Contribution of Cardiac and Arterial Baroreceptors to Enhanced Vasopressin Release During Hemorrhage with Autonomic Blockade (43863)

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Abstract. **During episodes of blood loss, several apparently redundant mechanisms are activated to maintain arterial blood pressure. This study was designed to examine one such compensatory mechanism involving enhanced vasopressin release during hemorrhage when the autonomic nervous system (ANS) is pharmacologically blocked. First, to confirm that this compensatory mechanism exists in canines, conscious dogs were hemorrhaged under normal conditions and during ANS blockade. In dogs with intact cardiac nerves (intact, $n = 7$), hemorrhage at 0.8 ml/kg/min increased plasma vasopressin (PAVP) from 3.0 ± 0.7 to 6.6 ± 2.4 and 78 ± 50 pg/ml at blood losses of 10 and 20 ml/kg, respectively. At the same amount of blood loss during hemorrhage with ANS blockade, PAVP was enhanced significantly from 33 ± 17 to 230 ± 90 and 610 ± 270 pg/ml. ANS blockade did not, however, alter the hemorrhage-induced increases in plasma renin activity. Next, to examine the afferent mechanisms responsible for the enhanced PAVP response, cardiac-denervated dogs (CD, $n = 9$) were hemorrhaged with and without ANS blockade. Without blockade, PAVP increased from 3.7 ± 0.9 to 5.2 ± 0.8 and 26 ± 11 pg/ml at blood losses of 10 and 20 ml/kg. PAVP was significantly higher in response to hemorrhage with ANS blockade, increasing from 17 ± 6 to 76 ± 18 and 330 ± 80 pg/ml. The rise in PAVP in the CD dogs suggested that peripheral baroreceptors were involved in eliciting vasopressin release under these conditions. Therefore, the influence of arterial baroreceptors was examined by infusing norepinephrine during hemorrhage in order to maintain blood pressure constant. Under these conditions, PAVP increased significantly in the intact dogs at 10 ml/kg blood loss, but did not change in the CD dogs. These results demonstrate that the enhanced release of AVP during hemorrhage with ANS blockade can be mediated either by cardiac or arterial baroreceptors; however, the maximum response is elicited only when both sets of receptors are functioning normally.**

[P.S.E.B.M. 1995, Vol 208]

Hemorrhage initiates a number of compensatory pressor mechanisms including autonomic nervous system activation and the release of vasoactive peptide hormones such as vasopressin and angiotensin. Complicated interactions have been reported among these pressor mechanisms (1–3). Of particular interest is the principle of redundancy in blood pressure regulation: ablation or inactivation of one mechanism is accompanied by compensatory adjustments in other mechanisms (4).

For example, there is general agreement that the autonomic nervous system is the dominant mechanism for blood pressure maintenance during acute changes in blood pressure such as occurs during hypotensive hemorrhage (1, 2, 5). However, after acute blockade of the autonomic nervous system, vasopressin release at a given level of blood loss is enhanced in conscious rabbits (6) and dogs (7). This enhanced vasopressin release appears to be the primary compensatory mech-

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Received May 10, 1994. [P.S.E.B.M. 1995, Vol 208]
Accepted October 3, 1994.

0037-9727/95/2084-0361$10.50/0

anism initiated to maintain arterial blood pressure during hemorrhage in autonomically blocked conscious rats (8) and dogs (9). An equivalent mechanism appears to exist in humans as well. In particular, Puritz *et al.* (10), have reported that patients with progressive autonomic failure secrete inappropriately high levels of vasopressin, suggesting that vasopressin may be involved in maintaining blood pressure in these patients. It is also important to note that the pressor effect of vasopressin is attenuated by baroreceptor-mediated reflexes (i.e., the pressor effect of vasopressin is greater after baroreceptor denervation or autonomic blockade) (11, 12). Wang *et al.* (13) also have demonstrated that the pressor effect of vasopressin is greater in dogs that lack functional cardiac innervation. Cumulatively, these data provide support for the notion that vasopressin plays an important role in maintaining blood pressure when the autonomic nervous system is not functioning normally. However, the afferent mechanisms responsible for the enhanced secretion of vasopressin and renin during hemorrhage with autonomic blockade have not been examined in detail.

Although controversial (7), several studies have indicated that receptors in the heart are more important than peripheral baroreceptors in the stimulation of vasopressin release in response to hemorrhage under normal conditions (14–17). Consequently, it was of interest to determine whether similar mechanisms may also be involved in the augmented hormonal response to hemorrhage with autonomic blockade.

Therefore, the present study was designed, first, to determine whether plasma vasopressin (PAVP) and plasma renin activity (PRA) are enhanced during hemorrhage with autonomic blockade in conscious dogs. After confirming that PAVP, but not PRA, was enhanced under these conditions, additional experiments were then performed to determine the contribution of cardiac and arterial baroreceptors to this augmented response.

Materials and Methods

All procedures in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 85-23, 1985.

Surgical Preparation. Female mongrel dogs (15–20 kg body wt) were anesthetized with pentobarbital sodium (30 mg/kg, iv), with supplements administered as needed. A left thoracotomy was performed at the level of the fourth intercostal space during positive pressure ventilation. Catheters were placed in the descending aorta, left atria, and pulmonary artery; an ultrasonic transit-time flow probe (Transonic Systems, Inc., Ithaca, NY) was placed around the ascending aorta. Nine dogs underwent cardiac denervation (CD) by the intrapericardial technique of Randall *et al.* (18), as modified by Fater *et al.* (19). Similar surgical procedures were performed in seven other dogs, but the heart was not denervated (intact). Penicillin (500,000 U/day, im) was administered postoperatively for 3 days, and catheters were flushed with sodium heparin (1000 U/ml) every other day. Dogs were allowed 2 weeks to recover from surgery before experimentation. They were fed every afternoon with a meal that provided 65 mEq of sodium per day. Free access to water was provided at all times.

Tests for Cardiac Denervation. The initial test for cardiac denervation was performed during the surgical operation by electrical stimulation of the ansae subclaviae and thoracic vagi as described by Randall *et al.* (18). Changes in heart rate of less than six beats per minute (bpm) in response to these stimulations were considered positive tests for denervation. Approximately 1 week after surgery, methoxamine (50 μg/kg) and then nitroglycerin (24 μg/kg) were administered intravenously to raise and lower arterial blood pressure, respectively. The absence of heart rate changes (±6 bpm) was taken to indicate that the cardiac efferent nerves had been effectively eliminated. In addition, lack of changes in heart rate and arterial pressure following intra-atrial injection of veratridine (25 μg) indicated that afferent cardiac pathways responsible for eliciting the Bezold-Jarisch reflex had been effectively eliminated. Previous studies by Fater *et al.* (19) have shown that this cardiac denervation technique also interrupts the cardiac afferent nerves responsible for the diuresis and natriuresis that normally occur during left atrial distension in normal dogs. Fater *et al.* (19) have also shown that dogs undergoing cardiac denervation remain denervated for at least 4 weeks following surgery. All experiments in the present study were performed within 4 weeks from the date of surgery.

Hemorrhage Protocol. On the day of the experiment, the dog was placed in a Pavlov-type stand. Indwelling fluid-filled catheters were connected to pressure transducers (Statham P23xL). Cardiac output was measured with an ultrasonic transit-time flowmeter (T101; Transonic Systems Inc., Ithaca, NY). The flow system and probes were checked for accuracy *in vitro* between each implantation. Hemodynamic variables were monitored continuously and digitized by a PDP 11/34 computer (Digital Equipment Corp., Maynard, MA) at a rate of 100 samples per second. Minute averages of each hemodynamic variable were displayed on a video screen during the experiment and stored on magnetic disk for later analysis.

After the hemodynamic variables had been stable for 30 min, the dog was given heparin sodium (3000 U, iv) and then hemorrhaged via the pulmonary arterial catheter at a rate of 0.8 ml/kg/min (pump model 375A; Sage Instruments, Cambridge, MA). Blood samples

were obtained 5 min before hemorrhage and at 10, 20, and 30 ml/kg blood loss in experiments without autonomic blockade. In experiments with autonomic blockade, blood samples were obtained at 5, 10, and 20 ml/kg blood loss. The volume of blood samples obtained prior to hemorrhage was replaced with an equal volume of isotonic, isoncotic dextran (26 ml). Blood samples obtained during the hemorrhage were considered part of the hemorrhage and were obtained during the 2 min immediately preceding the blood losses indicated above.

When the experiment was completed, the shed blood was filtered and reinfused into the dog, along with the packed cells from the blood samples; the cells were suspended in isotonic saline before infusion. The dogs were allowed to recover for 5 days between experiments.

Autonomic blockade in intact dogs. To determine the effect of autonomic blockade on the magnitude of the increase in PAVP and PRA during hemorrhage, intact dogs ($n = 7$) were hemorrhaged under normal conditions and, on another day, under autonomic blockade. Thirty minutes into a baseline period, autonomic blockade was produced with hexamethonium chloride (20 mg/kg, iv given over 5 min in 5 ml of 5% dextran followed by a continuous infusion of 0.3 mg/kg/min) plus methscopolamine bromide (1 μg/kg/min, iv). This regimen abolished changes in heart rate in response to repeated intravenous injections of nicotine (10 μg/kg) over a 2-hr period of autonomic blockade in intact dogs (20, and personal observations).

Twenty minutes after the initiation of autonomic blockade, mean arterial pressure (MAP) was reduced by approximately 21 mm Hg. This effect was reversed by infusion of norepinephrine (NE), which was adjusted until MAP remained at ±10 mm Hg from the baseline level recorded prior to blockade (the dose of NE ranged from 0.05 to 0.20 μg/kg/min). Once an effective dose was established in a particular dog, the same dose of NE was used for the entire hemorrhage. Blood samples were obtained for measurement of PAVP and PRA at 25 min into the baseline period, at 15 min into autonomic blockade, and at 25 min after MAP was stabilized by infusion of NE. This last sample was used as the control sample for the hemorrhage portion of the experiment. The volume of each prehemorrhage blood sample was replaced with an equal volume of isotonic, isoncotic dextran (26 ml). After blood pressure was stable for 30 min, hemorrhage was initiated as described previously. These experiments will be referred to as TAB/NMAP (total autonomic blockade/normalized mean arterial blood pressure).

Autonomic blockade in CD dogs. To investigate whether cardiac receptors contribute to the augmented secretion of vasopressin during hemorrhage, cardiac-denervated dogs ($n = 9$) were hemorrhaged twice as described above, once under normal conditions and, on a separate day, under autonomic blockade.

Hemorrhage during autonomic blockade with constant arterial blood pressure. To investigate whether arterial baroreceptors contribute to the augmented rise in PAVP induced by hemorrhage during autonomic blockade, norepinephrine was infused intravenously to maintain arterial blood pressure constant during hemorrhage both in intact ($n = 5$) and in cardiac-denervated ($n = 8$) dogs under autonomic blockade. In this series of experiments, the rate of administration of NE was continually adjusted (between 0.4 and 0.55 μg/kg/min) to maintain MAP constant during hemorrhage. These experiments will be referred to as TAB/CMAP (total autonomic blockade/constant mean arterial blood pressure).

Chemical Analyses. Plasma concentrations of arginine vasopressin were determined by radioimmunoassay as described by Wang *et al.* (17). The standard curve was prepared by adding known amounts of USP Posterior Pituitary Reference Standard to vasopressin-free plasma obtained from dogs several hours after acute hypophysectomy and was extracted in the same manner as the unknowns. Plasma was extracted with Sep-Pak C18 cartridges (Waters Associates, Milford, MA). Recovery using this method ranged from 65%–75%. Plasma renin activity was determined according to the method of Haber *et al.* (21) using antiserum and standards purchased from Clinical Assays (Cambridge, MA). Plasma osmolality (P_{Osm}) was determined by freezing point depression (Model 3MO; Advanced Micro-Osmometer, Needham Heights, MA).

Statistical Analyses. Hemodynamic variables from each experiment were averaged for consecutive 4-min periods. The 4-min periods reported reflect the 4 min of data obtained immediately preceding an indicated blood loss. Grouped data are expressed as mean ± SE. Data were analyzed by a two-way analysis of variance for repeated measurements of the same variable. Within each treatment group, Dunnett's test was used to determine which means differed statistically from control means. A series of completely randomized F tests was used to determine the experimental periods at which significant differences existed between groups (22). The concentration of vasopressin in plasma demonstrated a nonhomogenous variance and therefore these values were transformed logarithmically prior to statistical analyses. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of Autonomic Blockade Before Hemorrhage. Twenty minutes after starting autonomic blockade, mean arterial pressure (MAP) decreased and PAVP increased significantly both in the intact dogs and in the CD dogs (Table I). When MAP was

Table I. Effect of Autonomic Blockade Before Hemorrhage

	Baseline	TAB	TAB/NMAP
MAP (mm Hg)			
Intact	104 ± 5	83 ± 6[a]	103 ± 6
CD	101 ± 7	79 ± 7[a]	102 ± 5
PAVP (pg/ml)			
Intact	3.0 ± 1.0	86 ± 19[a]	33 ± 17[a]
CD	3.0 ± 1.1	31 ± 7[a,b]	17 ± 6[a]
PRA (ngAI/ml/h)			
Intact	1.5 ± 0.3	3.3 ± 0.9[a]	1.7 ± 0.6
CD	0.6 ± 0.1	4.0 ± 2.3[a]	2.1 ± 1.0
LAP (mm Hg)			
Intact	3.0 ± 1.3	−1.4 ± 1.6[a]	3.5 ± 0.9
CD	7.6 ± 1.8	3.6 ± 1.1[a]	1.1 ± 1.1[a,b]
CO (l/min)			
Intact	1.9 ± 0.4	1.5 ± 0.4[a]	2.4 ± 0.3
CD	1.7 ± 0.3	1.5 ± 0.3[a]	2.2 ± 0.4
HR (bpm)			
Intact	131 ± 13	141 ± 5	135 ± 10
CD	112 ± 4	98 ± 3[a,b]	140 ± 11[a]

Note. After a 30-min baseline period, total autonomic blockade (TAB) was produced by administering hexamethonium bromide and methscopolamine bromide intravenously. After the initial fall in mean arterial blood pressure (MAP), norepinephrine (NE) was administered to return MAP back to control levels (TAB/NMAP). Intact = cardiac nerves intact (n = 7); CD = cardiac-denervated (n = 9); PAVP = plasma arginine vasopressin; PRA = plasma renin activity; LAP = left atrial pressure; CO = cardiac output; HR = heart rate. Values are mean ± SEM.

[a] $P < 0.05$ vs baseline within group.

[b] $P < 0.05$, change from baseline, CD vs change from baseline, intact.

returned to control levels by infusion of norepinephrine, PAVP levels were 33 ± 17 pg/ml in the intact dogs and 17 ± 6 pg/ml in the CD dogs; these values were statistically greater than the control values. Plasma renin activity was elevated 20 min after starting autonomic blockade in both intact and cardiac-denervated dogs. Unlike PAVP though, when MAP was returned to control levels, PRA also returned to control levels. Left atrial pressure (LAP) and cardiac output (CO) decreased during autonomic blockade in both groups of dogs. In both groups of dogs, the decrease in CO was reversed with infusion of NE; however, LAP returned to levels not significantly different than control values only in the intact dogs. Autonomic blockade did not alter heart rate significantly in the intact dogs, but significantly decreased heart rate in the CD dogs. However, heart rate in both groups of dogs was comparable after blood pressure was normalized to control values by infusion of NE.

Three dogs were used to determine the effect of NE infusion on PAVP and MAP during autonomic blockade over a time period greater than that required for the hemorrhage experiments. In these experiments, NE was infused to return MAP to control levels and was maintained for 90 min, during which time MAP and PAVP remained stable (108 ± 7 mm Hg and 36 ± 20 pg/ml, respectively, at 90 min).

Hemorrhage with Autonomic Blockade in Intact Dogs. Under normal conditions, when 10 and 20 ml/kg of blood was removed, PAVP increased from 3.0 ± 0.7 to 6.6 ± 2.4 and 78 ± 50 pg/ml, respectively (Fig. 1A). During hemorrhage with autonomic blockade, the same amounts of blood loss produced much greater increases in PAVP, from a control value of 33 ± 17 to 230 ± 90 and 610 ± 270 pg/ml. The same amounts of blood loss also produced significant increases in PRA; however, there was no significant difference in the PRA response between normal and autonomic blockade conditions (Table II). The only significant change detected in P_{Osm} was at 10 ml/kg blood loss under autonomic blockade conditions.

Under normal conditions, MAP was well maintained until 20 ml/kg blood loss, but fell significantly with further blood loss (Table III). Conversely, hemorrhage during autonomic blockade decreased MAP after a blood loss of only 5 ml/kg. Hemorrhage-induced decreases in MAP were significantly greater during autonomic blockade than under normal conditions. Under normal conditions, heart rate (HR) did not change significantly at 20 ml/kg blood loss; however, during autonomic blockade, HR decreased modestly, but significantly, at the same level of blood loss. Left atrial pressure, cardiac output, and stroke volume (SV) decreased progressively during hemorrhage under both conditions.

Hemorrhage with Autonomic Blockade in Cardiac-Denervated Dogs. PAVP levels also were augmented in the CD dogs during hemorrhage with autonomic blockade (Fig. 1B). During autonomic blockade, hemorrhage (10 and 20 ml/kg blood loss) caused PAVP levels to increase more in CD dogs with autonomic blockade (from 17 ± 6 to 76 ± 18 and 330 ± 80 pg/ml) than in CD dogs under normal conditions (from 3.7 ± 0.9 to 5.2 ± 0.8 and 26 ± 11 pg/ml). Hemorrhage-induced increases in PRA were similar under both conditions. P_{Osm} increased significantly at 20 and 30 ml/kg blood loss under normal conditions, but was unaltered during hemorrhage with autonomic blockade.

As in the intact dogs, MAP declined more in CD dogs with autonomic blockade than in CD dogs under normal conditions (Table III). Heart rate increased significantly during hemorrhage under normal conditions, but was unaltered during hemorrhage with autonomic blockade. Under normal conditions, LAP, CO, and SV decreased significantly; but only LAP and CO decreased significantly during hemorrhage with autonomic blockade.

Hemorrhage with Autonomic Blockade and Constant Blood Pressure in Intact Dogs. When arterial blood pressure was maintained constant in intact dogs during autonomic blockade, hemorrhage (20 ml/kg blood loss) caused PAVP levels to increase from 16 ± 5 to 470 ± 220 pg/ml (Fig. 2); PRA also increased

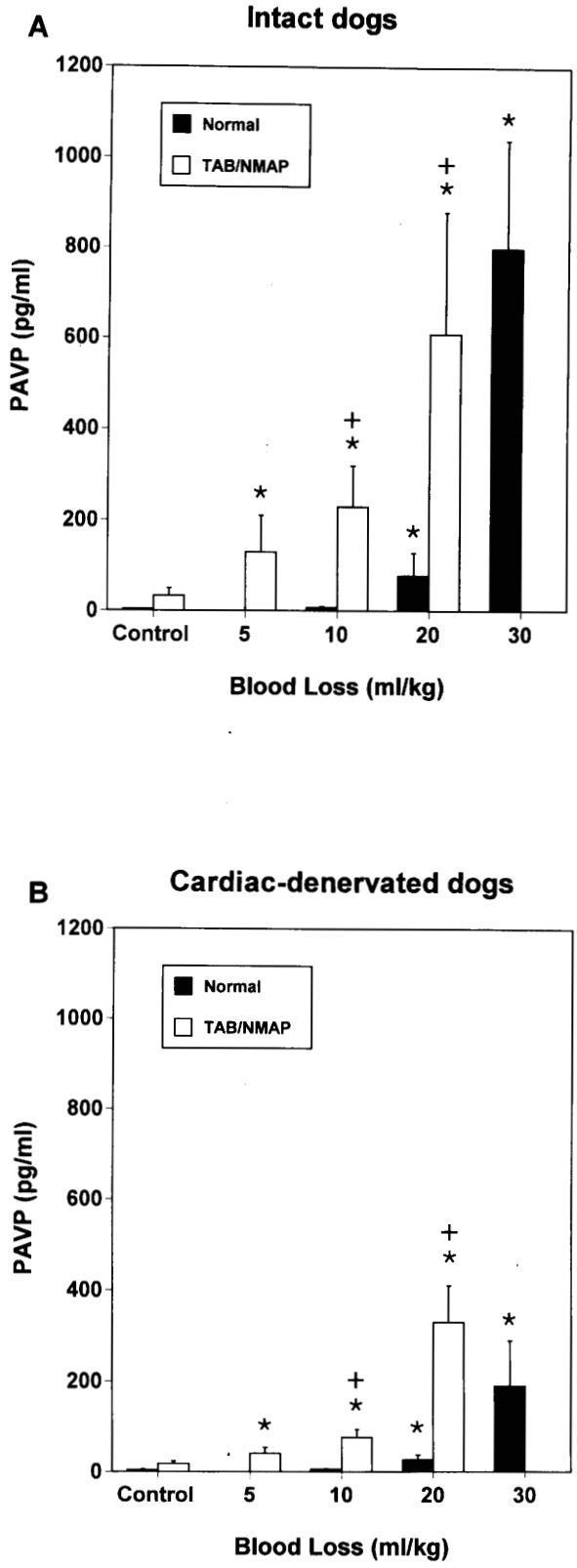

Figure 1. Changes in plasma vasopressin concentration (PAVP) in intact (Panel A) and cardiac-denervated (Panel B) dogs in response to hemorrhage under normal conditions and with total autonomic blockade. Mean arterial blood pressure was returned to preblockade levels prior to the hemorrhage by infusing norepinephrine (TAB/NMAP). $^{*}P < 0.05$ vs control. $^{+}P < 0.05$ vs normal conditions at corresponding level of blood loss.

significantly and no changes were detected in P_{Osm} (Table II). Under these conditions, LAP, CO, and SV decreased significantly during hemorrhage, and heart rate increased significantly (Table III).

Hemorrhage with Autonomic Blockade and Constant Blood Pressure in Cardiac-Denervated Dogs. When MAP was maintained constant by infusion of NE in CD dogs, PAVP, PRA, and P_{Osm} did not change during hemorrhage (Fig. 2 and Table II). As in the intact dogs, LAP, CO, and SV decreased significantly; however, in the CD dogs, HR increased markedly during hemorrhage (Table III), probably due to the hypersensitivity of the denervated heart to norepinephrine (23).

Discussion

The increase in PAVP that occurs during hemorrhage in conscious dogs is augmented when the autonomic nervous system is pharmacologically blocked. Under normal conditions in intact dogs, PAVP did not change significantly at 10 ml/kg blood loss. However, when the autonomic nervous system was pharmacologically blocked, already elevated PAVP levels increased approximately 4-fold at 5 ml/kg blood loss and 7-fold at 10 ml/kg blood loss. These results demonstrate that a smaller blood loss is required to elicit an increase in PAVP during autonomic blockade and that, at any given amount of blood loss, PAVP levels are markedly higher under autonomic blockade than under normal conditions. These results are consistent with those of Oliver *et al.* (6), who demonstrated that the increase in PAVP during hemorrhage is enhanced by autonomic blockade in conscious rabbits. The present study not only confirms the existence of this response in dogs, but also examines the afferent mechanisms involved in this response.

Cardiac receptors appear to be a major component of the afferent mechanism responsible for the increase in PAVP, which is routinely observed during hemorrhage under normal conditions in conscious animals (14–17). The present results concur with this concept. For example, the substantial rise in PAVP that occurred during hemorrhage at 30 ml/kg in the intact dogs was blunted in the CD dogs (Fig. 1), indicating the importance of cardiac receptors during hemorrhage. We therefore hypothesized that cardiac receptors were also responsible for the enhanced PAVP response to hemorrhage with autonomic blockade and that ablation of the cardiac nerves would thwart this response.

However, PAVP levels were also enhanced in CD dogs during hemorrhage with autonomic blockade. Under these conditions, it appears that greater unloading of peripheral baroreceptors may have been responsible for the enhanced PAVP response. During autonomic blockade in the CD dogs, MAP decreased sig-

Table II. Changes in PRA and P_{Osm} Elicited by Hemorrhage in Conscious Dogs

	Blood loss (ml/kg)				
	Control	5	10	20	30
PRA (ngAI/ml/hr)					
Intact					
Normal	2.2 ± 0.5		5.8 ± 1.3	9.2 ± 2.2[a]	13 ± 4[a]
TAB/NMAP	1.7 ± 0.6	5.0 ± 2.4	6.0 ± 1.5	12 ± 4[a]	
TAB/CMAP	3.2 ± 1.0	8.0 ± 2.3[a]	7.9 ± 2.0[a]	8.8 ± 1.6*	
CD					
Normal	1.5 ± 0.5		2.9 ± 0.8	7.4 ± 2.2[a]	11 ± 3[a]
TAB/NMAP	2.1 ± 1.0	3.0 ± 1.1	4.8 ± 1.3	11 ± 2[a]	
TAB/CMAP	1.7 ± 0.8	1.7 ± 0.6	1.5 ± 0.5	1.8 ± 0.5	
P_{Osm} (mOsm/kg H_2O)					
Intact					
Normal	301 ± 2		303 ± 2	302 ± 2	303 ± 2
TAB/NMAP	304 ± 5	303 ± 5	301 ± 4[a]	305 ± 4	
TAB/CMAP	303 ± 5	304 ± 4	303 ± 6	305 ± 5	
CD					
Normal	301 ± 1		302 ± 1	303 ± 2[a]	304 ± 2[a]
TAB/NMAP	299 ± 2	300 ± 2	300 ± 2	301 ± 2	
TAB/CMAP	298 ± 1	299 ± 1	300 ± 2	299 ± 1	

Note. Conscious intact (n = 7) and cardiac-denervated dogs (CD, n = 9) were hemorrhaged at a rate of 0.8 ml/kg/min under normal conditions and during total autonomic blockade. In the experiments labeled TAB/NMAP, arterial blood pressure was returned to pre-TAB values by constant infusion of norepinephrine (NE). Dogs were also hemorrhaged with TAB and simultaneous, regulated infusion of NE in order to maintain blood pressure constant during hemorrhage (TAB/CMAP; intact, n = 5; CD, n = 8). CD = cardiac-denervated; PRA = plasma renin activity; P_{Osm} = plasma osmolality. Values are mean ± SEM.
[a] $P < 0.05$ vs control.

nificantly by 5 ml/kg blood loss, providing a greater reduction in arterial baroreceptor input and, thus, a greater reflex-induced increase in PAVP.

To examine the possibility that the enhanced PAVP response in CD dogs was, indeed, mediated by peripheral baroreceptors, arterial blood pressure was maintained constant by a regulated infusion of NE in CD dogs during hemorrhage with autonomic blockade. PAVP did not change during hemorrhage under these conditions. When input to arterial baroreceptors was controlled in CD dogs, the enhanced PAVP response to hemorrhage with autonomic blockade was abolished. Interestingly, when similar experiments were performed in intact dogs, PAVP was significantly elevated after removal of 10 ml/kg of blood. This response was probably attributable to cardiac receptor-mediated reflex stimulation of vasopressin release caused by a significant fall in cardiac filling pressure, which occurred earlier in dogs under autonomic blockade than it did in dogs under normal conditions. Taken together, these results demonstrate that the enhanced release of vasopressin induced by hemorrhage during autonomic nervous system blockade is mediated both by cardiac and arterial baroreceptors.

The results indicating that cardiac receptors are primarily responsible for the increase in PAVP that occurs during hemorrhage under these conditions are in direct contrast to the conclusion of Shen *et al.*, who stated that cardiac receptors are not major regulators of AVP release in response to progressive hemorrhage (7). It has been suggested that the relative contribution of cardiac or arterial baroreceptors on vasopressin release may depend on the experimental conditions in which vasopressin release is stimulated (24). For example, when hypotension was produced by thoracic inferior vena caval occlusion in conscious dogs with acute, reversible cardiac nerve blockade with intrapericardial procaine, PAVP increased significantly (24). Although O'Donnell *et al.* demonstrated that cardiac receptors have a partial role in stimulating vasopressin release, they concluded that the principal stimulus for vasopressin secretion after acute cardiac nerve blockade arose from unloading arterial baroreceptors (24). It should be noted that the increase in PAVP in the dogs with cardiac nerve blockade was in response to a mean decrease in blood pressure of at least 24 mm Hg. Shen *et al.* (7) also observed that PAVP increased significantly in splenectomized CD dogs when hemorrhaged at 0.5 ml/kg/min. Again, it should be noted that the increase in PAVP did not become significant until blood pressure decreased significantly (approximately 20 mm Hg). Likewise, in the present experiments whenever MAP decreased significantly, PAVP increased significantly, regardless of the state of cardiac innervation. Results such as these have led others to conclude that cardiac receptors are not the major regulators of AVP in response to hemorrhage (7).

However, in the present study, significant increases in PAVP also occurred in the intact dogs when

Table III. Hemodynamic Changes Elicited by Hemorrhage in Conscious Dogs

	Blood loss (ml/kg)				
	Control	5	10	20	30
MAP (mm Hg)					
Intact					
Normal	113 ± 3		113 ± 4	110 ± 4	90 ± 7[a]
TAB/NMAP	103 ± 6	91 ± 6[a]	95 ± 6[a,b]	80 ± 5[a,b]	
TAB/CMAP	104 ± 9	102 ± 5	101 ± 5	104 ± 6	
CD					
Normal	101 ± 5		98 ± 5	93 ± 7[a]	85 ± 6[a]
TAB/NMAP	102 ± 5	88 ± 3[a]	84 ± 3[a,c]	68 ± 5[a,c]	
TAB/CMAP	108 ± 5	106 ± 4	110 ± 6	109 ± 6	
LAP (mm Hg)					
Intact					
Normal	4.8 ± 0.4		3.6 ± 0.7	2.5 ± 0.5[a]	0.2 ± 0.1[a]
TAB/NMAP	3.5 ± 0.9	1.4 ± 0.7[a]	1.0 ± 1.0[a,b]	0.0 ± 0.6[a]	
TAB/CMAP	1.2 ± 1.9	0.2 ± 1.9	−0.8 ± 1.5[a]	−1.8 ± 2.2[a]	
CD					
Normal	5.3 ± 1.8		3.6 ± 1.7	2.2 ± 1.6[a]	0.8 ± 1.3[a]
TAB/NMAP	1.1 ± 1.1	0.0 ± 0.9[a]	−0.4 ± 0.7[a]	−1.8 ± 0.4[a]	
TAB/CMAP	0.1 ± 0.7	−1.4 ± 0.9	−2.5 ± 0.7[a]	−4.2 ± 0.9[a]	
HR (bpm)					
Intact					
Normal	111 ± 8		107 ± 10	124 ± 11	126 ± 20
TAB/NMAP	135 ± 10	133 ± 10	132 ± 10	130 ± 10*	
TAB/CMAP	144 ± 7	151 ± 11	148 ± 9	153 ± 8*	
CD					
Normal	110 ± 4		113 ± 4	119 ± 4[a]	85 ± 6[a]
TAB/NMAP	140 ± 11	139 ± 11	137 ± 10	134 ± 10	
TAB/CMAP	144 ± 5	151 ± 7	162 ± 8[a]	188 ± 8[a]	
CO (l/min)					
Intact					
Normal	2.9 ± 0.5		2.5 ± 0.3	2.1 ± 0.3[a]	1.6 ± 0.2[a]
TAB/NMAP	2.4 ± 0.3	2.2 ± 0.3	1.9 ± 0.3[a]	1.5 ± 0.3[a]	
TAB/CMAP	1.9 ± 0.4	1.5 ± 0.4[a]	1.3 ± 0.4[a]	1.0 ± 0.3[a]	
CD					
Normal	2.5 ± 0.6		2.4 ± 0.5	2.2 ± 0.6[a]	1.8 ± 0.6[a]
TAB/NMAP	2.2 ± 0.4	1.9 ± 0.4	1.9 ± 0.3	1.7 ± 0.2[a]	
TAB/CMAP	2.4 ± 0.1	2.1 ± 0.2[a]	2.0 ± 0.2[a]	1.8 ± 0.2[a]	
SV (ml)					
Intact					
Normal	27 ± 5		25 ± 4	19 ± 5[a]	14 ± 4[a]
TAB/NMAP	18 ± 3	16 ± 2	15 ± 2[a]	12 ± 2[a]	
TAB/CMAP	14 ± 3	11 ± 3	9 ± 3[a]	7 ± 2[a]	
CD					
Normal	21 ± 4		20 ± 4	17 ± 4[a]	13 ± 4[a]
TAB/NMAP	15 ± 3	14 ± 2	14 ± 2	13 ± 1	
TAB/CMAP	17 ± 1	14 ± 6[a]	12 ± 1[a]	10 ± 1[a]	

Note. Conscious intact (n = 7) and cardiac-denervated (CD, n = 9) dogs were hemorrhaged at a rate of 0.8 ml/kg/min under normal conditions and during total autonomic blockade (TAB). In the experiments labeled TAB/NMAP, arterial blood pressure was returned to pre-TAB values by constant infusion of norepinephrine (NE). Dogs were also hemorrhaged with TAB and simultaneous, regulated infusion of NE in order to maintain blood pressure constant during hemorrhage (TAB/CMAP; intact, n = 5; CD, n = 8). MAP = mean arterial pressure; LAP = left atrial pressure; HR = heart rate; CO = cardiac output; SV = stroke volume. Values are mean ± SEM.
[a] $P < 0.05$ vs control.
[b] $P < 0.05$ vs intact normal.
[c] $P < 0.05$ vs CD normal.

MAP did not change or was maintained constant with norepinephrine, indicating that cardiac receptors are important in regulating PAVP. It appears that when splenectomized dogs are hemorrhaged, blood pressure drops more rapidly than under normal conditions. Shen *et al.* reported decreases in MAP in intact dogs of approximately 20% and 50% at 20 and 30 ml/kg blood loss, respectively (7), compared with approximately 3% and 20% at equal levels of blood loss in the present experiments. The more rapid decrease in blood pressure in that study (7) may have elicited a greater arterial baroreceptor-mediated reflex release of AVP and,

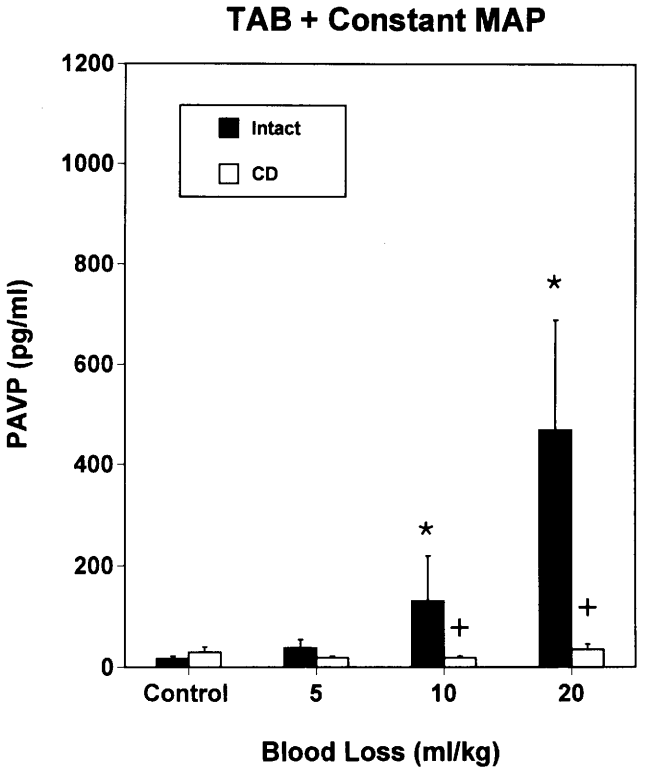

Figure 2. Changes in plasma vasopressin concentration (PAVP) in intact and cardiac-denervated (CD) dogs in response to hemorrhage with total autonomic blockade and constant mean arterial blood pressure. Blood pressure was maintained constant throughout the hemorrhage by continual regulation of an iv infusion of norepinephrine (TAB/CMAP). $^{*}P < 0.05$ vs control. $^{+}P < 0.05$ vs intact at corresponding level of blood loss.

thus, masked the impact of cardiac receptors. Consistent with this notion is the finding that when the complicating influences of sinoaortic reflexes were eliminated, cardiac denervation inhibited the release of AVP in response to hemorrhage (7). Taken together, these data concur with the notion that, depending on the circumstances (and in particular, the rate of change of blood pressure), both cardiac and arterial baroreceptors are important in regulating vasopressin release during hemorrhage. It appears that cardiac receptors are important in regulating PAVP during episodes of minor hypotension and that arterial baroreceptor input is important during bouts of severe hypotension.

A study by Zhu *et al.* (25) in which NE infusion increased PAVP even when peripheral and cardiac reflex influences were effectively eliminated suggested that NE may have a direct central stimulatory effect on AVP release. It is possible that, in the present study, the higher prehemorrhage control levels of PAVP during autonomic blockade may have been caused by a direct effect of NE on AVP release. However, any direct effect that infused NE may have had on PAVP was relatively minor compared with the effect of hemorrhage in these experiments. Additionally, in control experiments in which NE was infused for 90 min after autonomic blockade, MAP and PAVP remained constant, suggesting that any direct effect of NE alone on PAVP remained constant during the hemorrhage experiments.

In the current study, hemorrhage elicited similar increases in PRA in intact and CD dogs under normal conditions, thus confirming previous data reported by Goetz *et al.* (14) and Wang *et al.* (17). In both groups of dogs, hemorrhage with autonomic blockade elicited increases in PRA to levels that were not different from those measured during hemorrhage under normal conditions. These results are in contrast to the results obtained in conscious rabbits (6), in which PRA levels were enhanced by autonomic blockade during hemorrhage. The rabbit and dog protocols were very similar, suggesting that the difference in the PRA response may simply be attributable to species differences. These results are also consistent with several studies that have shown that the increase in PRA that occurs during acute blood loss is largely independent of baroreceptor input (15, 26), and may result from local hemodynamic changes on the renal barostat (27).

It is interesting to note that PRA did not change when CD dogs were hemorrhaged during autonomic blockade and constant MAP (Table II), but PRA increased significantly under similar conditions in the intact dogs. The basal and hemorrhage-induced changes in cardiovascular parameters were very similar in both groups of dogs under these conditions, suggesting that the input to the intrarenal baroreceptor was similar in both groups of dogs. It is possible, though, that subtle differences in the local hemodynamic and hormonal response to norepinephrine may have provided different inputs for renin release in these two groups.

To summarize, specific blockade of the autonomic nervous system during hemorrhage in conscious dogs confirms that there is a compensatory rise in PAVP, but not in PRA, under these conditions. The enhanced PAVP response can be elicited either by cardiac or arterial baroreceptor-mediated reflex activation of vasopressin release in response to falling cardiac filling pressure or arterial blood pressure, respectively. However, the maximal PAVP response under these conditions requires the unhindered function of both cardiac and arterial baroreceptors.

The authors with to express their gratitude to Dr. Kenneth L. Goetz and the late Dr. Bin C. Wang for their guidance and support during this project. We also thank Pamela Geer, Gloria Flora-Ginter, and Gregory Goetz for their expert technical assistance, and Catherine Leadley for her assistance in preparing this manuscript. This study was supported by National Heart, Lung, and Blood Institute Grant HL-13623 and HL-35830.

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