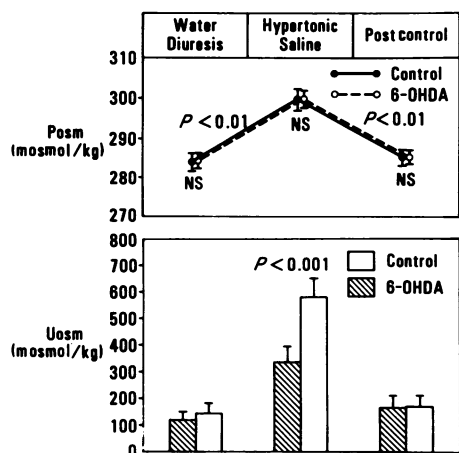


FIGURE 1 Posm (above) and Uosm (below) response to intravenous hypertonic saline. Results are expressed as ± 1 SEM. The values for the control water diuresis period are the mean of three values. The values for the experimental hypertonic saline period are the peak Uosm and the highest measured values of Posm achieved after saline administration.

During the postcontrol period, both groups of animals returned to the base-line levels of Posm and Uosm.

Fig. 2 shows the mean arterial pressure and inulin clearance results for animals in this protocol. Mean arterial pressure was equivalent for both injection groups during the control period of water diuresis and did not change significantly in either group during the administration of hypertonic saline. The postcontrol values also were comparable. Furthermore, inulin clearance was equivalent for both groups during base-line water diuresis and was not changed significantly with the administration of hypertonic saline. Inulin clearances were also comparable during the postcontrol water diuresis in the two groups of rats; however, there

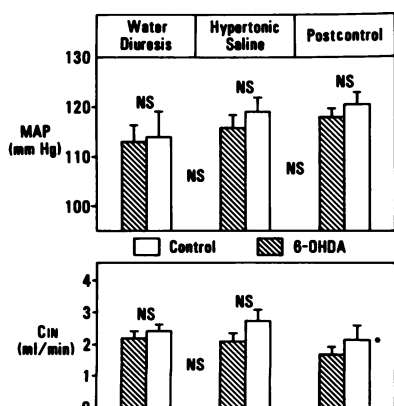


FIGURE 2 Mean arterial pressure (MAP) and inulin clearance (CIN) values for animals during the hypertonic saline studies. All results are expressed as ± 1 SEM and are the mean of three values during each collection period. * $P < 0.05$, postcontrol vs. hypertonic saline period for control-injected group.

was a modest but significant fall in inulin clearance in the control-injected group.

The results of the plasma ADH levels are shown in Fig. 3. These results were obtained from four separate groups of animals. Base-line measurements before intravenous hydration of Posm, Hct, and blood pressure were not different in 6-OHDA-injected or control-injected rats used in this protocol as compared with those rats used to assess maximal Uosm after hypertonic saline administration. Thus, base-line hydration appeared comparable in all rats used in these protocols. In both the control and 6-OHDA-treated rats, plasma ADH levels were drawn during water diuresis or after the hypertonic saline infusion. The water-diuresing rats of both groups had base-line ADH levels as measured by radioimmunoassay that were not significantly different from zero. The control-injected animals had a mean ADH response 20 min after hypertonic saline administration of $7.6 \mu\text{IU/ml}$, whereas the 6-OHDA-injected rats had an ADH response after hypertonic saline of $2.9 \mu\text{IU/ml}$, a value significantly less ($P < 0.005$) than in the control animals. At the time plasma ADH levels were obtained, Posm were comparable in the control vs. 6-OHDA-injected animals (290 vs. 289 mosmol/kg H_2O , respectively).

Hyperoncotic dextran administration. The change in BV induced by hyperoncotic dextran administered intraperitoneally is shown in Fig. 4. Both injection groups showed a decrease of $\sim 10\%$ in BV after dextran at the time at which peak Uosm was obtained.

The response in peak Uosm to the dextran administration is shown in Fig. 5 for both groups. Equivalently low Uosm were present in both groups during water diuresis. However, control-injected rats concentrated their urine to a mean of $753 \text{ mosmol/kg } \text{H}_2\text{O}$

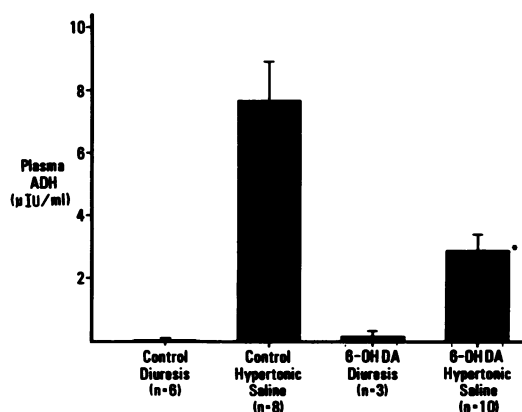


FIGURE 3 Plasma ADH levels in water-diuresing animals before and after hypertonic saline infusion. The results in the control rats are depicted in the left two bars and the 6-OHDA results are shown in the right two bars. Values are expressed in microinternational units per milliliter. * $P < 0.005$, 6-OHDA posthypertonic saline vs. control posthypertonic saline.

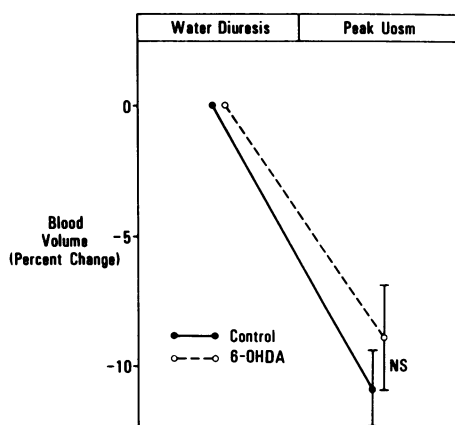


FIGURE 4 Change in BV (percent) induced by intraperitoneal administration of hyperoncotic dextran. Results are expressed as ± 1 SEM. The control values during the water diuresis are plotted as zero percent on the left and the percent BV decrement at the time of the peak increase in Uosm is shown on the right.

after dextran, whereas 6-OHDA-injected rats concentrated their urine to only 358 mosmol/kg H_2O ($P < 0.001$). There was no significant difference in mean Posm between the control and 6-OHDA animals in response to intraperitoneal dextran (290 vs. 288 mosmol/kg H_2O control and 291 vs. 289 mosmol/kg H_2O 6-OHDA, respectively).

Mean arterial pressure and inulin clearance for each group are shown in Fig. 6. Both injection groups had equivalent mean arterial pressure and inulin clearance during water diuresis and after dextran administration.

The plasma ADH results for the hyperoncotic dextran

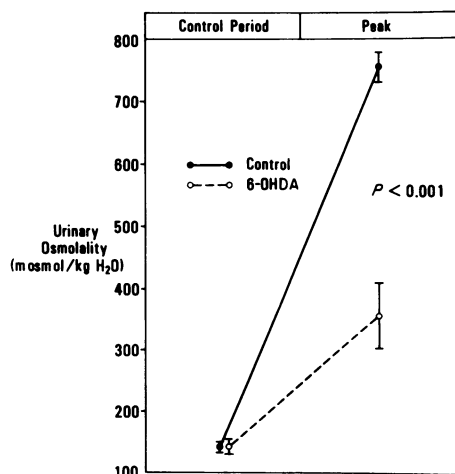


FIGURE 5 Response of Uosm to dextran administration in buffer-injected controls and in 6-OHDA-injected animals. Results are expressed as ± 1 SEM. Peak refers to the highest Uosm achieved after dextran.

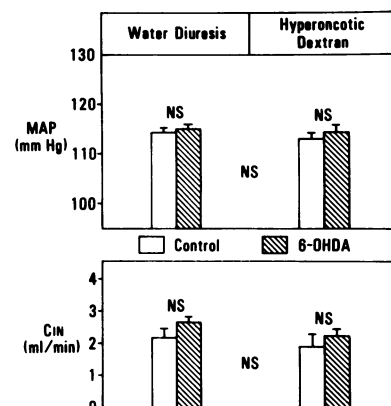


FIGURE 6 Mean arterial pressure (MAP) and inulin clearance (CIN) during water diuresis and in response to intraperitoneal hyperoncotic dextran. Results are expressed as ± 1 SEM. Data for both periods represents the mean of three values obtained during each period.

study are displayed in Fig. 7. As in the previous protocol, four groups of animals are represented here. There was no detectable difference in base-line blood pressure, Hct, or Posm between rats used in this protocol vs. those rats used to determine Uosm after hyperoncotic dextran, which indicates comparable base-line states of hydration. In this protocol, all animals had ADH levels drawn by the summed sampling technique outlined in Methods. Thus, the ADH values are mean values over 30 min rather than peak levels. Over this time interval there was no difference in the decrease in BV at any of the sample points (20, 30, 40, 50, and 60 min) between the two groups of animals, which suggests that cumulative BV changes were similar in the control and 6-OHDA-injected groups in which ADH was measured. Animals in both injection groups undergoing a water diuresis had ADH levels

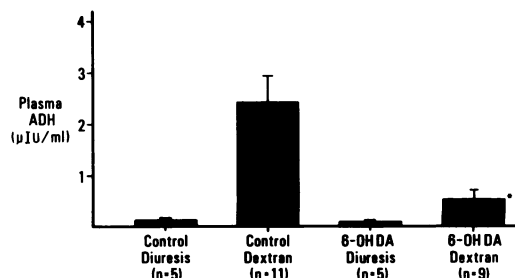


FIGURE 7 Plasma ADH levels for water-diuresing (columns labeled "control diuresis" and "6-OHDA diuresis") and dextran-stimulated (columns labeled "control dextran" and "6-OHDA dextran") animals. Results are expressed as ± 1 SEM. Neither water-diuresing group had ADH levels significantly different from zero. Values are expressed in microinternational units per milliliter. * $P < 0.05$, 6-OHDA post-dextran vs. control postdextran.

not significantly different from zero. Control-injected rats given dextran had a mean response to ADH of 2.4 μ IU/ml ($P < 0.05$), whereas 6-OHDA-injected rats had a significantly smaller response of 0.69 μ IU/ml ($P < 0.05$).

DISCUSSION

Recent *in vivo* studies have demonstrated that catecholamines effect renal water excretion by altering baroreceptor-mediated release of ADH (8, 9). Studies, however, investigating an additional role of catecholamines as potential central nervous system neurotransmitters for ADH release have produced conflicting results. Electrophysiologic studies of cells of the supraoptic or paraventricular nuclei after microiontophoretic application of norepinephrine or dopamine have concluded that catecholaminergic neurotransmitters inhibit electrical activity of these cells (29) or inhibit more cells than they excite (30). These results would suggest that these transmitters inhibit ADH release. In contrast, studies with direct injection of norepinephrine into the supraoptic nucleus of cats combined with measurement of plasma ADH by bioassay have concluded that norepinephrine stimulates ADH release (16). Similar results have been obtained with injection of norepinephrine and dopamine into the supraoptic nucleus of rats (31).

In our study an experimental protocol was designed to further examine the role of brain catecholamines in modulating the central release of ADH. Rather than the acute application or injection of catecholamines into the ventricles or directly into the brain, a chronic method of depleting brain catecholamines was used by intraventricular administration of 6-OHDA. This agent is an isomer of norepinephrine which is actively taken up by nerve cells and eventually incorporated into amine storage granules. One important advantage of this method of administration of 6-OHDA is that extracerebral catecholamine depletion does not occur, therefore simplifying the interpretation of the results. In the dosage schedule used in our study (two daily intraventricular injections of 200–250 μ g of 6-OHDA), a 60–80% depletion of brain catecholamines has been reported by other investigators (20, 22). In our investigation a 67% decrease in brain catecholamines occurred without concomitant extracerebral catecholamine depletion, as documented by the demonstration of no alteration in heart catecholamines. Of equal importance is the observation that there was no difference in behavior between control-injected and 6-OHDA-injected rats treated comparably to those used in the acute experimental protocols. Changes in weight, mean food intake, and serial blood pressures were identical in the two groups of animals. Although

water intake and urine volumes were slightly decreased in 6-OHDA-injected vs. control-injected rats, serum osmolality and pituitary ADH content were not different in the two groups of animals. Taken together, these results indicate that the observed differences in response to osmotic and nonosmotic stimuli were not a result of nonspecific effects of the 6-OHDA, such as chronic dehydration, resulting in depletion of pituitary ADH content.

Arterial blood pressure and renal hemodynamics were monitored before and after producing the osmotic and nonosmotic stimuli in both control-injected and 6-OHDA-injected rats. The mean arterial blood pressure, renal hemodynamics, and urinary diluting capacity were no different in the control and treated animals either before or after the stimulus for ADH release. These comparable findings in the control and 6-OHDA animals allowed for changes in U_{osm} to be monitored as an index of the end organ response to ADH. A sensitive and specific radioimmunoassay for ADH was used to monitor plasma changes in this hormone in response to osmotic and nonosmotic stimuli (26).

It was also important to test both the osmotic and nonosmotic response to ADH release because there is now evidence that these stimuli are mediated through different pathways. As discussed earlier, there is experimental evidence that both high (arterial) (26, 32, 33) and low (atrial) (34, 35) pressure baroreceptors are involved in mediating a variety of nonosmotic stimuli. These pathways, however, are clearly not involved in the osmotic stimulation of ADH release because Verney (2) originally demonstrated that baroreceptor denervation does not alter the ADH response to the injection of intracarotid hypertonic solutions. Moreover, there is now evidence in man that the osmotic release of ADH can be virtually abolished, while at the same time the nonosmotic release of ADH is perfectly normal (36). It is thus possible that catecholamines might be important as neurotransmitters in either the osmoreceptor or nonosmotic baroreceptor-mediated changes in ADH release, or both. Lastly, to examine the best sensitivity for osmotic and nonosmotic ADH release, the studies were performed in conscious rats.

The results of our study demonstrated that the same osmotic stimulus with hypertonic saline was associated with an attenuated release of ADH in the 6-OHDA animals as compared with the vehicle-treated control animals. The diminished release of ADH was associated with an appropriately lower U_{osm} in the 6-OHDA animals which could not be accounted for by differences in systemic or renal hemodynamics. Similar results were obtained when hyperoncotic dextran was used to cause hypovolemia and stimulate the nonosmotic release of ADH. This maneuver produced at least a 10% decrease in BV in both groups of animals;

our results, therefore, confirm earlier studies in the rat which indicate that such a fall in BV consistently stimulates ADH release (37). As in the hypertonic saline studies, the attenuated release of ADH in response to the nonosmotic hypovolemic stimulus was associated with an appropriately lower U_{osm} in the 6-OHDA-treated animals as compared with control animals. These different effects on ADH release and U_{osm} in response to the nonosmotic stimulus also could not be attributed to differences in measured systemic or renal hemodynamics.

It was not possible in our study to compare quantitatively the ADH response to an osmotic vs. a non-osmotic stimulus because different temporal relationships for measuring ADH were necessary in the two groups. The ADH values also were drawn at the time of peak U_{osm} and, thus, do not necessarily reflect maximal plasma levels. Moreover, the variability in the maximal response in the nonosmotic hypovolemic studies necessitated a method of measuring the mean plasma ADH level over a period of 30 min. On the other hand, the timing of the osmotic stimulus was consistently predictable, allowing ADH values in the hypertonic saline studies to be measured at a constant interval after the peak osmotic stimulus. In each case, however, it must be emphasized that the timing of collection for plasma ADH measurements was the same in the control and 6-OHDA groups and therefore could not explain the differences in responses. It should also be mentioned that further studies will be necessary to incriminate the specific catecholamine for the osmotic and nonosmotic stimuli because 6-OHDA is known to deplete both brain norepinephrine and dopamine. Moreover, as with all drug inhibitors, it is possible that the effect of 6-OHDA might alter ADH release in response to osmotic and nonosmotic stimuli by an as yet undefined mechanism. In this regard, Hoffman et al. (38) have suggested that 6-OHDA attenuates the response of ADH to intraventricular angiotensin II by directly altering central neuron integrity.

In conclusion, our results demonstrate that brain catecholamine depletion with 6-OHDA attenuates both the acute osmotic (hypertonic saline) and nonosmotic (hypovolemia secondary to intraperitoneal dextran) stimulation of ADH release. This attenuation could not be attributable to either alterations in arterial blood pressure or glomerular filtration rate, or the base-line pituitary ADH depletion. The failure to abolish totally the ADH release to these stimuli may be because of the fact that the brain catecholamines were only 67% depleted. Alternatively, other, perhaps secondary, pathways for ADH release may exist which are independent of hypothalamic catecholamines. In either case, a critical role of brain catecholamines in ADH release to osmotic and nonosmotic stimuli is implicated by our results.

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