Contribution of Plasma Vasopressin Concentration and Blood Pressure to Norepinephrine-Induced Diuresis in Conscious Dogs (43530)

JIA L. ZHU[1] AND ROBERT J. LEADLEY, JR.[2]
Division of Experimental Medicine, St. Luke's Hospital and Foundation, Kansas City, Missouri 64111

Abstract. **Infusion of norepinephrine (NE) into humans and experimental animals induces diuresis by mechanisms that are not completely understood. Two series of experiments were performed to determine whether changes in plasma levels of vasopressin or changes in mean arterial pressure (MAP) are important factors in NE-induced diuresis in conscious dogs. First, plasma vasopressin (PAVP) levels were measured in normal and cardiac-denervated conscious dogs during a 30-min intravenous infusion of NE (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$). When NE was administered to normal dogs, urine flow increased from 0.3 ± 0.1 to 0.9 ± 0.4 ml/min. PAVP did not decrease, in spite of increases in mean arterial pressure (from 103 ± 4 to 123 ± 6 mm Hg) and left atrial pressure (from 5.2 ± 0.9 to 8.6 ± 1.4 mm Hg). The same dose of NE infused into cardiac-denervated dogs significantly increased urine flow (from 0.2 ± 0.1 to 0.8 ± 0.3 ml/min) and MAP (from 107 ± 5 to 147 ± 10 mm Hg), and decreased PAVP (from 1.8 ± 0.3 pg/ml to 1.2 ± 0.3 pg/ml). In the second series of experiments, NE was infused into cardiac-denervated dogs for 40 min. During the final 20 min of NE infusion, nitroprusside was infused to offset the pressor effect of NE by returning MAP to the initial control level. Urine flow increased during the first 20 min in which NE alone was given; however, when MAP was returned to the control level by nitroprusside infusion, urine flow also returned to the control level. PAVP increased from a control value of 3.6 ± 0.6 to 18.9 ± 3.8 pg/ml 15 min after the NP infusion had begun. We conclude that a decrease in PAVP is not required to elicit diuresis during NE infusion in normal conscious dogs and that the pressor effect of NE appears to play a major role in NE-induced diuresis.**

[P.S.E.B.M. 1993, Vol 202]

Earlier studies examining the effects of catecholamines on renal water excretion indicated that pressor doses of intravenously infused norepinephrine (NE) increase the renal excretion of solute-free water (see Ref. 1 for review). Subsequent observations indicated that the diuretic response did not occur in anesthetized dogs after a combination of acute carotid sinus denervation and acute vagotomy (2) and that the diuretic response was absent in hypophysectomized dogs (3). Based on these results, in addition to the well-known inhibitory effects of left atrial and arterial baroreceptor stimulation on vasopressin secretion (see Ref. 4 for review), it was suggested that NE increases free water clearance indirectly by eliciting a reflex reduction in vasopressin secretion (1). Unfortunately, this theory could not be confirmed conclusively because quantitative techniques for measuring vasopressin were not available when these studies were performed.

More recently, plasma vasopressin has been measured during the intravenous infusion of NE, but the results have not been consistent. For example, an infusion of NE decreased plasma vasopressin in normally hydrated, anesthetized dogs (5) and in young people

[1] Current address: Bristol-Myers Squibb, Pharmacology Research Institute, F1.4600, P.O. Box 4000, Princeton, NJ 08543-4000.

[2] To whom requests for reprints should be addressed at (current address) Cardiovascular Biology, Rhône-Poulenc Rorer, Room 2250A, NW4, 500 Arcola Road, Collegeville, PA 19426.

Received November 27, 1991. [P.S.E.B.M. 1993, Vol 202]
Accepted September 1, 1992.

0037-9727/93/2022-0217$3.00/0
Copyright © 1993 by the Society for Experimental Biology and Medicine

after overnight dehydration (6). However, infusion of NE into human subjects in another study did not alter plasma vasopressin (7). Furthermore, when the α_2-adrenergic receptor agonist BHT-933 was infused into conscious rats whose basal plasma vasopressin levels had been elevated, plasma vasopressin levels were unchanged (8). The first purpose of this study, then, was to determine whether infusion of NE into conscious, normally hydrated dogs causes changes in plasma vasopressin levels that may be responsible for the NE-induced diuresis. To eliminate the possible effect of cardiac receptor activation on vasopressin release (9, 10), NE was also infused into dogs with chronically denervated hearts.

Although the changes in plasma vasopressin levels were not consistent in the previously mentioned studies (5–8), mean arterial blood pressure increased significantly in each of these studies, which suggests that the pressor effect of NE might be the major factor in NE-induced diuresis. Consequently, the second purpose of this study was to examine the contribution of changes in mean arterial pressure to the diuresis induced by NE.

Materials and Methods

Surgical Preparation. Female mongrel dogs (16–24 kg) were assigned randomly to one of two groups, sham-operated (normal) or cardiac-denervated, before undergoing surgery. The dogs were anesthetized with pentobarbital sodium (25–30 mg/kg iv), and supplements were given as needed. The chest was entered aseptically through the fourth intercostal space on the left side. Catheters were placed in the descending aorta and left atria as described previously (11). An ultrasonic transit-time flow probe (Transonic Systems, Inc., Ithaca, NY) was placed around the ascending aorta.

The heart of each animal in the cardiac-denervated group was surgically denervated by the intrapericardial method of Randall *et al.* (12) as modified by Fater *et al.* (11). The chest was then closed and the catheters and the flow probe cable were tunneled subcutaneously to the back and exteriorized. A chest tube was placed in the pleural cavity to allow removal of residual fluid and air postoperatively. Similar surgical procedures were performed on sham-operated dogs; the pericardium was opened and catheters were inserted, but the heart was not denervated. Tests for cardiac denervation were performed during the surgery and 1 week postoperatively, as described previously (11). Morphine sulfate (15 mg im) was given to relieve postoperative pain.

Penicillin (300,000 units im daily) was given for 3 days after surgery. Catheters were flushed every other day with saline and filled with sodium heparin (1,000 units/ml) to prevent clotting. The dogs were allowed at least 2 weeks to recover before experiments were performed. They were fed a diet that provided 65 mEq sodium/day and were allowed water *ad libitum*.

Experimental Protocols. On the day of the experiment a Foley catheter was inserted in the dog's bladder and the dog was placed in a Pavlov-type stand. Cardiovascular catheters were connected to pressure transducers (Statham P23xL). Cardiac output was measured with an ultrasonic transit-time flow meter (T101; Transonic Systems). Hemodynamic variables were monitored continuously on an oscillographic recorder and digitized by a PDP 11/34 computer (Digital Equipment Corp., Maynard, MA) at a rate of 100 samples/sec. Minute averages of each hemodynamic variable were displayed on a video terminal during the experiment and stored for later analysis.

In the first series of experiments, the effects of intravenous NE on plasma vasopressin levels and renal function were examined in normal dogs ($n = 6$). After a 30-min control period, NE was infused through a temporary leg vein catheter at a rate of 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 30 min; a 30-min recovery period followed. Similar experiments were performed using cardiac-denervated dogs ($n = 6$).

Blood samples were collected 5 min before the end of the control period, 15 and 28 min after the start of the NE infusion, and 5 min before the end of the recovery period. Blood samples were replaced with an equal volume (18 ml) of iso-oncotic dextran in isotonic saline. Urine samples were collected at 10-min intervals throughout the experiment.

In the second series of experiments, the possible contribution of the pressor effects of NE to the NE-induced diuresis was evaluated by returning arterial blood pressure to control levels during the latter half of a NE infusion period. The same cardiac-denervated dogs as those used in the first series of experiments were used in these experiments. The interval between experiments on each dog was at least 2 days.

After a 30-min control period, NE was infused into cardiac-denervated dogs at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 40 min. During the last 20 min of the infusion, nitroprusside was administered intravenously at a rate of 0.3–0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ to return arterial blood pressure to the control level and maintain it at this level throughout the remainder of the infusion. A 20-min recovery period followed. Blood samples were collected 5 min before the end of the control period, 18 and 38 min after the infusion of NE had started, and 5 min before the end of the recovery period. Time control experiments were conducted using an identical protocol, except that the dextrose vehicle alone was infused.

Chemical Analyses. Aliquots of fresh urine and plasma were used to measure osmolality and electrolytes. The remaining plasma was stored at −70°C for subsequent analysis of vasopressin and NE. Arginine vasopressin was extracted from plasma with Sep-Pak C18 cartridges (Waters Chromatography Division, Millipore Corp., Milford, MA) and measured by radio-

immunoassay as described by Goetz *et al.* (13) and modified by Wang *et al.* (9). Plasma NE concentration was measured by the single radioenzymatic method (Cat-A-Kit; Amersham Corp., Arlington Heights, IL). Plasma sodium and urinary sodium and potassium concentrations were measured by flame photometry (model 943; Instrumentation Laboratory, Lexington, MA). Plasma and urine osmolality were measured by freezing point depression (model 3MO; Advanced Instruments, Needham Heights, MA).

Statistical Analyses. Hemodynamic variables from each dog were averaged over consecutive 10-min periods. Control data were obtained during the 10-min period immediately preceding NE infusion. Results shown in the tables represent data obtained in the final 10 min of the corresponding experimental period. All data are expressed as mean ± SE. Data were analyzed by two-way analysis of variance for repeated measurements. Within each group, Dunnet's test was used to determine which means differed statistically from control means. A series of completely randomized F tests were used to determine which means differed significantly between groups (14). A value of $P < 0.05$ was considered to be statistically significant.

Results

Infusion of NE into Normal Conscious Dogs. The infusion of NE into normal dogs ($n = 5$) increased circulating levels of NE greater than 10-fold from the control level of 188 ± 32 pg/ml (Fig. 1). Urine flow increased from 0.3 ± 0.1 to 0.9 ± 0.4 ml/min (Fig. 1 and Table I). Nonsignificant increases in sodium excretion and osmolar and free water clearance, and decreases in potassium excretion and urine osmolality are shown in Table I. Plasma vasopressin levels did not change significantly during the NE infusion period (Fig. 1), in spite of increases in mean arterial blood pressure (from 103 ± 4 to 123 ± 6 mm Hg) and left atrial pressure (from 5.2 ± 0.9 to 8.6 ± 1.4 mm Hg; Table II). Total peripheral resistance, cardiac output, heart rate, and stroke volume remained relatively constant throughout the experiment (Table II). One animal had an unusually high urine flow; thus, the results obtained using this animal were excluded from the statistical analyses. It is interesting to note, however, that this dog's urine flow increased from 0.8 to 6.9 ml/min in response to NE infusion. As with the other dogs, the plasma vasopressin level did not change substantially in response to NE infusion (0.5 to 0.6 pg/ml).

Infusion of NE into Cardiac-Denervated Conscious Dogs. The infusion of NE into cardiac-denervated dogs ($n = 6$) also increased circulating levels of NE greater than 10-fold (Fig. 1). Urine flow increased from 0.2 ± 0.1 to 0.8 ± 0.3 ml/min (Fig. 1 and Table I). Like the normal dogs, sodium excretion and osmolar and free water clearance increased, and potassium and urine

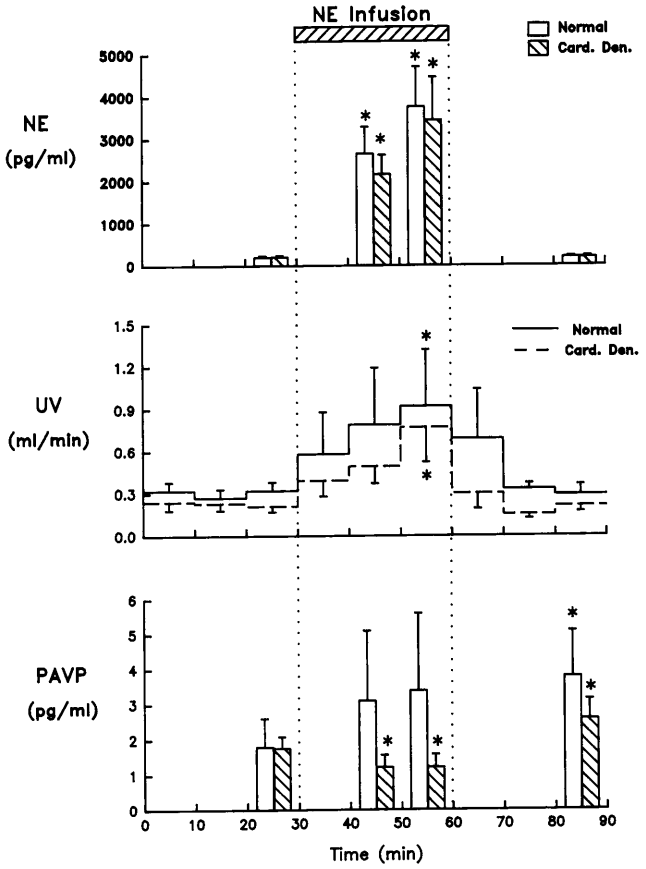

Figure 1. Renal and hormonal response to norepinephrine infusion in normal and cardiac-denervated conscious dogs. NE was infused at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 30 min in normal ($n = 5$) and cardiac-denervated ($n = 5$) conscious dogs. UV, urine flow; PAVP, plasma vasopressin. *$P < 0.05$ vs data collected between 20 and 30 min (within groups).

osmolality decreased; however, only the changes in osmolar clearance and urine osmolality were significant (Table I). Unlike the normal dogs though, plasma vasopressin levels decreased significantly (from 1.8 ± 0.3 pg/ml to 1.2 ± 0.3 pg/ml) in the cardiac-denervated dogs during NE infusion (Fig. 1). Also, mean arterial blood pressure, cardiac output, and heart rate increased significantly more when NE was infused into cardiac-denervated dogs than when it was infused into the normal dogs (Table II). Left atrial pressure and stroke volume decreased significantly when NE was given to the cardiac-denervated dogs. Total peripheral resistance did not change significantly.

Infusion of NE and Nitroprusside into Cardiac-Denervated Dogs. Plasma levels of NE increased greater than 10-fold during the first 20 min of the 40-min period of NE infusion (from 220 ± 40 to 2200 ± 130 pg/ml). During the initial 20 min of the infusion period, when only NE was given, mean arterial pressure, total peripheral resistance, cardiac output, and heart rate increased significantly, and left atrial pressure and stroke volume decreased significantly (Fig. 2 and

Table I. Renal and Electrolyte Effects of Norepinephrine Infusion in Normal and Cardiac-Denervated Conscious Dogs[a]

	Control	Norepinephrine	Recovery
UV (ml/min)			
Normal	0.3 ± 0.1	0.9 ± 0.4[b]	0.3 ± 0.1
CD	0.2 ± 0.1	0.8 ± 0.3[b]	0.2 ± 0.1
C_{Osm} (ml/min)			
Normal	1.3 ± 0.2	1.6 ± 0.4	1.4 ± 0.2
CD	0.9 ± 0.1	1.3 ± 0.2[b]	0.8 ± 0.1
C_{H_2O} (ml/min)			
Normal	−1.0 ± 0.2	−0.7 ± 0.4	−1.1 ± 0.2
CD	−0.7 ± 0.1	−0.5 ± 0.2	−0.6 ± 0.1
$U_{Na}V$ (μEq/min)			
Normal	46 ± 11	79 ± 39	70 ± 19
CD	23 ± 9	55 ± 29	19 ± 5
U_KV (μEq/min)			
Normal	29 ± 4	21 ± 5	25 ± 6
CD	20 ± 4	17 ± 5	18 ± 3
U_{Osm} (mOsm/kg H_2O)			
Normal	1317 ± 212	1050 ± 309	1628 ± 229
CD	1350 ± 170	670 ± 130[b]	1340 ± 200
P_{Osm} (mOsm/kg H_2O)			
Normal	300 ± 3	300 ± 3	300 ± 3
CD	298 ± 2	297 ± 1	297 ± 1
P_{Na} (μEq/ml)			
Normal	146 ± 1	146 ± 1	146 ± 1
CD	145 ± 1	145 ± 1	146 ± 1

[a] Norepinephrine was infused at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 30 min into normal (n = 5) and cardiac-denervated ([CD] n = 6) conscious dogs. Abbreviations used in table: UV, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, sodium excretion; U_KV, potassium excretion; U_{Osm}, urine osmolality; P_{Osm}, plasma osmolality; P_{Na}, plasma sodium.
[b] $P < 0.05$ vs control.

Table III). During the latter half of the infusion period, when nitroprusside was added, mean arterial pressure decreased promptly (within 3 min) to control levels. Plasma levels of NE remained elevated (2840 ± 340 pg/ml) during the period in which both NE and nitroprusside were infused.

The renal response to NE infusion during the first 20-min period (Table IV) was similar to that observed in the cardiac-denervated dogs during the 30-min NE infusion described in the previous section (Table I). However, when nitroprusside was given to return arterial pressure to control values during the final 20 min of the NE infusion, urine flow and the other renal parameters also returned to control or, as with osmolar clearance, below control values. There was no significant change in plasma vasopressin levels during the initial 20 min of the NE infusion (Fig. 2). However, during the latter half of the infusion, when blood pressure was returned to the control value with nitroprusside, plasma vasopressin increased to 18.9 ± 3.8 pg/ml. This change was reversed during the recovery period, when plasma vasopressin level declined to a value not significantly different from control. In the time control experiments, in which dextrose vehicle only was infused, there were no significant changes in any variable measured (Fig. 2 and Tables III and IV).

Discussion

In this study, intravenously administered NE caused an increase in urine flow in normal conscious dogs without altering plasma vasopressin levels. Consequently, the diuresis was not dependent upon a decline in plasma vasopressin in these experiments. Consistent with these results is the finding of Goldsmith *et al.* (7), who observed no change in plasma vasopressin levels when a smaller dose of NE was infused into humans.

Our results, however, are not consistent with the hypothesis of Berl *et al.* (2), who proposed that NE increases urine flow and free water excretion by arterial baroreflex-mediated inhibition of vasopressin secretion. Although vasopressin concentration was not measured by Berl *et al.*, later experiments by Kimura *et al.* (5) demonstrated that NE infusion reduces plasma vasopressin levels in anesthetized dogs. We were able to detect significant decreases in plasma vasopressin only when NE was given to cardiac-denervated dogs, not when it was given to normal dogs. The difference between our results from normal conscious dogs and those of Kimura *et al.*'s may be due to the confounding effects of acute surgical stress (15) or anesthesia (16).

Plasma vasopressin did not decline during NE in-

Table II. Hemodynamic Effects of Norepinephrine Infusion in Normal and Cardiac-Denervated Conscious Dogs[a]

	Control	Norepinephrine	Recovery
MAP (mm Hg)			
Normal	103 ± 4	123 ± 6[b]	99 ± 3
CD	107 ± 5	147 ± 10[b,c]	101 ± 6
LAP (mm Hg)			
Normal	5.2 ± 0.9	8.6 ± 1.4[b]	4.3 ± 0.7
CD	5.5 ± 1.1	2.0 ± 0.7[b,c]	5.6 ± 0.9
TPR (PRU)			
Normal	2.7 ± 0.8	2.9 ± 0.3	2.5 ± 0.2
CD	2.1 ± 0.3	2.2 ± 0.4	2.0 ± 0.3
CO (liter/min)			
Normal	2.3 ± 0.3	2.5 ± 0.4	2.5 ± 0.3
CD	3.0 ± 0.3	4.4 ± 0.6[b,c]	3.0 ± 0.4
HR (bpm)			
Normal	84 ± 10	87 ± 14	93 ± 12
CD	113 ± 7	210 ± 15[b,c]	123 ± 6
SV (ml)			
Normal	26 ± 2	28 ± 3	26 ± 2
CD	28 ± 5	21 ± 3[b]	25 ± 5

[a] Norepinephrine was infused at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 30 min into normal ($n = 5$) and cardiac-denervated ([CD] $n = 6$) conscious dogs. Abbreviations used in table: MAP, mean arterial pressure; LAP, left atrial pressure; TPR, total peripheral resistance; PRU, peripheral resistance unit; CO, cardiac output; HR, heart rate; SV, stroke volume.
[b] $P < 0.05$ vs control.
[c] $P < 0.05$ vs normal.

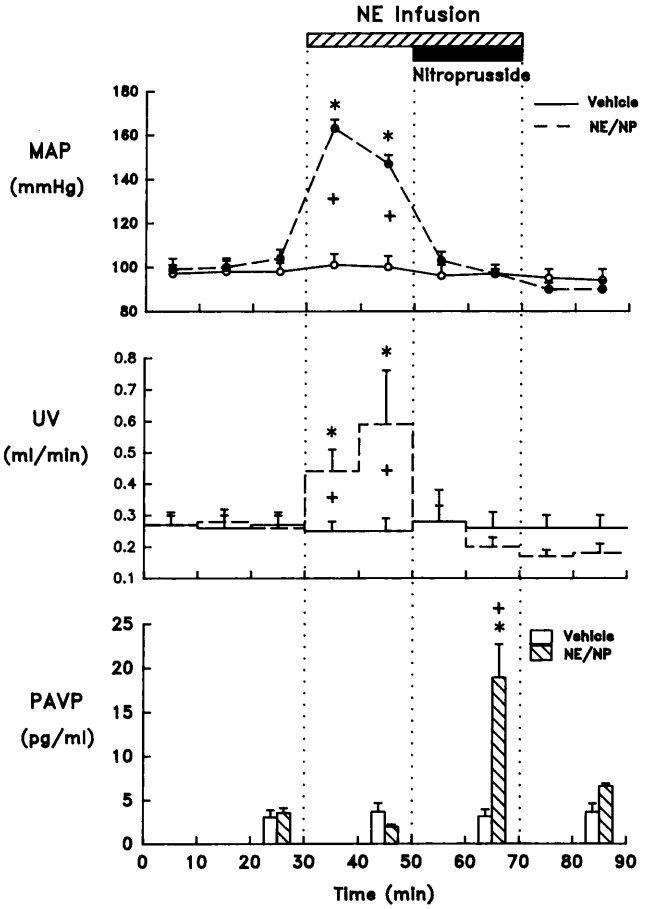

Figure 2. Pressor, renal, and vasopressin response to NE and combined NE-nitroprusside infusion in cardiac-denervated dogs. NE was infused intravenously at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 40 min into cardiac-denervated dogs. After 20 min into the NE infusion period, nitroprusside (0.3–0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 20 min) was administered to return blood pressure to control levels. MAP, mean arterial pressure; UV, urine flow; PAVP, plasma vasopressin. *$P < 0.05$ vs data collected between 20 and 30 min (within groups). †$P < 0.05$ vs vehicle infusion.

fusion in normal conscious dogs, in spite of large increases in arterial pressure and left atrial pressure, which would be expected to inhibit vasopressin secretion reflexly via arterial and atrial baroreceptors, respectively. This suggests that the reflex inhibitory effect on vasopressin release is counteracted by a direct stimulatory effect of NE on vasopressin release (see below). On the other hand, infusion of NE increased arterial blood pressure significantly more in the cardiac-denervated dogs than in the normal dogs. The enhanced increase in arterial blood pressure in the cardiac-denervated dogs was attributable to the marked increase in heart rate because total peripheral resistance did not change. The greater increase in arterial blood pressure observed in the cardiac-denervated dogs conceivably provided a greater reflex inhibition of vasopressin secretion via sinoaortic baroreceptors. Consequently, plasma vasopressin levels decreased significantly during NE infusion in the cardiac-denervated dogs.

Likewise, the significant increase in plasma vasopressin level 25 min after the NE infusion was stopped (Fig. 1) may also be attributable to influences originating from arterial baroreflexes. For example, elevation of arterial pressure for 30 min may have caused a resetting of baroreceptor input to a higher set point. Once the NE infusion was terminated, the decline in pressure decreased baroreceptor input to a greater extent than normal for the given level of pressure. It is possible that this unloading of arterial baroreceptors reflexly increased vasopressin release.

The relatively large increase in plasma vasopressin concentration that was measured during combined NE and nitroprusside infusion in cardiac-denervated dogs may be explained in a similar fashion (Fig. 2). In this case, however, the decrease in blood pressure was more abrupt, thus providing a more rapid decrease in arterial baroreceptor input and, conceivably, a more potent reflex-mediated stimulus for vasopressin release. It is also possible that the decrease in stroke volume during combined NE and nitroprusside infusion may have decreased sinoaortic baroreceptor activity. That is, although nitroprusside infusion returned blood pressure to control levels, stroke volume remained below control levels, probably due to nitroprusside-induced venodilation and venous pooling. It is possible, then, that the nitroprusside-induced decrease in stroke volume (Table III), and probably pulse pressure, may have stimulated vasopressin release reflexly by decreasing sinoaortic

Table III. Hemodynamic Effects of Norepinephrine and Norepinephrine Plus Nitroprusside Infusion in Cardiac-Denervated Conscious Dogs[a]

	Control	NE	NE + NP	Recovery
MAP (mm Hg)				
Vehicle	99 ± 5	101 ± 5	98 ± 5	94 ± 5
NE/NP	104 ± 4	148 ± 4[b]	98 ± 3	91 ± 4
LAP (mm Hg)				
Vehicle	5.3 ± 1.5	5.1 ± 1.4	4.2 ± 1.1	3.9 ± 1.1
NE/NP	5.4 ± 1.5	3.1 ± 1.5[b]	1.6 ± 1.1[b]	4.9 ± 1.6
TPR (PRU)				
Vehicle	3.2 ± 1.0	4.0 ± 1.5	3.3 ± 0.8	3.7 ± 1.1
NE/NP	2.5 ± 0.3	2.7 ± 0.3[b]	2.4 ± 0.3	2.5 ± 0.3
CO (liter/min)				
Vehicle	2.2 ± 0.3	2.2 ± 0.4	2.2 ± 0.3	2.0 ± 0.3
NE/NP	2.6 ± 0.2	3.6 ± 0.3[b]	2.6 ± 0.2	2.3 ± 0.2
HR (bpm)				
Vehicle	116 ± 4	122 ± 4	122 ± 4	119 ± 5
NE/NP	122 ± 4	223 ± 10[b]	216 ± 10[b]	136 ± 6
SV (ml)				
Vehicle	19 ± 3	18 ± 3	18 ± 3	17 ± 3
NE/NP	21 ± 2	16 ± 1[b]	12 ± 1[b]	17 ± 2[b]

[a] Norepinephrine was infused intravenously at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 40 min into cardiac-denervated ($n = 6$) conscious dogs. After 20 min into the NE infusion period, nitroprusside ([NP] 0.3–0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$, iv, for 20 min) was administered to return blood pressure to control levels. Abbreviations used in table: MAP, mean arterial pressure; LAP, left atrial pressure; TPR, total peripheral resistance; PRU, peripheral resistance unit; CO, cardiac output; HR, heart rate; SV, stroke volume.
[b] $P < 0.05$ vs. control.

baroreceptor activity. Alternatively, it is possible that the difference in vasopressin levels in cardiac-denervated dogs during the recovery period in the first series of experiments (2.6 ± 0.6 pg/ml; Fig. 1) and the levels obtained during combined NE and nitroprusside infusion (18.9 ± 3.8 pg/ml; Fig. 2) may have been due to the difference in blood sampling times between the two protocols. For example, in the cardiac-denervated dogs given both NE and nitroprusside, the blood sample for vasopressin was taken only 18 min after blood pressure was abruptly returned to the control level by nitroprusside. In the dogs receiving NE only, the blood samples were obtained 25 min after the infusion of NE had been terminated. The half-life of vasopressin in dogs is approximately 1–4 min (17). Consequently, the difference in plasma vasopressin in these two situations may be attributable to the greater time allowed for metabolic clearance of vasopressin in the first series of experiments.

Another possible explanation for the relatively large increase in plasma vasopressin level when nitroprusside was infused along with NE (Fig. 2) is that NE may have a direct stimulatory effect on vasopressin release. In the normal dogs, this stimulatory effect may have been masked by inhibitory influences originating from arterial and atrial baroreceptors in response to the pressor effects of NE. Evidence supporting the direct effect of NE on vasopressin release includes the localization of α_2-adrenoreceptors in the area postrema and in the subfornical organ (18, 19), areas with neural projections to the vasopressin-secreting neuroendocrine cells of the hypothalamus (see Ref. 4 for review). Central administration of NE has also been shown to stimulate vasopressin release by activating α_1-adrenoceptors in the anterior hypothalamus (20).

It is highly unlikely that nitroprusside exerted a direct effect on vasopressin release. Earlier studies by Brooks *et al.* (21) demonstrated that an infusion of nitroprusside does not alter plasma vasopressin in dogs with cardiac and arterial baroreceptor denervation. Furthermore, Zucker and his colleagues (22) demonstrated that intracarotid administration of nitroprusside into conscious dogs does not evoke changes in plasma vasopressin or arterial pressure.

Likewise, osmotic influences played no role in altering plasma vasopressin in this study. Intravenous infusion of NE did not alter plasma osmolality; plasma sodium and plasma osmolality were constant throughout these experiments (Tables I and IV). It is also unlikely that NE-induced changes in clearance would have been responsible for the changes in plasma vasopressin levels in these experiments. Although vasopressin secretion was not directly measured in this study, it is doubtful that NE-induced changes in clearance of this peptide would have increased plasma vasopressin concentration in one set of experiments and reduced it in another.

It is tempting to argue that the decrease in urine flow during combined NE and nitroprusside infusion may be due to the relatively large increase in plasma vasopressin concentration (Fig. 2). However, urine flow, urine osmolality, and free water clearance (Table IV) were not significantly different from control values during this experimental period. Moreover, in all experimental groups, the changes in urine flow paralleled the changes in osmolar clearance, indicating that the increase in urine flow was due to a solute diuresis. Taken together, these data indicate that changes in plasma vasopressin concentration are relatively unimportant in regulating urine flow in this situation.

Although some evidence suggests that NE is capable of inhibiting the hydro-osmotic effects of vasopressin intrarenally (23–27), results from the present study do not support the concept that an intrarenal mechanism is the major factor responsible for NE-induced diuresis in conscious dogs. For example, if NE inhibited vasopressin-induced renal water reabsorption in conscious dogs, urine flow should have increased during NE infusion, regardless of the prevailing arterial blood pressure. However, when mean arterial pressure was returned to the control level by nitroprusside during NE infusion, urine flow also returned to the control level (Fig. 2). In addition, infusion of NE into the renal

Table IV. Renal and Electrolyte Effects of Norepinephrine and Norepinephrine Plus Nitroprusside Infusion in Cardiac-Denervated Conscious Dogs[a]

	Control	NE	NE + NP	Recovery
UV (ml/min)				
Vehicle	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
NE/NP	0.3 ± 0.1	0.6 ± 0.2[b]	0.2 ± 0.3	0.2 ± 0.1
C_{Osm} (ml/min)				
Vehicle	1.1 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2
NE/NP	1.1 ± 0.1	1.4 ± 0.2[b]	0.6 ± 0.1[b]	0.9 ± 0.1
C_{H_2O} (ml/min)				
Vehicle	−0.8 ± 0.2	−0.9 ± 0.2	−0.9 ± 0.2	−0.9 ± 0.2
NE/NP	−0.8 ± 0.1	−0.8 ± 0.2	−0.5 ± 0.2	−0.7 ± 0.1
$U_{Na}V$ (μEq/min)				
Vehicle	24 ± 8	28 ± 8	36 ± 6	44 ± 4[b]
NE/NP	43 ± 16	68 ± 14	21 ± 7	21 ± 5
U_KV (μEq/min)				
Vehicle	42 ± 11	40 ± 8	42 ± 9	37 ± 11
NE/NP	25 ± 5	25 ± 7	24 ± 4	23 ± 3
U_{Osm} (mOsm/kg H_2O)				
Vehicle	1220 ± 50	1570 ± 390	1560 ± 290	1470 ± 270
NE/NP	1400 ± 280	890 ± 150[b]	1220 ± 260	1360 ± 160
P_{Osm} (mOsm/kg H_2O)				
Vehicle	298 ± 1	297 ± 2	298 ± 1	298 ± 1
NE/NP	296 ± 1	296 ± 1	296 ± 1	297 ± 1
P_{Na} (μEq/ml)				
Vehicle	146 ± 1	146 ± 1	145 ± 1	146 ± 1
NE/NP	146 ± 1	146 ± 1	146 ± 1	146 ± 1

[a] Norepinephrine was infused intravenously at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 40 min into cardiac-denervated ($n = 6$) conscious dogs. After 20 min into the NE infusion period, nitroprusside ([NP] 0.3–0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$, iv, for 20 min) was administered to return blood pressure to control levels. Abbreviations used in table: UV, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, sodium excretion; U_KV, potassium excretion; U_{Osm}, urine osmolality; P_{Osm}, plasma osmolality; P_{Na}, plasma sodium.
[b] $P < 0.05$ vs control.

arteries of anesthetized dogs does not produce a diuresis (2). Furthermore, recent evidence indicates that the inhibition of the tubular action of vasopressin by α-adrenoceptor agonists is species specific (28). Specifically, NE did not reverse the decrease in urine flow and increase in urinary osmolality caused by the infusion of vasopressin in conscious dogs (28). These results imply that the NE-induced diuresis observed in the present study was elicited primarily by the elevation in arterial blood pressure, a finding that is consistent with the well-known phenomenon of pressure diuresis (29).

In conclusion, these results demonstrate that a decrease in plasma vasopressin is not responsible for the diuretic response to NE infusion in normal conscious dogs. In addition, when the pressor effects of NE were abolished by the infusion of nitroprusside, NE did not increase urine flow, indicating that the pressor effect of NE appears to play a major role in NE-induced diuresis in conscious dogs.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-13623 and HL-35830. Our grateful thanks to Dr. Kenneth L. Goetz and the late Dr. Bin C. Wang for their advice and guidance during these studies. We also express our gratitude to Pamela Geer, Gloria Flora-Ginter, Barbara Roberts, and Gregory Goetz for their excellent technical assistance, and to Catherine Leadley for her assistance in the preparation of the manuscript.

1. Schrier RW, Berl T, Harbottle JA, McDonald KM. Catecholamines and renal water excretion. Nephron **15**:186–196, 1975.
2. Berl T, Cadnapaphornchai P, Harbottle JA, Schrier RW. Mechanism of suppression of vasopressin during alpha-adrenergic stimulation with norepinephrine. J Clin Invest **53**:219–227, 1974.
3. Schrier RW, Berl T. Mechanism of effect of alpha adrenergic stimulation with norepinephrine on renal water excretion. J Clin Invest **52**:502–511, 1973.
4. Bisset GW, Chowdrey HS. Control of release of vasopressin by neuroendocrine reflexes. Q J Exp Physiol **73**:811–872, 1988.
5. Kimura T, Wang BC, Crofton JT, Share L. Effect of norepinephrine on plasma vasopressin concentration and renal water metabolism. Neuroendocrinology **31**:276–281, 1980.
6. Shimamoto K, Miyahara M. Effect of norepinephrine infusion on plasma vasopressin levels in normal human subjects. J Clin Endocrinol Metab **43**:201–204, 1976.
7. Goldsmith SR, Dodge-Brown D, Pentel P. Effects of infused norepinephrine and angiotensin-II on vasopressin levels in humans. Am J Med Sci **295**:513–516, 1988.
8. Gellai M, Ruffolo RR Jr. Renal effects of selective α_1- and α_2-adrenoceptor agonists in conscious, normotensive rats. J Pharmacol Exp Ther **240**:723–728, 1987.
9. Wang BC, Sundet WD, Hakumaki MOK, Goetz KL. Vasopressin and renin responses to hemorrhage in conscious, cardiac-denervated dogs. Am J Physiol **245**:H399–H405, 1983.
10. Wang BC, Flora-Ginter G, Leadley RJ Jr, Goetz KL. Ventricular receptors stimulate vasopressin release during hemorrhage. Am J Physiol **254**:R204–R211, 1988.
11. Fater DC, Schultz HD, Sundet WD, Mapes JS, Goetz KL. Effects of left atrial stretch in cardiac-denervated and intact conscious dogs. Am J Physiol **242**:H1056–H1064, 1982.

12. Randall WC, Kaye MP, Thomas JX, Barber MJ. Intrapericardial denervation of the heart. J Surg Res **29**:101–109, 1980.
13. Goetz KL, Wang BC, Hakumaki MOK, Fater DC, Geer PG, Sundet WD. Cardiovascular, renal, and humoral effects of applying local anesthetic to the atria of conscious dogs. Proc Soc Exp Biol Med **167**:101–109, 1981.
14. Bruning JL, Kintz BL. F-tests for simple effects. In: Computational Handbook of Statistics. Glenview, IL: Scott-Foresman, Vol **2**: p55, p132, 1977.
15. Moran WH, Miltenberger FW, Shuayb WA, Zimmerman B. The relationship of antidiuretic hormone secretion to surgical stress. Surgery **56**:99–108, 1964.
16. Oyama T, Sata K, Kimura K. Plasma levels of antidiuretic hormone in man during halothane anesthesia and surgery. Can Anaesth Soc J **18**:614–620, 1971.
17. Weitzman RE, Fisher DA. Arginine vasopressin metabolism in dogs. I. Evidence for a receptor-mediated mechanism. Am J Physiol **235**:E591–E597, 1978.
18. Beleslin DB, Strbac M. Noradrenaline-induced emesis: Alpha-2 adrenoceptor mediation in the area postrema. Neuropharmacology **26**:1157–1165, 1987.
19. Bruning G, Kaulen P, Baumgarten HG. Quantitative autoradiographic localization of α_2-antagonist binding sites in rat brain using ^{3}H-idazoxan. Neurosci Lett **83**:333–337, 1987.
20. Brooks DP, Share L, Crofton JT. Central adrenergic control of vasopressin release. Neuroendocrinology **42**:416–420, 1986.
21. Brooks VL, Keil LC, Reid IA. Role of renin-angiotensin system in the control of vasopressin secretion in conscious dogs. Circ Res **58**:829–838, 1986.
22. Zucker IH, Gorman AJ, Cornish KG, Huffman LJ, Gilmore JP. Influence of left ventricular receptor stimulation on plasma vasopressin in conscious dogs. Am J Physiol **245**:R792–R799, 1983.
23. Krothapalli RK, Duffy WB, Senekjian HO, Suki WN. Modulation of the hydro-osmotic effect of vasopressin on the rabbit cortical collecting tubule by adrenergic agents. J Clin Invest **72**:294–298, 1983.
24. Krothapalli RK, Suki WN. Functional characterization of the alpha adrenergic receptor modulating the hydroosmotic effect of vasopressin on the rabbit cortical collecting tubule. J Clin Invest **73**:740–749, 1984.
25. Ribeiro CP, Ribeiro-Neto F, Field JB, Suki WN. Prevention of α_2-adrenergic inhibition on ADH action by pertussis toxin in rabbit CCT. Am J Physiol **253**:C105–C112, 1987.
26. Teitelbaum I, Strasheim A, Berl T. Adrenergic control of cAMP generation in rat inner medullary collecting tubule cells. Kidney Int **35**:647–653, 1989.
27. Gellai M. Modulation of vasopressin antidiuretic action by renal α_2-adrenoceptors. Am J Physiol **259**:F1–F8, 1990.
28. Brooks DP, Gellai M, DePalma PD, Edwards RM. Modulation of vasopressin antidiuretic action by α_2-adrenoceptors is species specific. Am J Physiol **261**:R1242–R1246, 1991.
29. Hall JE, Guyton AC, Coleman TG, Mizelle HL, Woods LL. Regulation of arterial pressure: Role of pressure natriuresis and diuresis. Fed Proc **45**:2897–2903, 1986.