Acute increases in arterial blood pressure do not reduce plasma vasopressin levels stimulated by angiotensin II or hyperosmolality in rats

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Stocker, Sean D., Jennifer C. Schiltz, and Alan F. Sved. Acute increases in arterial blood pressure do not reduce plasma vasopressin levels stimulated by angiotensin II or hyperosmolality in rats. Am J Physiol Regul Integr Comp Physiol 287: R127–R137, 2004. First published February 26, 2004; 10.1152/ajpregu.00526.2003.—The present study sought to determine whether an acute increase in arterial blood pressure (ABP) reduces plasma vasopressin (VP) levels stimulated by ANG II or hyperosmolality. During an intravenous infusion of ANG II (100 ng·kg⁻¹·min⁻¹), attenuation of the ANG II-evoked increase in ABP with diazoxide or minoxidil did not further enhance plasma VP levels in rats. When VP secretion was stimulated by an infusion of hypertonic saline, confusion of the α-adrenergic agonist phenylephrine (PE) significantly increased ABP but did not reduce plasma VP levels. In fact, plasma VP levels were enhanced. The enhancement of plasma VP levels cannot be explained by a direct stimulatory action of PE, as plasma VP levels of isosmotic rats did not change during a similar infusion of PE. An infusion of endothelin-1 in hyperosmotic rats significantly raised ABP but did not reduce plasma VP levels; rather, VP levels increased as observed with PE. In α-chloralose-anesthetized rats infused with hypertonic saline, inflation of an aortic cuff to increase ABP and stimulate arterial baroreceptors did not reduce plasma VP levels. In each experiment, plasma oxytocin levels paralleled plasma VP levels. Collectively, the present findings suggest that an acute increase in ABP does not inhibit VP secretion.

baroreceptor; vasopressin; oxytocin

In electrophysiological studies performed in anesthetized rats, magnocellular neurosecretory cells in the hypothalamus are commonly divided into two classes based on the ability of an increase in arterial blood pressure (ABP) to inhibit the activity of some neurons but not others (32, 35). Putative vasopressin (VP) neurons exhibit a bursting or continuous activity during hemorrhage (60) and are inhibited by an increase in ABP. On the other hand, putative oxytocin (OT) neurons show intermittent bursts of activity immediately before milk ejection (59, 60), are excited by CCK (36), but are insensitive to increases in ABP (32, 35). Indeed, the inhibition of neuronal activity by an acute increase in ABP is a distinguishing characteristic of putative VP neurons in electrophysiological studies performed in anesthetized rats (32, 35).

Because an acute increase in ABP inhibits the electrophysiological activity of putative VP neurons in anesthetized rats, similar increases in ABP may inhibit or mask VP secretion. For example, increases in circulating ANG II stimulate the release of VP (21, 46). However, an infusion of exogenous ANG II also increases ABP, and it has been postulated that the true efficacy of ANG II to stimulate VP release has been underestimated due to the ANG II-evoked increase in ABP (28, 29, 46). Consistent with this notion, intravenous infusion of ANG II at relatively low doses in anesthetized rats has been reported to increase the activity of putative VP neurons, whereas an infusion of larger doses of ANG II produced larger increases in ABP and was associated with a decrease in the activity of these neurons (29). Moreover, the ANG II-evoked increase in the activity of putative VP supraoptic neurons with pressor doses of ANG II was enhanced in acutely sinoaortic-denervated rats (28). However, in marked contrast to these observations, increases in ABP do not appear to inhibit VP secretion stimulated by ANG II. Complete removal of cardiopulmonary and arterial baroreceptor afferents by electrolytic lesion of nucleus tractus solitarius in rats did not enhance plasma VP levels during an intravenous infusion of pressor and nonpressor doses of ANG II (42). Therefore, the impact of ANG II-evoked increases in ABP on circulating VP levels remains unclear due to the conflicting observations between the vast amount of electrophysiological data and the little data on plasma VP levels that exist under these conditions. Indeed, there are very little data to demonstrate that the well-known inhibition of putative VP neuronal activity by increases in ABP leads to a reduction in plasma VP levels.

Therefore, the present study sought to determine whether acute increases in ABP inhibit VP secretion stimulated by ANG II or hyperosmolality in baroreceptor-intact rats. Because the secretion of VP is regulated coordinately with the ingestion of water to maintain body fluid homeostasis (40, 52) and an acute increase in ABP inhibits thirst stimulated by ANG II and hyperosmolality (13, 37, 49, 50), the present experiments utilized similar protocols to evaluate the impact of acute increases in ABP on circulating VP levels. The first set of experiments examined whether an acute increase in ABP inhibits VP secretion stimulated by ANG II. A pressor dose of ANG II was administered intravenously, and the pressor response was attenuated by administration of selective arteriolar vasodilators. If the ANG II-evoked increase in ABP inhibits VP secretion, then plasma VP levels should be enhanced compared with control rats when the increase in ABP is attenuated. A second set of experiments was performed to determine whether increases in ABP inhibit VP secretion stimulated by hyperosmolality. In each experiment, plasma levels of OT were analyzed along with VP because electrophysiological studies indicate that putative OT neurons, in contrast to putative VP neurons, are characteristically insensitive to increases in ABP (32, 35). Thus plasma OT levels were hypothesized to be unaffected by an increase in ABP.
Methods

Animals

Adult male Sprague-Dawley rats (Harlan Laboratories) weighing 325–400 g were housed individually in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle (lights on at 8:00 AM). Tap water and Purina Laboratory Chow (no. 5001) were available ad libitum except where noted. Animal care and experimental procedures were performed with approval of the Institutional Animal Care and Use Committee of the University of Pittsburgh. In all experiments, an adequate depth of anesthesia was assessed by absence of withdrawal reflex or pressor response to foot pinch. At the end of experiments, rats were euthanized with an overdose of urethane (experiments in unanesthetized rats) or potassium chloride (experiments in anesthetized rats).

Studies in Unanesthetized Rats

At least 48 h before experiments, catheters were implanted in the left femoral artery (Silastic, 0.025-in. ID and 0.037-in. OD, Fisher Scientific; or Microcathaneh tube, 0.012-in. ID and 0.025-in. OD, Braintree Scientific) and vein (Silastic, 0.025-in. ID and 0.037-in. OD, Fisher Scientific) while rats were anesthetized with halothane (2–3% in 100% O2). For ANG II experiments, an additional catheter (Silastic) was placed in the right jugular vein. All catheters were tunneled subcutaneously to exit between the scapulas and were filled with heparinized saline (arterial, 1,000 U/ml; venous, 40 U/ml). Rats were injected with an antibiotic (Dual-Cillin; 30,000 U im) and fitted with an infusion harness (Harvard Apparatus) that allowed the catheters to extend ~3 in. above the rat’s back while protected by a steel spring. The catheters extended 1 in. outside the spring and were plugged with steel obturators. On days of experiments, the catheters were connected to arterial and venous lines that extended outside the cage. Body weights and 24-h water intakes were monitored daily, and there was no significant change in these variables from presurgical values at any time. Experiments began 2 days later.

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

Effect of Increases in ABP on Plasma VP and OT Levels Stimulated by ANG II

The first series of experiments was designed to determine whether attenuation of the ANG II-evoked increase in ABP during an intravenous infusion of ANG II would enhance plasma VP levels. Water bottles were removed from the cages, and baseline MAP and HR were recorded for 20 min. Rats were then infused with hypertonic saline (HS, 1 M NaCl, 2 ml/h iv) for 60 min. After 60 min, rats were coinfused with a solution containing HS (2 ml/h iv) and either phenylephrine (PE, 8 μg·kg⁻¹·min⁻¹ iv; n = 10) or endothelin-1 (ET; 250 ng·kg⁻¹·min⁻¹ iv for 10 min, then 50 ng·kg⁻¹·min⁻¹ iv for the remainder of the test; n = 6) to raise ABP. Control rats received SLN (n = 5) instead of PE or ET. In addition, separate groups of rats were treated identically except that the HS infusion was replaced by SLN (2 ml/h). These groups will be referred to as “first infusion plus the subsequent infusion” (e.g., “HS + PE” or “SLN + PE”).

Blood samples (1.5 ml) were collected into microcentrifuge tubes containing heparin (20 U) as described above at baseline (5 min before any infusion) and 60, 75, and 105 min after the initiation of the first infusion. Samples were immediately centrifuged (10,000 g), and the plasma was stored at −80°C until analyzed for VP and OT concentrations. Posmol was determined as described above.

Studies in unanesthetized rats. A separate group of experiments, an inflatable cuff was placed around the descending aorta, as a nonpharmacological tool to increase ABP in the carotid arteries and aortic arch. Rats were instrumented with arterial and venous catheters in the brachial and femoral vessels while anesthetized with halothane (2–3% in 100% O2). NaCl (2 M; 1 ml/h iv) was infused through the femoral venous catheter, whereas the remaining drugs were administered through the brachial venous catheter. The trachea was then cannulated, and rats were ventilated with halothane (2–3%) in 100% O2 (small animal respirator; Harvard Apparatus, South Natick, MA). An inflatable cuff was placed around the descending aorta either proximal (n = 7) to the renal arteries through a retroperitoneal incision or distal (n = 7) to the renal arteries through a midline incision; these groups will be referred to as “proximal aortic cuff” or “distal aortic cuff,” respectively. At least 1 day before use, the inflatable cuff was

R128

ACUTE INCREASES IN ABP DO NOT REDUCE PLASMA VP LEVELS

and the respective vasodilator” (e.g., “ANG II + CPT + DZX”). In all experiments, drugs were dissolved in isotonic saline. Additional control rats did not receive CPT but instead were infused with SLN for 10 min before 100 ng·kg⁻¹·min⁻¹ ANG II (ANG II; n = 8). The dose of ANG II was selected from a previous study that examined the influence of ANG II-evoked increases in ABP on vasopressin secretion in chronically baroreceptor-denervated rats with electrolytic lesion of nucleus tractus solitarius (42). This dose significantly increases ABP, plasma VP levels, and the ingestion of water, whereas lower doses of ANG II do not consistently increase VP. Furthermore, the increase in ABP with this dose of ANG II has been demonstrated to inhibit the evoked thirst through the activation of arterial baroreceptor afferents (13, 37, 49, 50).

In each animal, blood samples (1.5 ml) were collected from the arterial catheter into microcentrifuge tubes containing heparin (20 U) at baseline and 15 and 45 min after initiation of the ANG II infusion. 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. In addition, plasma protein concentration was measured by protein refractometry, and plasma osmolality (Posmol) was measured from two 20-μl aliquots by freezing point depression using a micro-osmometer (model 3360, Advanced Instruments; Norwood, MA). In this and subsequent experiments, the first blood sample was replaced with an equal volume of isotonic saline, whereas subsequent samples were replaced with red blood cells from the previous sample resuspended in heparinized saline (40 U/ml) warmed at 37°C.

Effect of Increases in ABP on Plasma VP and OT Levels Stimulated by Hyperosmolality

Studies in unanesthetized rats. A second series of experiments was designed to determine whether increases in ABP reduce plasma VP levels stimulated by increases in plasma osmolality. Water bottles were removed from the cages, and baseline MAP and HR were recorded for 20 min. Rats were then infused with hypertonic saline (HS, 1 M NaCl, 2 ml/h iv) for 60 min. After 60 min, rats were coinfused with a solution containing HS (2 ml/h iv) and either phenylephrine (PE, 8 μg·kg⁻¹·min⁻¹ iv; n = 10) or endothelin-1 (ET; 250 ng·kg⁻¹·min⁻¹ iv for 10 min, then 50 ng·kg⁻¹·min⁻¹ iv for the remainder of the test; n = 6) to raise ABP. Control rats received SLN (n = 5) instead of PE or ET. In addition, separate groups of rats were treated identically except that the HS infusion was replaced by SLN (2 ml/h). These groups will be referred to as “first infusion plus the subsequent infusion” (e.g., “HS + PE” or “SLN + PE”).

Blood samples (1.5 ml) were collected into microcentrifuge tubes containing heparin (20 U) as described above at baseline (5 min before any infusion) and 60, 75, and 105 min after the initiation of the first infusion. Samples were immediately centrifuged (10,000 g), and the plasma was stored at −80°C until analyzed for VP and OT concentrations. Posmol was determined as described above.

Studies in anesthetized rats. In a separate group of experiments, an inflatable cuff was placed around the descending aorta, as a nonpharmacological tool to increase ABP in the carotid arteries and aortic arch. Rats were instrumented with arterial and venous catheters in the brachial and femoral vessels while anesthetized with halothane (2–3% in 100% O2). NaCl (2 M; 1 ml/h iv) was infused through the femoral venous catheter, whereas the remaining drugs were administered through the brachial venous catheter. The trachea was then cannulated, and rats were ventilated with halothane (2–3%) in 100% O2 (small animal respirator; Harvard Apparatus, South Natick, MA). An inflatable cuff was placed around the descending aorta either proximal (n = 7) to the renal arteries through a retroperitoneal incision or distal (n = 7) to the renal arteries through a midline incision; these groups will be referred to as “proximal aortic cuff” or “distal aortic cuff,” respectively. At least 1 day before use, the inflatable cuff was

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ACUTE INCREASES IN ABP DO NOT REDUCE PLASMA VP LEVELS

R129

constructed from Tygon microbore tubing (OD 0.060 in. × ID 0.020 in.) tied at one end, and the open end was connected to a 1-ml syringe filled with SLN. The tied end was placed into boiling water, and SLN was pushed into the tubing with the syringe to expand and soften the tied end, resulting in an inflatable portion. During experiments, the tied end was wrapped around the aorta and secured to the remainder of the tubing with a suture. After the cuff was implanted, the incision was closed with suture. Then halothane was terminated, and anesthesia was maintained by α-chloralose (70 mg/kg initial bolus of a 30 mg/ml stock solution dissolved in 3% sodium borate and supplemented hourly by 20 mg/kg iv). The total volume of α-chloralose given during the course of an experiment was 0.9–1.2 ml. Additionally, rats were treated with a muscle relaxant (0.5 mg/kg D-tubocurarine, supplemented hourly with 0.2 mg/kg iv), and no manipulations or procedures were performed for at least 30 min. In all experiments, rats were anesthetized with halothane for ~1 h before α-chloralose.

Baseline femoral and brachial MAP and HR (triggered via brachial pulsatile ABP signal) were recorded for 10 min. Then rats were infused with 2 M NaCl (1 ml/h) for 55 min through the femoral venous catheter. At 60 min, the cuff was inflated for 15 min. Control rats were implanted with an inflatable cuff on the descending aorta either proximal (n = 4) or distal (n = 4) to the renal arteries; however, the cuff was not inflated. An additional group of rats received an infusion of PE (8 µg·kg⁻¹·min⁻¹ iv; n = 7) instead of inflation of the aortic cuff. Blood samples (2.0 ml) were collected into microcentrifuge tubes containing heparin (25 U) from the brachial arterial line at baseline, at the end of the 2 M NaCl infusion, 12 min after ABP was measured, and 10 min after the cuff was deflated or the PE infusion was terminated. Samples were immediately centrifuged (10,000 g, 1 min) and stored at ~80°C until plasma VP and OT levels and plasma renin activity (PRA) were determined by radioimmunoassay. Plasma VP and OT levels did not differ from rats infused with 2 M NaCl (1 ml/h).

Determination of Plasma VP, OT, and PRA

Plasma VP and OT levels were determined by radioimmunoassay as described previously (38). Briefly, samples were extracted using C₁₈ Sep-Pak Vac Cartridges (1 ml, 50 mg; Waters, Milford, MA), and the extract was frozen, dried using a Speed Vac (Savant Instruments), and reconstituted in buffer (50 mM Na₂PO₄, 25 mM EDTA, 0.9% NaCl, 0.5% bovine serum albumin, 0.1% sodium azide, pH 7.4); 200-µl aliquots were used for radioimmunoassays. Samples were incubated for 16–24 h at 4°C with a rabbit polyclonal antibody to either VP (final dilution 1:300,000) or OT (1:450,000). The characteristics of these antibodies, which were generously donated by Dr. J. Fernstrom (Pittsburgh, PA), have been described previously (15). Samples were then incubated for 16 h at 4°C with ~3,200 counts/min of ¹²⁵I-labeled VP or OT (New England Nuclear-DuPont, Boston, MA). Subsequently, antibodies were precipitated using a second antibody procedure (38), and tubes were centrifuged (3,000 g, 25 min), the supernatant was aspirated, and the remaining pellets were counted in a gamma counter (1470 Wizard, Wallac, Gaithersburg, MD). Values of VP and OT were calculated from standard curves generated with known values of synthetic VP or OT (Bachem, Torrance, CA) that were extracted identically to plasma samples from buffer containing 0.5% bovine serum albumin. Duplicate plasma VP samples and single plasma OT samples were analyzed, and values were expressed as picograms per milliliter plasma. All samples that were compared statistically were analyzed within the same assay. Intra- and interassay variation is <10%. The sensitivity of the VP and OT assay is ~0.5 pg/sample (~2.5 pg/ml plasma) and ~1.0 pg/sample (~5.0 pg/ml plasma), respectively.

PRA was determined by radioimmunoassay as described previously (41, 54) except that incubation times were 1 h.

Statistical Analysis

All data are expressed as means ± SE. MAP and HR were analyzed by a two-way ANOVA with repeated measures. When significant F values were obtained for the factor group, an ANOVA was performed at each time followed by a layered Bonferroni post hoc test. When significant F values were obtained for the time factor, an ANOVA with repeated measures was performed followed by paired t-tests corrected by a layered Bonferroni. For experiments using an aortic cuff, brachial and femoral MAP and HR values are expressed as the change from the 60-min point for each rat and averaged. Change in brachial and femoral MAP and HR are analyzed as described above.

Plasma VP and OT levels, and PRA values were log transformed and analyzed similar to MAP and HR. Posmol and plasma protein were analyzed by a two-way ANOVA with repeated measures as described for MAP and HR. A P value of <0.05 was considered to be significant in all statistical tests.

RESULTS

Effect of Increases in ABP on Plasma VP and OT Levels Stimulated by ANG II

Infusion of 100 ng·kg⁻¹·min⁻¹ ANG II significantly increased plasma VP and OT levels and MAP above baseline values (Fig. 1). Similarly, ANG II + CPT significantly increased plasma VP and OT levels and MAP above baseline values (Fig. 1), and these increases in VP, OT, and MAP were not significantly different from those increases in rats treated with ANG II only.

To prevent the increase in ABP associated with the infusion of ANG II, rats were treated with either DZX or MXD in addition to the infusion of ANG II + CPT. DZX or MXD markedly attenuated the ANG II-evoked increase in ABP, but plasma VP and OT levels did not differ from rats infused with ANG II + CPT (P > 0.7 from overall ANOVAs at each time; Fig. 1). Rats treated with ANG II + CPT + MXD displayed a significantly lower MAP than those treated with ANG II + CPT after 5 min, and it remained significantly lower throughout the test. Furthermore, MAP of rats treated with ANG II + CPT + MXD was not different from baseline values from 30 to 45 min (Fig. 1C). Similarly, rats treated with ANG II + CPT + DZX displayed a significantly lower MAP than those treated with ANG II + CPT; however, MAP of rats treated with ANG II + CPT + DZX was significantly elevated compared with baseline values through the initial 30 min (Fig. 1C).

As expected, rats infused with ANG II only or ANG II + CPT displayed a significant bradycardia throughout the infusion of ANG II (Fig. 1D). In contrast, HR of rats treated with ANG II + CPT + DZX or ANG II + CPT + MXD was not different from baseline values for the initial 30 min, and thereafter HR was elevated significantly above baseline values (Fig. 1D).

Posmol did not differ between any treatment groups infused with ANG II at baseline, 15 min, or 45 min (Table 1; P > 0.2 from overall ANOVA). Similarly, plasma protein did not differ between treatment groups (data not shown; P > 0.1 from overall ANOVA).
Effect of Increases in ABP on Plasma VP and OT Levels Evoked by Hyperosmolality

PE-induced increases in ABP on plasma VP and OT levels. To determine whether VP and OT secretion stimulated by hyperosmolality would be inhibited by an increase in ABP, unanesthetized rats were infused with HS, and then ABP was raised by an infusion of PE. The infusion of HS did not alter MAP from baseline values but significantly raised plasma VP levels to ~16 pg/ml at 60 min, and these levels did not differ between rats subsequently receiving either SLN (HS + SLN) or PE (HS + PE) (Fig. 2A). As expected, the infusion of PE in HS + PE rats significantly raised MAP (Fig. 2C); however, this was associated with an unexpected increase in plasma VP levels (Fig. 2A). Indeed, rats treated with HS + PE displayed significantly higher MAP and plasma VP levels than rats treated with HS + SLN (Fig. 2, A and C). Similarly, plasma OT levels of HS + PE rats were significantly higher than those of HS + SLN rats (Fig. 2B). While the infusion of PE significantly raised MAP in SLN + PE group, plasma VP and OT levels did not change from baseline or 60-min values (Fig. 2, A–C). This apparent potentiation of plasma VP and OT levels by PE in rats infused with HS occurred without any difference in \( P_{\text{osmol}} \) between rats treated with HS + SLN and HS + PE (Table 2).

Not surprisingly, the infusion of PE caused a significant bradycardia in rats treated with either HS + PE or SLN + PE (Fig. 2D). HR of rats treated with either HS + SLN or HS + SLN did not change from baseline values at any time (\( P > 0.7 \) from overall ANOVA).

Effect of ET-induced increases in ABP on plasma VP and OT levels. To examine whether increases in ABP induced by a pressor agent other than PE would reduce plasma VP levels, unanesthetized rats were infused with HS, and ABP was raised by an infusion of ET. Similar to the results obtained with PE, the infusion of ET significantly raised ABP above baseline values in HS + ET rats and those values of HS + SLN rats (Fig. 3C); however, this was again associated with an unexpected increase in plasma VP and OT levels by the infusion of ET occurred with a significantly greater increase in \( P_{\text{osmol}} \) compared with HS + SLN rats (Table 2). The infusion of SLN + ET did not alter plasma VP levels or \( P_{\text{osmol}} \) (Fig. 3A); however, plasma OT levels were elevated significantly above those values of SLN + SLN rats at 75 and 105 min (Fig. 3B). As expected, the infusion of ET caused a

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**Table 1.** \( P_{\text{osmol}} \) of rats infused with ANG II (100 ng·kg\(^{-1}\)·min\(^{-1}\) iv)

<table>
<thead>
<tr>
<th>( P_{\text{osmol}} ) mmol/kg H(_2)O</th>
<th>( n )</th>
<th>Baseline</th>
<th>15 min</th>
<th>45 min</th>
</tr>
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<tbody>
<tr>
<td>ANG II only</td>
<td>8</td>
<td>292±1</td>
<td>294±1</td>
<td>293±1</td>
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<tr>
<td>ANG II + CPT</td>
<td>10</td>
<td>294±1</td>
<td>294±1</td>
<td>295±2</td>
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<tr>
<td>ANG II + CPT + DZX</td>
<td>9</td>
<td>294±1</td>
<td>297±1</td>
<td>297±1</td>
</tr>
<tr>
<td>ANG II + CPT + MXD</td>
<td>8</td>
<td>292±1</td>
<td>293±2</td>
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</tbody>
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Values are means ± SE. Rats were infused with captopril (CPT; 0.33 mg·min/iv) for 10 min and then coinfused with CPT plus ANG II (100 ng·kg\(^{-1}\)·min\(^{-1}\)). One min later, diazoxide (DZX; 10 mg/kg) or minoxidil (MXD; 2 mg/kg) was injected iv and administered over the first 5 min. Control rats received the infusion of ANG II only or ANG II + CPT. \( P_{\text{osmol}} \), plasma osmolality.
significant bradycardia in both HS + ET and SLN + ET rats (Fig. 3D).

**Aortic cuff-induced increases in ABP on plasma VP and OT levels.** Because PE- and ET-induced increases in ABP did not inhibit, but rather enhanced, plasma VP and OT levels stimulated by hyperosmolality, a second set of experiments was performed to raise ABP nonpharmacologically by inflation of a cuff placed around the descending aorta proximal or distal to the renal vessels. Because this experiment was conducted in α-chloralose-anesthetized rats, an additional group of rats in which ABP was raised by an infusion of PE was used for purposes of comparison. The infusion of 2 M NaCl caused a significant increase in plasma VP and OT levels (Fig. 4, A and B) and \( P_{\text{osmol}} \) in all treatment groups (Table 3), and there were no significant differences in these parameters among treatment groups at the end of the 2 M NaCl infusion (\( P > 0.3 \) from overall ANOVAs). Inflation of the proximal aortic cuff produced a significant and sustained increase in brachial MAP and a significant decrease in HR compared with control rats (Fig. 5, A and C); however, plasma VP levels of rats with an inflated proximal cuff were not different from those of control rats or those levels before cuff inflation (Fig. 4A). However, inflation of the proximal aortic cuff caused a significant increase in PRA levels (Fig. 4C), presumably resulting from the decrease in renal perfusion pressure, as reflected by the decrease in femoral MAP (Fig. 5B). Because increases in PRA levels (via circulating ANG II) increase the activity of putative VP neurons and plasma VP levels (14, 21), this experiment was repeated using an aortic cuff placed distal to the renal vessels. Inflation of the distal aortic cuff produced a significant and sustained increase in brachial MAP (Fig. 5A) but did not change PRA (Fig. 4C). Again, plasma VP levels were not altered by cuff inflation (Fig. 4A) despite the increase in brachial MAP. Similarly, plasma OT levels were not affected by inflation of the proximal or distal aortic cuff (Fig. 4B).

An inflation of PE in rats anesthetized with α-chloralose significantly increased brachial MAP (Fig. 6C) and femoral MAP (data not shown) and significantly decreased HR (Fig. 6D). These changes in brachial MAP and HR were similar to those of rats during inflation of a proximal aortic cuff (\( P > 0.5 \) from overall ANOVA). However, plasma VP and OT levels
were not different from control rats or rats with an inflated proximal aortic cuff (Fig. 6, A and B).

The infusion of 2 M NaCl significantly raised P osmol in all groups, but P osmol did not differ among treatment groups at any time (Table 3).

**DISCUSSION**

The inhibition of neuronal firing rates of putative VP neurons by acute increases in ABP in anesthetized rats is well established (32, 35). Indeed, it is used as a distinguishing characteristic of putative VP neurons in electrophysiological studies. However, there are very little data to demonstrate that the well-known inhibition of putative VP neuronal activity by brief increases in ABP correlates with a reduction in plasma VP levels. Furthermore, the infusion of ET in hyperosmotic rats produced a significantly greater elevation in MAP compared with isosmotic rats at 90 and 105 min. *Significant difference between HS + ET and HS + SLN rats (P < 0.05). The infusion of ET significantly elevated MAP and plasma OT levels at 75 and 105 min (P < 0.05) but had no effect on plasma VP levels. Furthermore, the infusion of ET in hyperosmotic rats produced a significantly greater elevation in MAP compared with isosmotic rats at 90 and 105 min. *Significant difference between HS + ET and HS + SLN rats (P < 0.05). Note the scales of the y-axis are different for plasma VP and OT levels.

**Increases in ABP Do Not Reduce Plasma VP Levels During an Infusion of ANG II**

In the present study, attenuation of the ANG II-evoked increase in ABP by treatment with a vasodilator drug (DZX or MXD) did not enhance plasma VP levels during an infusion of a pressor dose of ANG II, as would be expected if the ANG II-evoked increase in ABP inhibited VP secretion. DZX and MXD act by opening K+ channels (31) and may produce actions other than countering the ANG II-induced increase in ABP. However, no other known effects of these drugs can explain the current data. For example, although DZX may lead to the retention of sodium and water (31), it did not influence P osmol in the present studies. Furthermore, while the infusion of ANG II may increase atrial pressure (4), which together with an increase in ABP inhibits VP secretion, DZX or MXD attenuated the increased ABP and therefore would have removed at least one of these two inhibitory signals. Thus VP levels should have increased when the increase in ABP was attenuated despite potentially elevated atrial pressure, although this assumes that changes in atrial and arterial pressure interact in a linear fashion. Moreover, elimination of arterial baroreceptor and cardiac afferents by electrolytic lesion of nucleus tractus solitarius in rats did not further enhance plasma VP levels compared with control rats during intravenous infusion of pressor doses of ANG II (42). Although neural plasticity may contribute to changes in responses in chronically deafferented animals, the results of these chronic lesion experiments are fully consistent with the present data. Thus we believe that changes in atrial pressure likely do not explain the lack of an effect on VP levels when the ANG II-evoked increase in ABP is attenuated in rats. Together, these observations suggest VP secretion in rats is not inhibited by the ANG II-evoked increase in ABP.

Consistent with these observations in rats, complete surgical removal of sinoaortic arterial baroreceptor afferents plus car- diopulmonary afferents in dogs did not enhance plasma VP levels during infusions of pressor doses of ANG II (3). On the
Although MAP of dogs receiving ANG II plus hydralazine was not reported (2). In this regard, preliminary experiments indicate that MAP of rats treated with ANG II plus hydralazine transiently drops below baseline values (Stocker and Sved, unpublished observations), thereby providing a hypotensive stimulus for VP secretion. Therefore, baroreceptor denervation and attenuation of the ANG II-evoked increase in ABP with nitroprusside or hydralazine may not be equivalent in dogs. The reason for these apparent differences between dogs and rats will likely remain unclear until parallel experiments controlling all of the relevant variables are performed in both species; however, the discrepancies between dogs and rats may reflect real species differences in the regulation of VP secretion.

**Increases in ABP Do Not Reduce Plasma VP Levels During Hyperosmolality**

Additional experiments were conducted to determine whether acute increases in ABP reduce plasma VP levels during hyperosmolality. Despite a large increase in MAP of hyperosmotic rats infused with PE or ET, plasma VP levels did not decrease; rather plasma VP levels were enhanced by either infusion. The potentiation of plasma VP levels in hyperosmotic rats by PE and ET has been replicated in Sprague-Dawley rats from a different supplier (Zivic Laboratories, Zelienople, PA) under a similar protocol (47). Furthermore, preliminary experiments suggest that an infusion of PE also enhances VP secretion in water-deprived rats (Schiltz and Sved, unpublished observations).

The enhancement of plasma VP levels in hyperosmotic rats by PE and ET cannot be explained by direct stimulatory action(s) because plasma VP levels of isosmotic rats infused with PE or ET did not increase from baseline values. Similar observations have been reported in dogs (58). This may further suggest that increases in ABP do not inhibit basal VP secretion (although the sensitivity of the VP radioimmunoassay in the present study does not permit an accurate detection of a decrease in plasma VP from baseline levels). Whereas the

**Fig. 4. Mean ± SE plasma VP (A) and OT (B) levels and plasma renin activity (PRA; C) of α-chloralose-anesthetized rats infused with 2 M NaCl (solid line) for 55 min. Then an aortic cuff placed proximal or distal to the renal vessels was inflated from 0 to 15 min (dashed line). The infusion of 2 M NaCl significantly increased plasma VP and OT levels (P < 0.025). Inflation of the aortic cuff placed proximal or distal to the renal vessels significantly raised MAP (see Fig. 5); however, plasma VP and OT levels of either group were not different from those of control rats at 12 min (P > 0.75 from overall ANOVA). Inflation of the proximal aortic cuff significantly increased PRA above those of control rats or those with an inflated distal aortic cuff (*P < 0.05). Once the proximal aortic cuff was deflated, PRA values were not different between groups (P > 0.25 from overall ANOVA). Baseline plasma VP and OT levels and PRA values were not different between treatment groups (P > 0.4 from overall ANOVA). Note the scales of the y-axis are different for plasma VP and OT levels.

**Table 3. Baseline MAP and HR and P_{osmol} at −60, −5, 12, and 25 min in α-chloralose-anesthetized rats infused with 2 M NaCl (1 ml/h) for 55 min**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Proximal Aortic Cuff (n=7)</th>
<th>Distal Aortic Cuff (n=7)</th>
<th>PE (8 μg kg⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Baseline MAP, mmHg</td>
<td>113±6</td>
<td>117±2</td>
<td>110±3</td>
<td>112±2</td>
</tr>
<tr>
<td>Baseline HR, beats/min</td>
<td>369±12</td>
<td>370±8</td>
<td>351±8</td>
<td>369±5</td>
</tr>
<tr>
<td>P_{osmol}, mOsmol/kgH₂O</td>
<td>−60 min</td>
<td>301±2</td>
<td>300±2</td>
<td>304±1</td>
</tr>
<tr>
<td></td>
<td>−5 min</td>
<td>322±3</td>
<td>320±2</td>
<td>324±1</td>
</tr>
<tr>
<td></td>
<td>12 min</td>
<td>320±3</td>
<td>320±1</td>
<td>323±1</td>
</tr>
<tr>
<td></td>
<td>25 min</td>
<td>319±2</td>
<td>318±2</td>
<td>322±2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline mean arterial pressure (MAP) and heart rate (HR) were not different between treatment groups (P > 0.2 from overall ANOVA). The infusion of 2 M NaCl (1 ml/h) significantly increased P_{osmol} above baseline values for the remainder of the test (P < 0.01). In addition, P_{osmol} was not significantly different between treatments groups at any time (P > 0.2 from overall ANOVA).
infusion of PE did not alter P\textsubscript{osmol} in hyperosmotic rats, the infusion of ET in hyperosmotic rats was associated with a significantly larger increase in P\textsubscript{osmol}, which is consistent with the action of ET to decrease renal blood flow and urine osmolality (1, 16, 39), thereby increasing P\textsubscript{osmol} and consequently VP secretion. Because elevations in P\textsubscript{osmol} and ABP increase the permeability of the blood-brain barrier (27), the possibility that PE or ET additionally may act in the central nervous system (CNS) to enhance the VP release cannot be excluded. In this regard, direct application of norepinephrine and \(\alpha_1\)-agonists has been reported to excite hypothalamic neurons in vitro (33, 62) and in vivo (8). Similarly, norepinephrine or PE injected into the supraoptic nucleus (61) or added to the perfusate of hypothalamic explants increases VP secretion (23, 34); however, the site of action for PE in studies using perfused hypothalamic explants was not determined.

Despite the excitatory action of norepinephrine and \(\alpha_1\)-agonists centrally, intracarotid injections of PE failed to excite supraoptic neurons (30). Nevertheless, the present findings suggest that PE- or ET-induced increases in ABP do not inhibit VP secretion stimulated by hyperosmolality in rats. Still, these surprising findings must be interpreted cautiously; because of this unexpected response to PE and ET in the hyperosmotic rats, it may simply be that under these conditions a pressure-induced decrease in VP secretion was obscured by other actions of the drug. At the very least, these studies highlight some of the problems for future experiments investigating similar questions.

Inflation of a cuff placed proximal or distal to the renal vessels increased brachial MAP, reflecting an increased stretch of the aortic arch and carotid sinuses, but did not reduce circulating VP levels in chloralose-anesthetized, hyperosmotic rats. Although inflation of the proximal aortic cuff decreased renal perfusion pressure and presumably increased plasma ANG II levels, circulating ANG II is not a powerful stimulus for VP secretion unless plasma levels of ANG II are extremely high (43). Moreover, the decrease in renal perfusion pressure may have activated renal afferents that are capable of stimulating VP secretion (5, 44). Together, these additional stimuli may have masked any reduction in VP levels caused by increased ABP. However, plasma VP levels were not reduced when ABP was increased significantly by inflation of the distal aortic cuff; although the pressor response was smaller compared with the proximal aortic cuff and did not evoke a baroreceptor reflex bradycardia. Unfortunately, it was not possible to elicit a larger increase in ABP with distal cuff inflation. Interestingly, an infusion of PE in anesthetized hyperosmotic rats significantly raised ABP (and reflectively reduced HR) but did not affect plasma VP levels. However, unlike the results in unanesthetized rats where PE potentiated hyperosmotic-stimulated VP secretion, baseline and stimulated plasma VP levels and P\textsubscript{osmol} were much higher in \(\alpha\)-chloralose-anesthetized rats, which was probably a result from anesthesia and surgical procedures. However, it is noteworthy that essentially all of the data concerning the excitability of putative VP neurons during changes in ABP have been collected from anesthetized and surgically stressed rats in vivo (32, 35) in which circulating VP levels are certainly elevated (in excess of 100 pg/ml plasma; Sved, unpublished observations). While anesthesia may blunt the responsiveness of VP secretion, a reduction in circulating VP levels should have been observed if such a response occurred. Therefore, these findings are inconsistent with the notion that increases in ABP reduce plasma VP levels.

The present experiments were designed to permit detection of a decrease in plasma VP levels if it occurred. The radioimmunoassay, as performed, allowed for a sensitive measurement of changes in circulating VP levels in the range stimulated by ANG II or hyperosmolality. The half-life of circulating VP is only a few minutes (7), and therefore any decrease in secretion should be reflected by a decrease in plasma levels given the time course of these experiments. Plasma VP levels were analyzed no sooner than 10 min after initiation of the acute increase in ABP, thereby allowing sufficient time for a measurable decrease in plasma VP levels. In an analogous manner, decreases in plasma VP levels have been reported in hyperosmotic rats due to water ingestion under a similar time frame in our laboratory (20). Thus the present experiments had suffi-

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**Fig. 5.** Mean ± SE change (\(\Delta\)) in brachial MAP (A), femoral MAP (B), and HR (C) of \(\alpha\)-chloralose-anesthetized rats infused with 2 M NaCl (solid line) for 55 min. Then an aortic cuff placed proximal or distal to the renal vessels was inflated from 0 to 15 min (dashed line). Brachial MAP and HR were not different between treatment groups at baseline and 60 min (Table 3). Inflation of the proximal aortic cuff significantly increased brachial MAP and significantly decreased femoral MAP and HR compared with baseline values or those of control rats (\(P < 0.05\)). In addition, inflation of the distal aortic cuff significantly increased brachial MAP (\(P < 0.05\)) and decreased femoral MAP (\(P < 0.01\)) without any significant change in HR during the cuff inflation.
cient power and sensitivity to reliably detect a decrease in plasma VP levels had such a response occurred.

**Effect of Increased ABP on VP Secretion and Neuronal Activity**

Although increases in ABP inhibit the neuronal activity of putative VP neurons (32, 35), the present findings suggest that plasma VP levels are not reduced by an increase in ABP. This discrepancy may be explained by the duration of the hypertensive stimulus. First, the inhibition of putative VP neurons by an increase in ABP is based on increases in ABP lasting only tens of seconds (18, 32, 35), whereas the majority of studies examining the effects on circulating VP levels rely on a much longer time course, i.e., several minutes to tens of minutes (2, 3, 42). In this regard, the neuronal activity of putative VP neurons in vasopressin-deficient Brattleboro rats was inhibited initially by increased ABP, but this response was not sustained during several minutes of elevated ABP (26). Unfortunately, similar experiments have not been reported using standard rat strains. Thus the neuronal activity of putative VP neurons and VP secretion may be inhibited only briefly (i.e., seconds, not minutes) by increases in ABP. Consistent with this notion, the one paradigm in which increased ABP appears to inhibit VP secretion also relies on a very brief stimulus. Specifically, electrical stimulation of the parabrachial (55) or fastigial nucleus (9, 10) for 10 s increases ABP and plasma VP levels, and the increases in ABP appear to inhibit the stimulated VP secretion in chloralose-anesthetized rats. Interestingly, injection of PE to evoke an acute increase in ABP was capable of inhibiting fastigial nucleus stimulation-evoked VP secretion in this brief time frame (10). Therefore, increases in ABP lasting a few seconds may inhibit the activity of putative VP neurons and VP secretion, and this acute inhibitory mechanism may adapt or desensitize when an increase in ABP persists for minutes. For this reason, a reduction in plasma VP levels may not have been observed in the present series of experiments.

To summarize, although brief increases in ABP lasting several seconds appear to inhibit the activity of VP neurons and VP secretion, increases in ABP of a longer duration (i.e., several minutes) do not appear to inhibit VP secretion during increases in P_{osmol} or peripheral ANG II levels. Therefore, the well-known inhibitory effect of acute increases in ABP on putative VP neuronal activity may have little physiological impact on the organism.

**Increases in ABP Do Not Inhibit OT Secretion Stimulated by ANG II or Hyperosmolality**

Because electrophysiological studies indicate that putative OT neurons are insensitive to increases in ABP (32, 35), plasma OT levels in response to ANG II or hyperosmolality were hypothesized to be unaffected by increases in ABP. In each paradigm of the present study, increases in ABP did not reduce plasma OT levels. Instead, plasma OT levels were enhanced in rats treated with HS + PE or HS + ET. In this regard, norepinephrine and α1-adrenergic agonists have been demonstrated to stimulate or enhance OT secretion in hypothalamic-pituitary explants (23, 34). Curiously, increased ABP has been reported to inhibit OT secretion during brief electrical stimulation of the parabrachial nucleus, a paradigm in which increased ABP inhibits VP secretion (17).
Moreover, arterial hypotension produced by chlorisondamine or hydralazine increases plasma OT levels (38). Together, these observations suggest that OT neurons may be sensitive to changes in AP; however, presently no data exist examining the impact of prolonged changes in AP on the firing rates of OT neurons. Regardless, the present findings collectively suggest that prolonged increases in ABP do not inhibit OT secretion stimulated by ANG II or hyperosmolality, and these results parallel the effects of increases in ABP on VP secretion.

**Perspectives**

Hyperosmolality, hypovolemia, and hypotension each stimulate the ingestion of water and VP secretion from the posterior pituitary (22, 43, 52, 53, 56). The physiological significance of these responses is readily apparent. The ingestion of water restores normal blood volume and osmolality. Similarly, VP promotes water conservation by the kidney, thereby aiding in the maintenance of blood volume and osmolality (6), whereas higher circulating levels of VP stimulate vasoconstriction and thus aid in the maintenance of ABP (6, 43). Therefore, the ingestion of water and secretion of VP appear to be regulated coordinately to maintain body fluid homeostasis.

The present findings are at odds with this notion, as acute increases in ABP do not influence VP secretion and thirst similarly. Acute increases in ABP inhibit water intake and lengthen the latency to drink during increases in plasma osmolality or ANG II levels in rats (13, 37, 49, 50), but the present findings suggest that similar increases in ABP do not reduce circulating VP levels under these same conditions. Furthermore, complete removal of arterial and cardiopulmonary afferents by electrolytic lesion of nucleus tractus solitarius in rats enhanced water intakes and shortened the latency to drink but did not alter plasma VP levels during intravenous infusions ofpressor doses of ANG II (42). In dogs, surgical removal of arterial and cardiopulmonary afferents enhanced the dipsogenic response to pressor doses of ANG II but did not alter circulating VP levels (3, 24). Even though the regulation of VP secretion and thirst may differ between the rat and dog, particularly during hypovolemia (57), these observations suggest that increases in ABP do not affect VP secretion and thirst similarly. On the other hand, arterial hypotension increases VP secretion and thirst in rats. Whereas disruption of the peripheral renin-angiotensin system attenuates hypotension-induced thirst (11, 12, 45, 48, 49, 51), it does not greatly affect the increase in plasma VP levels (25). Together with the findings of the present study, these observations suggest that VP secretion and thirst are stimulated under similar conditions, but the mechanism(s) regulating and/or modulating each of these responses is distinct. This differential regulation of VP secretion and thirst may allow a redundant mechanism(s) that permits the proper regulation of body fluid homeostasis under a variety of conditions.

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