Arterial baroreceptors control blood pressure and vasopressin responses to hemorrhage in conscious dogs

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Thrasher, Terry N., and Lanny C. Keil. Arterial baroreceptors control blood pressure and vasopressin responses to hemorrhage in conscious dogs. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1843–R1857, 1998.—The goal of this study was to determine the role of arterial baroreceptors in the reflex control of arginine vasopressin (AVP), renin, and cortisol secretion in response to a 30-ml/kg hemorrhage in conscious dogs. The hormonal responses were measured in six dogs under four treatment conditions: 1) intact, 2) acute cardiac denervation (CD) by intrapericardial infusion of procaine, 3) after sinoaortic denervation (SAD), and 4) during combined SAD + CD. In the intact condition, mean arterial pressure (MAP) was maintained at control levels until blood loss reached 20 ml/kg and the absolute magnitude of the fall at 30 ml/kg was 35 ± 10 mm Hg. Similar responses were obtained during acute CD. In contrast, MAP fell earlier (at 5 ml/kg, P < 0.05) and to much lower levels in both the SAD and SAD + CD conditions. The individual slopes relating systolic pressure to plasma AVP, renin activity (PRA), and cortisol were used to compare the treatment effects using a 2 × 2 factorial analysis. There was a significant (P < 0.01) effect of SAD on the slope relating AVP to systolic pressure but no effect of CD and no SAD × CD interaction. In contrast, there was no effect of either SAD or CD on the relationship between PRA or plasma cortisol and systolic pressure. These results indicate that maintenance of blood pressure and the normal pattern of AVP secretion during hemorrhage depend on intact arterial baroreceptor reflexes.

HEMORRHAGE IS A POTENT STIMULUS to secretion of arginine vasopressin (AVP) (31, 32), renin (8), and ACTH (11). However, correct identification of the afferent receptors that initiate the increases in AVP, renin, and ACTH secretion has proven to be a long, contentious, and still unresolved question of regulatory physiology. Concerning AVP secretion, Share (31) reviewed the experimental literature in the 1974 Handbook of Physiology and concluded that unloading left atrial receptors accounted for most of the increase in plasma AVP during progressive hemorrhage together with a smaller contribution arising from the unloading of arterial baroreceptors after the onset of frank hypotension. Supporting this conclusion are electrophysiological measurements showing that activity of atrial type B receptors with vagal afferents varied directly with changes in blood volume in anesthetized dogs (14). Furthermore, functional studies in anesthetized dogs demonstrated that acute cervical vagotomy produced large, albeit transitory, increases in plasma AVP (33), that the hemorrhage-induced rise in plasma AVP is blunted by vagotomy but not carotid sinus denervation (30), and that significant increases in plasma AVP occur in response to “nonhypotensive” levels of hemorrhage in both anesthetized (6) and conscious dogs (16) and conscious sheep (17). More recently, Gotz et al. (13) reported that cardiac denervation (CD), but not sinoaortic denervation (SAD), resulted in a dramatic reduction in the AVP response to hemorrhage in conscious dogs. Similarly, Quail et al. (25) reported that acute CD using intrapericardial administration of procaine eliminated the increase in plasma AVP in response to hemorrhage in conscious rabbits, but SAD had no effect on the response. Thus the concept that a major component of the AVP response to hemorrhage is driven by unloading atrial receptors is supported by many lines of evidence and in three different species.

There are, however, many other observations that suggest different mechanisms. For example, Larrson et al. (19) measured responses in conscious goats and reported that hemorrhage volumes of 8 or 12 ml/kg had no effect on plasma AVP and that a 16-ml/kg hemorrhage caused a rise only in those goats which became hypotensive. However, central venous pressure fell in response to all levels of hemorrhage, indicating that unloading atrial receptors alone was not sufficient to stimulate an increase in AVP secretion. Furthermore, the observation that nonhypotensive hemorrhage stimulated an increase in plasma AVP in conscious sheep (17) used a bioassay to measure AVP, but urine function in the sheep undergoing hemorrhage indicated no change in free water clearance. Thus it is arguable whether the hemorrhage actually led to a rise in plasma AVP. Studies in rabbits have also shown that hemorrhage does not cause a significant rise in plasma AVP until there is a fall in blood pressure (7, 25). Studies of AVP responses to hemorrhage in rats have yielded conflicting results (32). In studies using the same radioimmunoassay for AVP, hemorrhage amounting to 10% of blood volume caused increases in plasma AVP in some experiments (5) but not in others (4), with little change in MAP. However, loss of 20% of blood volume consistently resulted in arterial hypotension and large increases in plasma AVP (4, 5). Previous studies from this laboratory have shown no increase in plasma AVP in conscious dogs in response to either hemorrhage or constriction of the thoracic vena cava until there was a fall in MAP (22, 24). Thus observations in dogs, rats, rabbits, and ruminants do not provide uniform support...
for the concept that unloading atrial receptors is sufficient to stimulate an increase in AVP secretion.

Studies in conscious primates and in human subjects also fail to support the concept that atrial receptors play a role in AVP secretion during hypovolemia (12). Arnauld et al. (2) demonstrated there was no increase in plasma AVP in conscious monkeys undergoing hemorrhage unless the animal became hypotensive. Furthermore, most studies of human responses to graded hypovolemia (e.g., tilt or lower body negative pressure) have failed to document an increase in plasma AVP unless the subject became hypotensive (see Ref. 37 for review). In one attempt to reconcile these apparent species differences, Gilmore (12) proposed that primates and quadruped mammals have evolved different mechanisms for the volemic control of AVP secretion, quadrupeds depending primarily on input from left atrial receptors and primates depending on input from arterial baroreceptors. However, this explanation appeared before studies of hemorrhage in conscious rabbits (7, 25) and dogs (22) demonstrated the same relationship between plasma AVP and arterial pressure as reported by Arnauld et al. (2) in monkeys.

Recently, various studies have questioned whether atrial receptors play any role in the stimulation of AVP secretion during hypovolemia. Wang et al. (38) reported that denervation of the cardiac ventricles alone was as effective in suppressing the AVP response to a 30-ml/kg hemorrhage as complete CD in dogs. Thus Wang and colleagues (38) argued that stimulation of ventricular hemorrhage as complete CD in dogs. Wang et al. (38) reported that cardiac receptors alone were not sufficient to stimulate an increase in AVP secretion. We also examined the effects of acute blockade of cardiac nerves by pericardial (PC) infusion of propranolol (referred to hereafter as CD) on plasma AVP and found no evidence of a change in baseline levels, suggesting that atrial receptors were not inhibiting AVP secretion in conscious, euovolemic dogs (23). Furthermore, we did not find any effect of acute CD on the increase in plasma AVP in response to graded hemorrhage in conscious dogs (22). Consequently, we concluded that cardiac receptors were not essential for the stimulation of AVP secretion during hypovolemia in dogs.

Renin secretion is controlled by a complex of both intrarenal mechanisms and reflexes arising from arterial and cardiac receptors (8). During progressive hemorrhage, renin secretion may increase in response to unloading atrial or arterial baroreceptors or to reductions in renal perfusion pressure which stimulate the renal vascular receptor mechanism. Reflex mechanisms also cause stimulation of ACTH secretion and a rise in plasma cortisol (11). Increases in plasma ANG II (26) and plasma AVP (40) have also been shown to stimulate ACTH and thus cortisol secretion. Thus several mechanisms could participate in the increase in ACTH secretion during hypovolemia.

The purpose of this study was to compare the increases in plasma AVP, plasma renin activity (PRA), and cortisol in response to graded hemorrhage in a single group of conscious dogs. To minimize any differences that arise from comparing responses to hemorrhage in different groups of animals, we studied the same dogs in four treatment conditions: 1) with cardiac and arterial baroreceptors intact, 2) during PC infusion of propranolol, 3) with combined SAD and acute CD. Because each animal was studied in each treatment condition, we were able to apply a 2 × 2 factorial analysis to determine the contribution of cardiac and arterial baroreceptor afferents in the stimulation of hormonal responses to hemorrhage.

METHODS

General procedures. Experiments were performed on nine adult mongrel dogs (4 male and 5 female) weighing between 19 and 25 kg. The dogs were housed in a room maintained at 22 ± 2°C and 70% humidity with a 12:12-h light-dark cycle. Each day, between 1600 and 1800, the dogs were administered oral prophylactic antibiotic treatment with sulfamethoxazole (800 mg) and trimethoprim (160 mg) and fed a mixture of dry chow and canned food sufficient to maintain a constant body weight. The food was always consumed within 10 min of presentation, and sodium intake on this diet averaged 2–3 meq·kg⁻¹·day⁻¹. Water was available ad libitum. The dogs were allowed into an outside run for at least 60 min two times each day for socialization and exercise.

Patency and sterility of the vascular catheters was maintained by filling them with a mixture of heparin (1,000 U/ml; Elkins-Sinn, Cherry Hill, NJ) and penicillin G potassium (20,000 U/ml; Eli Lilly, Indianapolis, IN), which was replaced a minimum of every 72 h. Even with the precaution of filling the catheters with an antibiotic, dogs implanted with chronic vascular catheters are at risk for blood-borne infections (unpublished observations). To ensure that the dogs were free of infection throughout all aspects of the study, complete blood counts and rectal temperature readings were performed on a weekly basis. In addition, rectal temperatures were taken on the morning before experiments. Rectal temperatures never exceeded 39°C, and white blood cell counts were within the normal reference range. On the basis of these measurements, the dogs were free of infection throughout the duration of the study.

Experimental design. The principal goal of this study was to compare hormonal responses to hemorrhage in the same group of dogs with cardiovascular afferents intact and after removal of cardiac and arterial baroreceptor reflexes. Cardiac reflexes were eliminated by PC infusion of procaine to produce acute CD. This method results in a modest sustained increase in heart rate (HR) and MAP but does not affect resting levels of plasma norepinephrine, AVP, PRA, or cortisol (23). The role of arterial baroreceptors was evaluated by surgical denervation of sinoaortic baroreceptors (SAD), which eliminates both arterial baroreceptors and chemoreceptors. Combining acute CD with SAD made it possible to evaluate responses in the absence of cardiac and arterial receptor input. This experimental design eliminated the variability which arises from comparisons between different groups of animals used in prior studies.

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Surgical procedures. In all surgical procedures, the dogs were sedated with acepromazine maleate (0.2 mg/kg iv; Tech America, Elwood, KS) and anesthetized with pentobarbital sodium (25 mg/kg iv; Fort Dodge Laboratories, Fort Dodge, IA). During the 7-day postoperative period after each surgical procedure, the dogs were treated with enrofloxacin (2.5 mg/kg Baytril; Mobay, Shawnee, KS) two times daily to provide antibacterial coverage and oxymorphone (Numorphan; DuPont Pharmaceuticals, Wilmington, DE) as required to provide analgesia. In the first surgery, the trachea was intubated and a right thoracotomy was performed at the fourth intercostal space, a 1-cm incision was made in the pericardium adjacent to the right atrial appendage, and a catheter (medical grade Tygon; 0.05 ID and 0.09 OD) was inserted into the right atrium and secured with a purse-string suture. The pericardium was closed with a suture. The PC catheter was constructed out of Tygon (as above) with a 6-in. Silastic extension at the distal end and a 1-in.-diameter Dacron flange at the junction between the Tygon and Silastic tubes. A 1-mm hole was made in the pericardium to allow insertion of the Silastic tip into the PC space, and the flange was sutured to the outside of the pericardium to secure the catheter in place. The chest was then closed, and negative intrapleural pressure was established to completely reflate the lungs. Additional Tygon catheters were introduced into the femoral artery and vein and advanced to the abdominal aorta and inferior vena cava, respectively. All catheters were tunneled subcutaneously to exit between the shoulder blades and were protected by placement in a pouch sewn to the underside of a nylon jacket (Alice King Chatham Medical Arts, Los Angeles, CA). At least 2 wk were allowed for recovery.

After completion of the experiments in the intact and acute CD conditions, the dogs were anesthetized as above and the carotid sinus region was exposed via a ventral midline neck incision. The internal carotid arteries were ligated and cut together with all other vessels originating from the external carotid artery proximal to the lingual artery. The adventitia between the cranial thyroid artery and the lingual artery was stripped and painted with 5% phenol in ethanol bilaterally. The dogs received antibiotics and analgesia as above, and at least 2 wk were allowed for recovery from this procedure. Finally, the dogs were anesthetized as above and aortic baroreceptors were exposed via a left thoracotomy at the fourth intercostal space. All visible nerves in the region of the aortic arch were cut, and the adventitia of the aorta to the level of the second thoracic artery was stripped. In addition, the adventitia of the brachiocephalic and subclavian trunks was stripped to the level of the second bifurcation of each vessel. Finally, the vessels were painted with phenol solution as above. The chest was then closed, and negative pressure was reestablished to insure complete expansion of the lungs. The dogs were treated with antibiotic and analgesia as above, and at least 3 wk were allowed before experimentation.

Verification of cardiac nerve blockade and effectiveness of SAD. Blockade of cardiac efferent nerves during PC infusion of procaine was tested by measuring HR responses to bolus administration of nitroglycerine (NG, 15 µg/kg; American Critical Care, McGaw Park, IL) and phenylephrine (PE, 5 µg/kg; Winthrop-Breen Laboratories, New York, NY) before and during acute CD. Cardiac afferent nerve blockade was tested by bolus injection of veratridine (V, 50 µg) into the right atrium before and during acute CD. Effectiveness of SAD was determined by measuring the HR responses to bolus injections of NG and PE at the doses stated above. At the end of each experiment using PC infusion of procaine, responses to V were retested to ensure blockade of cardiac afferents. After SAD, two dogs displayed measurable changes in HR in response to NG and PE. Consequently, the results from these two dogs were eliminated. A third dog died during denervation of the aortic arch. Therefore, the results are based on observations in six dogs (2 males and 4 females). There were no apparent gender differences in the responses to or tolerance of hemorrhage (female dogs were not used for experimentation during estrus).

After all studies were completed, the dogs were euthanized with an overdose of pentobarbital sodium to examine the hearts for evidence of inflammation or adhesion of the PC membrane to the epicardium in response to the PC catheter. Typically, there was a small area (maximum diameter of 1 cm) of adhesion of the PC membrane to the underlying epicardium where the atrial catheter passed through the pericardium. There was no adhesion between the pericardium and epicardial surface at the entry of the PC catheter. On the outer surface of the pericardium, the Dacron patch was completely enclosed in fibrous tissue, effectively preventing any leakage of fluid infused into the PC space. There was no evidence of generalized adhesions between the pericardium and the myocardium or irritation of the myocardial surface. Therefore, there was no visible barrier to the distribution of procaine throughout the region bounded by the PC sac.

Experimental protocols. Experiments were conducted between 0800 and 1300 in a quiet room with the dog in a sling (Alice King Chatham Medical Arts), which provided support but minimal restraint. The dog was allowed 30 min to settle down in the sling before the beginning of data collection. Each of the dogs underwent two 30-ml/kg hemorrhages, one during infusion of saline and again during infusion of procaine into the PC space. In addition, each dog underwent two time-control experiments, one time during PC infusion of saline and the other during PC infusion of procaine. The PC infusion of either 0.9% saline or 2% procaine hydrochloride (Abbott Laboratories, North Chicago, IL) involved an initial loading dose infused at a rate of 1 ml/min for 10 min followed by a maintenance infusion of 0.2 ml/min for the duration of the experiment. Each experiment consisted of a 20-min control period to establish baseline values for hemodynamic variables and plasma hormone levels, a 20-min equilibration period after initiation of PC infusion to allow hemodynamic variables to stabilize after acute CD, a 30-min period of hemorrhage during which time blood was removed at 1 ml·kg⁻¹·min⁻¹, and finally a 30-min period of sustained hypovolemia to measure the effectiveness of reflex mechanisms to restore MAP. Blood samples (10 ml each) were collected at the beginning and end of the control period, the end of the equilibration period, at 5-min intervals during the hemorrhage, which corresponds to hemorrhage volumes of 5, 10, 15, 20, 25, and 30 ml/kg, and 15 and 30 min after completion of the hemorrhage. The blood samples were immediately aliquoted into chilled tubes containing 0.3 M EDTA, for measurement of PRA, or heparin, for measurement of plasma sodium, protein, osmolality, cortisol, and AVP. The hemorrhage was performed by withdrawing blood from the femoral venous catheter into a blood transfusion pack unit (Fenwal Laboratories, Deerfield, IL) which contained 1,000 U heparin/100 ml blood. The blood samples taken during the control and equilibration periods were replaced with 10 ml of saline, but samples taken during the hemorrhage were considered part of the 30 ml/kg to be removed and were not replaced with saline. At the end of the acute CD experiments, 50 µg of V was injected into the right atrium to ensure that cardiac afferent nerves were indeed blocked. Finally, the PC infusion was stopped and the blood from the hemorrhage was reinfused into the dog. In the time-control experiments,
procedures identical to those outlined above were used, except that no hemorrhage occurred. An additional set of control experiments was conducted to estimate the systemic effects of procaine leaking into the vascular compartment. In these experiments, procaine was infused intravenously at the same rate and duration as infused into the PC space in the intact and SAD conditions. Experiments were typically performed at 7-day intervals, but there were never more than three experiments in a 2-wk period. The order of experiments was randomized within each stage of surgical preparation.

Methods of measurement. Arterial and right atrial pressures (RAP) were measured using Cobe transducers and recorded on a Grass model 7D polygraph. The pressure transducers were adjusted to heart level for each dog. The output from the polygraph was fed to a Buxco cardiovascular analyzer (model CVA-1, Buxco Electronics) connected to an online data acquisition system (Keithly, Series 500), and the digitized data were stored in an IBM-XT. Plasma sodium and potassium were determined by flame photometry (model 343, Instrumentation Laboratories), plasma osmolality was determined by freezing point depression (Advanced model 3W), and plasma protein was determined by refractometry. Plasma AVP was determined by RIA after extraction with bentonite (18, 36). Recovery of AVP averaged 70 ± 2%, and the values reported are not corrected for recovery. Synthetic AVP (357 U/mg) was used to prepare standards, and the minimum level of detectability was 0.3 pg AVP/ml. The intra- and interassay coefficients of variability were 9 and 12%, respectively. PRA was measured using an RIA for ANG I and expressed as nanograms of ANG I generated per milliliter of plasma during a 3-h incubation at pH 6.0 (RIANEN RIA kit; DuPont). Plasma cortisol was measured using a kit obtained from Diagnostic Products (Los Angeles, CA). The intra- and interassay coefficients of variability were 11 and 16%, respectively.

Data analysis. A two-way ANOVA with repeated measures on both treatment (intact, CD, SAD, and SAD + CD) and time (or hemorrhage volume) as factors was used to analyze the data (39). Differences were considered significant if P < 0.05. When a significant interaction between factors was detected, the simple main effect of each level of one factor was evaluated at all levels of the other factor. When simple main effects were significant, Newman-Keuls test was applied to compare the means. The individual slopes relating the plasma level of each hormone to systolic pressure were estimated using the least squares method (41). The slopes in each of the four treatment conditions were compared using a 2 × 2 factorial analysis (39). The values for plasma AVP during hemorrhage displayed excessive heterogeneity (Bartlett's test; see Ref. 41) and were transformed logarithmically before analysis.

RESULTS

Baroreflex tests. The changes in MAP and HR in response to PE (5 µg/kg iv), NG (15 µg/kg iv), and V (50 µg administered into right atrium) are shown in Fig. 1. In the intact condition, baroreflex responses induced by PE and NG were appropriate to the change in MAP, and V caused a simultaneous fall in both MAP and HR. During acute CD, HR responses to PE, NG, and V were all blocked, as well as the reflex hypotension induced by V acting on cardiac receptors. In the SAD condition, HR responses to NG and PE were absent but V induced a profound bradycardia and hypotension. Intravenous administration of procaine at the same rate given intrapericardially did not block the HR responses induced by PE and NG, nor did it prevent the hypotension or bradycardia induced by V. These results indicate that PC procaine specifically blocks cardiac afferent and efferent nerves, because equivalent amounts of procaine given intravenously did not prevent baroreflex-induced changes in HR or reflex hypotension in response to V. Furthermore, the lack of change in HR in response to increases and decreases in MAP in the SAD condition is evidence that the arterial baroreceptors were eliminated.

Time-control experiments. The hemodynamic and hormonal responses obtained during the time-control experiments are shown in Fig. 2. In the intact condition, there were no significant differences in any measured variable over the 100-min experiment. In the CD condition, both HR and MAP increased from control levels to 137 ± 7 beats/min and 122 ± 6 mmHg, respectively, 20 min after PC infusion of procaine began and remained at these new levels for the remainder of the experiment. There were no significant changes in either plasma AVP or PRA during acute CD, but plasma cortisol was significantly higher compared with levels in the intact condition from 45–65 min of infusion. In the chronic SAD condition, MAP was similar to the intact condition, albeit much more variable, but HR was elevated. Plasma AVP and plasma cortisol did not change during the experiment, but a small increase in PRA was observed at 45 min and PRA remained elevated for the remainder of the experiment. PC infusion of procaine in the SAD condition caused a greater increase in MAP compared with the CD condition, but the steady-state HRs were not different. There were no changes in either plasma AVP or PRA in the

![Fig. 1. Changes in mean arterial pressure (MAP, solid bars) and heart rate (HR, open bars) in response to intravenous boluses of phenylephrine (PE, 5 µg/kg) and nitroglycerine (NG, 15 µg/kg) and to right atrial administration of veratridine (V, 50 µg) are indicated for intact, cardiac denervated (CD), and sinoaortic denervated (SAD) treatment conditions and during intravenous infusion of procaine (Pro). Acute CD eliminated HR responses to PE and NG and both reflex hypotension and bradycardia in response to V, indicating blockade of both cardiac efferent and afferent nerves. SAD eliminated HR responses to PE and NG, indicating abolition of baroreceptor reflexes, but did not affect bradycardia and reflex hypotension in response to V, indicating normal cardiac neural pathways. Intravenous administration of procaine did not affect either baroreflex-mediated changes in HR or responses to V (n = 6) bpm, Beats/min.](http://ajpregu.physiology.org/doi/10.1210/jc.2017-00014/fig-1)
SAD + CD condition, but plasma cortisol was increased at 50 and 55 min compared with the intact condition. The responses to intravenous infusion of procaine are shown in Fig. 3. In the intact condition, procaine (intravenous) caused acute increases in both MAP and HR that subsequently declined to steady-state levels modestly but significantly higher than the control means. Plasma AVP clearly increased in some of the dogs, but the mean change at 40 min did not reach statistical significance. There was no change in PRA, but plasma cortisol was elevated at 40 min (P < 0.05) and drifted downward during the remainder of the infusion. A similar pattern of responses to intravenous administration of procaine was observed in the SAD condition. Both MAP and HR increased initially and then stabilized at lower levels during the maintenance phase of the infusion; plasma AVP and PRA were unchanged, and cortisol increased initially and then fell toward control. These results indicate that a component of the increase in MAP during PC infusion of procaine is probably due to uptake of the drug into the systemic circulation. The results also show that most if not all of the increase in plasma cortisol during PC infusion of procaine may be due to nonspecific effects of procaine entering the systemic circulation, because intravenous administration of procaine caused similar increases in cortisol. In contrast, administration of procaine by either the PC or intravenous route had no significant effect on plasma AVP and PRA.

Cardiovascular responses to hemorrhage. Changes in systolic, mean, and diastolic pressure and RAP, HR, and plasma AVP during the 30-ml/kg hemorrhage in the intact condition are shown in Fig. 4. A significant change in RAP was detected at 7.5 ml/kg and preceded changes in other measured variables. Statistically significant declines in systolic pressure and MAP occurred at hemorrhage volumes of 17.5 and 20 ml/kg, respectively, whereas significant increases in HR and plasma AVP occurred at 15 ml/kg of blood loss. The appearance of increases in HR and plasma AVP before significant decreases in either systolic pressure or MAP would appear to suggest lack of arterial baroreceptor participation. However, such a conclusion is erroneous because in each of the individual dogs, an obvious decline in systolic pressure always preceded any increase in plasma AVP and HR. To get a more accurate estimate of changes in arterial pressure, we defined a decline in systolic pressure (averaged into 1-min bins) as the first occurrence of a fall that was >5% below the animal’s individual control average and that continued to fall without recovery. Using these criteria, we found that systolic pressure fell after loss of 13 ± 2 ml/kg of blood (range = 6–18 ml/kg). With use of the same criteria for estimating a fall in MAP, a value of 20 ml/kg resulted

Fig. 2. Mean values (and representative SE) are shown for hemodynamic and hormonal variables during time-control experiments in the 4 treatment conditions (indicated at top). Control period, period of pericardial (PC) saline or procaine infusion, and sham hemorrhage (Hem) are indicated at top. Acute CD caused increases in MAP and HR. Plasma arginine vasopressin (AVP, in pg/ml) did not change during any treatments. Plasma renin activity (PRA) (in ng ANG 1·ml−1·h−1) increased significantly in SAD condition at 45 min (arrows) and remained elevated for duration of experiment. Plasma cortisol (µg/dl) was elevated between arrows (P < 0.05) in CD condition and also at 2 time points in SAD + CD condition (★) (n = 6).
(range = 9–30 ml/kg), the same as that obtained with the ANOVA approach. Because the decline in systolic pressure appears to mark the earliest evidence of arterial baroreceptor unloading, we chose to analyze

the hormonal responses as a function of systolic pressure rather than MAP.

The average MAP, HR, and RAP in each treatment condition during the 5 min preceding the hemorrhage are given in Table 1. The data in Fig. 5 show the changes from these control values during progressive hemorrhage. There was a significant effect of SAD on the ability to maintain MAP during hemorrhage but no effect of CD and no interaction between the SAD and CD conditions. In both the intact and CD conditions, a significant decline in MAP did not occur until blood loss reached 20 ml/kg. In contrast, MAP fell significantly after loss of 5 ml/kg of blood in both the SAD and SAD + CD conditions and the absolute decline in MAP was much greater compared with responses with arterial baroreceptors intact. In fact, in the SAD condition, the hemorrhage was stopped when MAP approached 40 mmHg. Thus in two dogs the hemorrhage was stopped at 25 ml/kg, and it was stopped at 27–27.5 ml/kg in the remaining four dogs.

A significant change in HR appeared only in the intact response to hemorrhage (Fig. 5). HR did not change in any of the other three conditions. The lack of
HR change in the SAD condition indicates that unloading cardiac receptors has no effect on mechanisms controlling HR during hemorrhage. The decline in RAP (Fig. 5) was not altered by either CD or SAD compared with the intact condition.

Arterial blood gases were measured in three of the six dogs in the intact and CD conditions (Table 2). Arterial hemoglobin was 97% saturated at the beginning of the hemorrhage in both treatments and did not change in response to hemorrhage.

![Graph](image)

**Fig. 5.** Hemorrhage-induced changes (Δ) in MAP, HR, and RAP from control levels immediately before hemorrhage are indicated for 4 treatment conditions (see Table 1 for control values immediately before hemorrhage). Arrows indicate first point differing significantly (P < 0.05) from control, and all points to right of arrow are significant. Representative SE are indicated (n = 6).

### Table 1. Control values preceding hemorrhage in 4 treatment conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>RAP (mmHg)</th>
<th>BW (kg)</th>
<th>Posm (mosmol/kg H2O)</th>
<th>Pprot (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>120 ± 2</td>
<td>97 ± 3</td>
<td>2.3 ± 0.7</td>
<td>22.4 ± 0.7</td>
<td>297 ± 2</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>CD</td>
<td>109 ± 7</td>
<td>122 ± 5*</td>
<td>1.8 ± 0.7</td>
<td>22.3 ± 0.9</td>
<td>296 ± 2</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>SAD</td>
<td>138 ± 5</td>
<td>132 ± 4*</td>
<td>2.3 ± 0.4</td>
<td>22.3 ± 0.9</td>
<td>295 ± 1</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>SAD + CD</td>
<td>138 ± 5</td>
<td>132 ± 4*</td>
<td>2.3 ± 0.4</td>
<td>22.3 ± 0.9</td>
<td>295 ± 1</td>
<td>6.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± 1 SE; n = 6. CD, cardiac denervation; SAD, sinoaortic denervation; MAP, mean arterial pressure (in mmHg); HR, heart rate (in beats/min); RAP, right atrial pressure (in mmHg); BW, body weight (in kg); Posm, plasma osmolality (in mosmol/kg H2O); Pprot, plasma protein concentration (in g/dl). *Significant differences (P < 0.05) compared with intact condition.

Hormonal responses to hemorrhage. The changes in plasma AVP in response to hemorrhage are plotted in the traditional method in Fig. 6A, i.e., as a function of blood volume removed. The ANOVA did not detect a significant treatment effect, but there was a significant effect of hemorrhage volume (P < 0.01) and a significant treatment × volume interaction (P < 0.01) (note that analysis did not include 30 ml/kg points because hemorrhage was stopped in dogs in SAD and SAD + CD conditions after loss of 25 or 27.5 ml/kg). Comparisons within treatments indicated that plasma AVP increased significantly after loss of 10 ml/kg in the SAD condition and after loss of 15 ml/kg in the intact, CD, and SAD + CD conditions. Significant treatment effects on plasma AVP were detected at the 25 ml/kg stage of hemorrhage. Plasma AVP in the SAD condition was greater than the response in either the CD or SAD + CD conditions but was not different from the intact response (P > 0.2). In contrast, there were no significant differences in plasma AVP among the intact, CD, and SAD + CD conditions at any level of hemorrhage. This analysis would appear to suggest that stimulation of AVP secretion during hemorrhage is enhanced by SAD.

In contrast, analyzing the relation between the log of the plasma AVP concentration versus the systolic pressure gives a different interpretation of the results (Fig. 6B; mean of individual slopes, intercepts, and r values obtained from data are shown at bottom of figure). Factorial analysis of the slopes indicated a significant main effect of SAD (P < 0.01) but no effect of CD and no significant SAD × CD interaction. These results indicate that most of the increase in plasma AVP during hemorrhage in the intact condition is due to unloading arterial baroreceptors. Cardiac receptors do not contribute significantly to the response because the slope is not affected by CD. It should be noted that removing the arterial baroreceptors does not prevent an increase in AVP secretion in response to hypovolemia, but the increase occurs at much lower levels of arterial pressure compared with the intact condition. The similarity

### Table 2. Blood oxygen saturation (%) before and during hemorrhage

<table>
<thead>
<tr>
<th>Hemorrhage Volume, ml/kg</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>98.0 ± 0.1</td>
<td>97.6 ± 0.2</td>
<td>98.0 ± 0.1</td>
<td>97.8 ± 0.2</td>
</tr>
<tr>
<td>CD</td>
<td>97.9 ± 0.4</td>
<td>97.4 ± 0.2</td>
<td>97.6 ± 0.2</td>
<td>97.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± 1 SE; n = 3.
of the slopes in the SAD and SAD + CD conditions suggests that the stimulus causing the increased secretion of AVP after removal of arterial baroreceptors is probably the same, and therefore not related to cardiac receptors. The changes in PRA as a function of hemorrhage volume are shown in Fig. 7A. The ANOVA detected a significant effect of treatment (P < 0.03) and hemorrhage volume (P < 0.01) and a significant treatment × volume interaction (P < 0.01). Subsequent analysis indicated a significant increase in PRA at 15 ml/kg in the SAD condition, whereas increases in PRA were not significant until 20 ml/kg of blood was removed in the intact, CD, and SAD + CD conditions. Furthermore, PRA in the SAD condition was greater than in all other treatment conditions at 15 ml/kg and exceeded the means in the intact and CD conditions at 20 and 25 ml/kg of hemorrhage. These results indicate that in the absence of arterial baroreceptors, renin secretion is stimulated earlier and to a greater extent in response to hemorrhage.

Fig. 6. A: means and SE for plasma AVP plotted as a function of hemorrhage volume in the 4 treatment conditions. First significant change within each treatment condition is indicated by arrows, and all points to right are different from control. At 25 ml/kg, plasma AVP in SAD condition was greater than levels in CD and SAD + CD conditions (*, P < 0.05). Note that means at 30 ml/kg are not included in analysis. B: same values for plasma AVP (converted to logs) and plotted against systolic pressure at each level of hemorrhage. Means of individual slopes (b), intercepts (I), and r values obtained by linear regression are indicated for each treatment condition at bottom. Factorial analysis of slopes indicated a significant effect of SAD (*P < 0.01), but no effect of CD and no SAD × CD interaction (n = 6).

Fig. 7. A: means (and SE) for PRA [ng angiotensin I (AI)·ml⁻¹·3 h⁻¹] plotted as a function of hemorrhage volume in each of the 4 treatment conditions. Arrows indicate first point within each treatment condition that differed (P < 0.05) from control. *, Differences (P < 0.05) among treatments at various levels of hemorrhage. B: same values for PRA plotted as a function of systolic pressure at each level of hemorrhage. Values for b, I, and r obtained by linear regression are indicated for each treatment condition at bottom. Factorial analysis of slopes indicated no effect of either SAD or CD on response and no SAD × CD interaction (n = 6).
to hemorrhage compared with the intact response. When the PRA responses are plotted as a function of systolic pressure, the responses appear similar and the slopes of the responses are not statistically different for any of the treatment conditions (Fig. 7B, mean slopes, intercepts, and r values are indicated at bottom of figure). Similar results were obtained when the PRA responses were plotted against MAP (data not shown). The fact that the PRA responses varied similarly with either systolic pressure or MAP, both of which are direct determinants of renal perfusion pressure, suggests that intrarenal mechanisms can account for the stimulation of renin secretion in each of the treatment conditions.

The changes in plasma cortisol plotted as a function of hemorrhage volume in each of the treatment conditions are shown in Fig. 8A. Significant increases were detected at 15 ml/kg in the CD, SAD, and SAD + CD conditions, but an increase in the intact condition did not occur until 20 ml/kg of blood loss. Furthermore, the response in the CD condition was greater than the intact response at 15 ml/kg of blood loss. The cortisol data were also plotted as a function of systolic pressure (Fig. 8B). Comparison of the slopes relating plasma cortisol to systolic pressure by factorial analysis indicated no effect of CD, a marginal effect of SAD (P = 0.059), and no CD × SAD interaction.

Recovery of MAP and hormonal responses after hemorrhage. The recovery of MAP and changes in plasma AVP, cortisol, and PRA during the 30 min after hemorrhage are shown in Fig. 9. MAP did not change significantly in either the intact or CD conditions, indicating that there was no measurable recovery from the levels reached at the end of hemorrhage. In contrast, significant increases in MAP were detected in both the SAD and SAD + CD conditions. However, MAP in the SAD condition remained significantly lower compared with the intact condition at all time points after hemorrhage.

A significant decline in plasma AVP was detected 30 min after the end of hemorrhage in the SAD condition (Fig. 9). The average value for plasma AVP fell by the same magnitude in the intact condition, but the change did not reach statistical significance (P = 0.06). There was no change in plasma AVP in either the CD or SAD + CD conditions during the 30-min recovery period. PRA did not change significantly after hemorrhage in any treatment condition. In contrast, plasma cortisol tended to rise during the recovery period but the increase reached statistical significance only in the CD condition.

DISCUSSION

The effects of SAD and acute CD on hemodynamic responses to hemorrhage in conscious dogs are similar to results obtained in an earlier study by Shen et al. (35). Acutely removing cardiac afferent and efferent neural connections had no effect on the ability to maintain MAP during progressive hemorrhage compared with the intact condition, but SAD caused marked impairment of mechanisms responsible for defending blood pressure (Fig. 5). It is remarkable that there was no hint of a decline in MAP in the intact state after loss of 15 ml/kg of blood (MAP was 99 ± 4% of control) but, after SAD, blood pressure declined significantly (7% below control) after loss of only 5 ml/kg and was 37% below control after removal of 15 ml/kg of blood. This comparison suggests that arterial baroreceptors must have detected some alteration in the arterial pressure wave and engaged reflex mechanisms to defend blood pressure very early in the hemorrhage, long before we were aware of a change in systolic, pulse, or mean...
arterial pressure. Equally important, this observation indicates that the concept of a “nonhypotensive” hemorrhage is untenable. Many previous studies have inferred a role for atrial or “low-pressure” receptors if a response to hypovolemia occurred in the absence of a change in MAP. However, the results obtained here indicate that in the SAD condition, a fall in MAP during hemorrhage occurred even earlier than a significant decline in RAP. Thus it would appear that even small reductions in blood volume cannot be assumed to selectively unload atrial receptors and not affect arterial baroreceptor firing.

We measured hemorrhage-induced increases in three hormones believed to be important in the maintenance of arterial pressure and blood volume restitution after hypovolemia. The control of AVP secretion will be discussed first. The prevailing view of volemic mechanisms controlling AVP secretion hypothesizes that in the normal euvoletic state, both atrial and arterial baroreceptors provide a tonically inhibitory signal to medullary neurons which, if released from inhibition, stimulate AVP secretion. In support of this concept is evidence that acute vagotomy or SAD in anesthetized dogs (33) and vagal cold block in conscious dogs (3) all lead to a large, transient increase in plasma AVP. In contrast, acutely blocking the cardiac nerves with PC procaine in either conscious rabbits (7, 25) or dogs (Refs. 22 and 23 and Fig. 2) has no effect on plasma AVP, and these observations are inconsistent with tonic inhibition of AVP secretion arising from atrial receptors.

Bishop et al. (3) have reported that vagal cold block in conscious SAD dogs results in much larger increases in plasma AVP compared with the increase in dogs with arterial baroreceptors intact. Therefore, it could be argued that because PC procaine causes a 15- to 20-mmHg increase in arterial pressure in dogs (see Fig. 2), the increased firing by arterial baroreceptors may have matched the reduction in inhibitory input from atrial receptors, leaving AVP secretion unchanged. However, if this were the case, acutely blocking the cardiac nerves in SAD dogs should cause a rise in plasma AVP, and no increase was observed (Fig. 2). Similarly, acute CD in SAD rabbits has no effect on plasma AVP (7, 25). Thus these results argue against the hypothesis that atrial receptors tonically inhibit AVP secretion in the euvoletic state. We have no ready explanation regarding the cause of the increase in plasma AVP in response to vagal cold block or vagotomy as observed in previous studies. One difference between PC procaine and vagal cold block is that the former eliminates afferent and efferent transmission in both parasympathetic and sympathetic nerves, whereas the latter blocks parasympathetic but not sympathetic efferent nerves. This fact, however, does not provide a plausible explanation for the differing effects on AVP secretion. Another difference is that vagal cold block of vagotomy eliminates the major afferent and efferent link between the thoracic and abdominal viscera and the medulla. Thus it is possible that the transient increase in plasma AVP measured immediately after vagal cold block or vagotomy is a nonspecific response to the acute interruption of impulse traffic in the nerve.

Another possible source of cardiac receptor input has been proposed by Wang et al. (38). They reported that ventricular denervation reduced the AVP response to hemorrhage to the same level as total CD in conscious dogs. To explain this effect, they hypothesized that ventricular mechanoreceptors with afferents in the vagus nerve were stimulated by hemorrhage and this
afferent signal led to direct stimulation of AVP secretion rather than removal of an inhibitory input. The experimental basis for this hypothesis was derived from an earlier study by Oberg and Thoren (20) that described the appearance of a reflex bradycardia in response to rapid hemorrhage in anesthetized cats. Oberg and Thoren (20) showed that the receptors mediating this response were located in the left ventricle, had afferents in the vagus, and were silent at rest. They proposed that the receptors were stimulated by distortion of ventricular muscle contracting around small ventricular volumes. Interestingly, they also noted that these same ventricular receptors were stimulated by large increases in ventricular pressure, as during aortic occlusion (20).

If stimulation of ventricular receptors is the cause of the massive release of AVP during hemorrhage, then CD in either intact or SAD conditions should result in markedly lower plasma AVP levels during hemorrhage. As noted above, Wang and colleagues (38) reported that surgical CD does reduce the AVP response to hemorrhage in conscious dogs. In contrast, a study by Shen et al. (34) also using conscious dogs as a model reported that neither chronic surgical CD nor acute CD had a significant effect on the AVP response to hemorrhage compared with intact dogs. Similarly, we observed no effect of CD on the AVP response to hemorrhage in conscious dogs (22). It is worth pointing out that MAP did not fall significantly in the ventricularly denervated dogs until the end of the 30-ml/kg hemorrhage (38). Thus it is possible that the smaller increase in plasma AVP observed by Wang et al. (38) in dogs with denervated ventricles is related to better maintenance of MAP rather than to removal of ventricular receptors.

Although Shen et al. (34) reported no effect of CD alone compared with intact dogs, they did observe that plasma AVP was significantly lower in dogs with combined SAD + CD after removal of 25 ml/kg of blood (94 ± 15 pg/ml) compared with the response in the SAD condition (214 ± 35 pg/ml). Thus Shen et al. (34) concluded that cardiac receptors play a modest role in the stimulation of AVP secretion but suggested that the response was apparent only after complicating influences of sinoaortic reflexes were eliminated. They did not propose an explanation as to how cardiac receptors could account for about one-half of the AVP response to hemorrhage in the SAD condition and apparently none in the intact condition.

In the present study, we also observed that at 25 ml/kg of blood loss, plasma AVP was significantly greater in the SAD condition compared with either CD or SAD + CD conditions (Fig. 6A). However, there were no significant differences in the AVP responses among the intact, CD, and SAD + CD conditions. Taken at face value, analysis of the present data as a function of blood volume removed would appear to provide another set of conflicting results that further confuse the role of cardiac and arterial baroreceptors in the control of AVP secretion. The confound seems to arise from the usual method of plotting plasma AVP against hemorrhage volume, a procedure which contains an implicit assumption that there are "volume-sensitive" receptors which influence mechanisms controlling AVP secretion. Furthermore, these volume-sensitive receptors have historically been equated with atrial receptors. Thus experimental data showing that CD reduces the AVP response to hemorrhage have been used to argue that cardiac receptors play a dominant role in the stimulation of AVP secretion during hemorrhage. In contrast, the apparent lack of effect of SAD on plasma AVP during hemorrhage (Ref. 13 and Fig. 6A) could be used to argue against participation of arterial baroreceptors in the stimulation of AVP secretion.

We are proposing to analyze the results in relation to systemic arterial pressure. This approach is based on the simple hypothesis that in the euvolemic state, the only tonically active signal inhibiting AVP secretion arises from the arterial baroreceptors. On the basis of this hypothesis, we predict that during graded progressive hemorrhage, there should be a good relationship between baroreceptor firing and plasma AVP. We chose systolic pressure as a good index of the load on the baroreceptors and found an exponential relationship between this variable and plasma AVP (Fig. 6B). If the hypothesis is correct, one would predict that blocking cardiac receptor input should have no effect on the relationship between systolic pressure and plasma AVP, and the experimental results support this prediction (Fig. 6B). However, removal of the arterial baroreceptors should lead to a marked alteration in the relationship between systolic pressure and plasma AVP, and SAD combined with acute CD should have no additional effect on this relationship. The experimental results also support this prediction. This hypothesis provides a coherent explanation for the lack of effect of CD on the AVP response to hemorrhage in our earlier study (22) and in the study by Shen et al. (34).

Our results differ from Shen et al. (34) only in regard to the importance of a cardiac contribution to AVP secretion during hemorrhage in SAD dogs. The principal methodological differences between the two studies are that Shen et al. used splenectomized dogs, a rate of hemorrhage that was 50% slower than ours (0.5 ml·kg⁻¹·min⁻¹ vs. 1 ml·kg⁻¹·min⁻¹), and lidocaine instead of procaine to anesthetize the cardiac nerves. Because splenic contraction can provide an infusion of red blood cells in response to sympathetic discharge, splenectomy removes one mechanism that normally participates in the defense of blood pressure. Thus they reported an average decline in MAP of 48 ± 2 mmHg after removing 30 ml/kg of blood in splenectomized, baroreceptor-intact dogs compared with a decline of 34 ± 5 mmHg in our intact dogs. The difference might have been larger, but the slower rate of hemorrhage allows more time for transcapillary movement of fluid into the vascular space, which means that the actual loss of circulating volume is less compared with a faster rate of hemorrhage. Nevertheless, these methodological differences are unlikely to account for the differences in experimental results.

In fact, our experimental results are similar if we use an analysis based on hemorrhage volume; the AVP
response in the SAD + CD condition was less than that in the SAD condition at 25 ml/kg of blood loss (see Fig. 6A). However, comparing the plasma AVP at the same systolic (or mean) arterial pressure at each level of hemorrhage indicated no difference between the SAD and the SAD + CD conditions (Fig. 6B). As argued in the preceding paragraphs, if cardiac receptors contribute a measurable component of the increase in plasma AVP in response to hemorrhage, then it should be apparent in the presence, as well as in the absence, of the arterial baroreceptors. If it is not demonstrable with arterial baroreflexes intact, then there is no basis to conclude that cardiac receptors contribute to the normal physiological increase in plasma AVP in response to hemorrhage. Thus we conclude that the normal pattern of AVP secretion in intact dogs in response to a 30 ml/kg-hemorrhage can be accounted for by the unloading of arterial baroreceptors.

We are not suggesting that atrial receptors play no role in the control of AVP secretion. Rather, we are proposing that in the euvolemic state, the activity of these receptors is insufficient to generate an inhibitory signal to the medullary neurons which participate in the control of AVP secretion; presumably the peripheral signal dies out at some medullary processing site. In contrast, increasing the load on atrial receptors does cause inhibition of AVP secretion (15). For example, we have shown that even large decreases in arterial pressure do not cause increases in plasma AVP if accompanied by a simultaneous increase in LAP (1). This suggests that when medullary processing centers receive conflicting signals indicating decreased load on arterial baroreceptors and increased load on atrial receptors, the dominant factor controlling AVP secretion is inhibitory input from cardiac receptors. Thus, starting from the euvolemic state, increasing blood volume can inhibit AVP secretion primarily via stimulating cardiac receptors, whereas decreasing blood volume stimulates AVP secretion via unloading the arterial baroreceptors.

The results obtained here and by others (34) show that there is still a substantial increase in plasma AVP in response to hemorrhage in dogs with combined SAD and CD. Furthermore, Schreihofer et al. (28) have reported that lesions of the nucleus of the solitary tract, which receives all known afferent input from peripheral baroreceptors, does not prevent the AVP response to hemorrhage in conscious rats. Thus there must be some additional mechanism which can cause the stimulation of AVP secretion during hemorrhage in the absence of cardiovascular reflexes. Because arterial baroreceptor denervation also removes peripheral chemoreceptors, the source of the signal must lie elsewhere. At present, one can only speculate as to the mechanism(s) which stimulate AVP secretion in the absence of cardiac and arterial baroreceptors. Whatever the case, it should be noted that the increases we measured in plasma AVP in dogs without arterial baroreceptors occurred at systolic (and mean) arterial pressures far below those observed in the intact condition, and thus the mechanism is largely irrelevant in the physiological control of AVP secretion in this species.

Multiple intrarenal and reflex mechanisms could participate in the stimulation of renin secretion during progressive hemorrhage (8). It is well known that stimulation of left atrial receptors leads to a reflex reduction in renal nerve activity and inhibition of renin secretion (15) and that administration of propranolol causes a fall in PRA (27). Therefore, it is plausible that atrial receptor firing leads to tonic inhibition of renal nerve activity in the euvolemic state and that unloading these receptors during hemorrhage could cause stimulation of renin secretion before a decline in renal perfusion pressure occurs. In the present experiments, we could not demonstrate an increase in PRA after acute CD (Fig. 2). However, in a previous study, we observed an increase in PRA during acute CD if renal perfusion pressure was maintained at control levels (21). Thus the lack of an increase in renin in response to acute CD is probably due to the simultaneous increase in arterial pressure and, thus, inhibition of intrarenal mechanisms controlling renin secretion.

If afferent signals from atrial receptors are important in the reflex stimulation of renin secretion in response to hemorrhage, it is reasonable to predict some attenuation of the increase in PRA in the CD condition. When the results were analyzed as a function of hemorrhage volume (Fig. 7A), the only significant treatment effect was an earlier and greater increase in PRA in the SAD condition compared with either the intact or CD condition. This analysis would appear to suggest that intrarenal mechanisms are more sensitive to hemorrhage in the absence of arterial baroreceptor input and that they do not depend on cardiac receptor input.

In contrast, a different interpretation emerges when the results are analyzed as a function of systolic (Fig. 7B) or mean arterial pressure (data not shown). Comparison of the slopes of the relationships between PRA and systolic pressure in each of the treatment conditions indicated no significant differences. Thus, for any given systolic pressure, the PRA was similar in each condition. Because removing baroreceptor or cardiac receptor influences on renal nerve activity had no effect on the renin response to hemorrhage as performed in this study, it is possible to conclude that intrarenal mechanisms, presumably the renal vascular receptor, could account for the renin response in each of the treatment conditions.

We are not suggesting that reflex mechanisms are unimportant in the control of renin secretion. For example, we previously demonstrated that gradual inflation of a cuff placed around the thoracic vena cava leads to a rise in PRA after 30 min in the absence of a significant change in MAP and thus renal perfusion pressure (24). Presumably, the stimulation of renin secretion arose from unloading atrial receptors in this condition, although we cannot rule out participation of arterial baroreceptors as discussed earlier. The apparent lack of reflex involvement in the present study may be explained by the fact that at a rate of blood loss of 1 ml·kg⁻¹·min⁻¹, the time difference between a signifi-
cant decline in RAP (7.5 ml/kg) and a decrease in MAP (20 ml/kg) is only 12.5 min. This time difference between activation of a reflex stimulus and a reduction in renal perfusion pressure (stimulating intrarenal mechanisms) may have been too narrow to detect a reflexly mediated increase in PRA, given a sampling frequency at 5-min intervals. Thus the importance of reflex stimulation of renin secretion may only be evident during gradual development of hypovolemia, whereas in the present study, the engagement of intrarenal mechanisms by the reduction in renal perfusion pressure masked any contribution arising from reflex mechanisms.

Stimulation of ACTH secretion has been reported to occur in response to unloading of atrial and arterial baroreceptors (11) and increases in plasma ANG II (26) and AVP (40). Because ACTH is the principle regulator of cortisol synthesis and secretion, hemorrhage-induced increases in plasma cortisol must reflect changes in the secretion of ACTH. In the time-control experiments (Fig. 2), we observed that acute CD caused a small but significant rise in plasma cortisol in dogs with intact arterial baroreceptors and also after SAD. Thus these data are compatible with the concept that atrial receptors tonically inhibit ACTH secretion (11). However, intravenous administration of procaine in the present study caused similar increases in plasma cortisol (Fig. 3), suggesting that the stimulus to ACTH secretion was a nonspecific response to procaine. In two previous studies from this laboratory (22, 23), we did not observe a rise in cortisol during PC infusion of procaine, and we have no explanation as to why the dogs in the current study did show a response. Given the inconsistent responses, we conclude that using procaine to induce acute CD is not a fruitful method for examining the role of cardiac receptors in the control of ACTH secretion.

In contrast, chronic SAD did not appear to have any effect on basal levels of cortisol. There was a marginally significant effect of SAD (P = 0.059) on the relationship between plasma cortisol and systolic pressure (Fig. 8B). Furthermore, in three of the animals, SAD reduced the slope of the relationship by >50%. However, in the other animals, there was no clear effect of SAD on the response. It may be that the larger increases in PRA, and thus plasma ANG II, in the SAD condition provided a compensatory stimulus to ACTH secretion. Nevertheless, the results clearly indicate that reflex responses arising from arterial and cardiac receptors did not play an essential role in the cortisol response to hemorrhage as performed in this study.

Given the high plasma concentrations of plasma AVP and PRA at the end of hemorrhage, one might expect some recovery in MAP in dogs with intact reflexes. To our surprise, only the SAD condition was associated with a significant increase in MAP during the 30 min after the hemorrhage and this increase was accompanied by a significant decrease in plasma AVP (from 199 ± 94 to 101 ± 45 pg/ml). Plasma AVP also tended to fall in the intact condition, but the decline did not reach statistical significance (P = 0.06). Because AVP has been shown to contribute to the maintenance of MAP during hemorrhage in dogs (29, 34) and has many effects on the cardiovascular system in many species (see Ref. 32 for review), it is puzzling that MAP increased in the SAD condition at the same time plasma AVP fell 50%. The study by Shen et al. (34) also reported an anomalous relationship between AVP and maintenance of MAP in SAD dogs. They noted that blockade of V1 receptors during hemorrhage in baroreceptor-intact or CD dogs led to a greater fall in MAP, as would be predicted if AVP is acting as a vasopressor hormone. In contrast, V1 receptor blockade had no effect on MAP during hemorrhage in SAD dogs, although plasma levels of AVP were equal to or higher than levels reported in the intact dogs. These unexplained observations suggest the possibility that the role of AVP in blood pressure maintenance may be linked to interactions between the hormone and baroreceptor reflexes rather than exclusively dependent on vascular smooth muscle during hemorrhage.

These results are significant because they emphasize the crucial importance of arterial baroreceptor reflexes in homeostatic responses to hypovolemia. With the reflexes intact, losing 15 ml/kg of blood, or ~17% of blood volume, had no effect on the ability to maintain MAP at normal levels. There was no discernible contribution of either AVP or the renin-angiotensin system to the defense of MAP, because plasma levels of both hormones were still at control values. Thus the remarkable ability to maintain constant blood pressure during significant loss of blood is surely due to activation of arterial baroreceptor reflexes. In the absence of the arterial baroreceptors (SAD condition), MAP began falling in response to loss of only 5 ml/kg, or ~6% of blood volume, and the dogs were markedly hypotensive (67 ± 5 mmHg) at 15 ml/kg of blood loss, although PRA and plasma AVP were dramatically elevated at this level of hemorrhage.

Because AVP is a powerful vasoconstrictor, the increase in plasma AVP in response to hemorrhage is generally considered an important component in the defense of blood pressure in dogs (32). However, comparison of patterns of AVP secretion in response to progressive hypovolemia before and after removal of arterial baroreceptors suggests that in the intact state, the reflex functions to prevent secretion of AVP as long as MAP is maintained within normal limits. That is, increases in plasma AVP associated with decreases in systolic pressure but not MAP are far below the pressor range (Fig. 4), but as the decline in MAP accelerates after loss of 20 ml/kg of blood, massive release of AVP is initiated. This analysis suggests that AVP plays only a limited role in the defense of blood pressure during continuous hemorrhage and only after reflex mechanisms have failed.

Considerations on intrapericardial infusion of procaine. A paper by Evans et al. (10) noted a number of potential problems associated with the use of intrapericardial procaine in rabbits, namely phrenic nerve blockade and subsequent hypoxia; and absorption of procaine into the general circulation. Furthermore,
they noted that intravenous infusion of procaine blocked the HR and MAP responses to right atrial injection of phenylbiguanide, a cardiac C fiber stimulant. We have no doubt that some of the procaine infused intrapericardially in dogs is absorbed into the general circulation. However, we are sure that at the dose used here, PC procaine does not affect respiration. We have never noticed any change in respiratory pattern or rate in conscious dogs during PC procaine and could not detect any change in arterial oxygen saturation (Table 2). Furthermore, even if the phrenic nerves are blocked in dogs during PC procaine, it will not affect normal respiration in this species (9). Intravenous infusion of procaine at the same dose infused intrapericardially does not prevent baroreceptor-mediated changes in HR in response to PE or NG, and it does not prevent the Bezold-Jarisch reflex (Fig. 1). Clearly, some of the increase in MAP during PC infusion of procaine can be accounted for by systemic absorption of the drug, because pressure increases during intravenous infusion of the drug. More importantly, any spillover that does occur has no effect on basal plasma AVP or PRA, although there is a small but significant increase in plasma cortisol. Therefore, the confounding effects of PC procaine that Evans et al. (10) noted in rabbits do not occur in dogs. Nevertheless, there are problems associated with PC procaine in dogs. A major problem is that MAP is elevated ~20 mmHg (Fig. 2), and the increase is maintained during infusions lasting up to 6 h (unpublished observations). The elevated MAP means that responses to hemorrhage or other maneuvers affecting blood pressure or volume occur at a different baseline compared with responses in the intact state. Another problem is that ~10% of the dogs infused pericardially with procaine (and lidocaine) become nauseous 40 to 80 min after beginning the infusion (unpublished observations). Because nausea is known to be a powerful stimulant to AVP secretion, experiments in which nausea occurs must be discarded. There is no obvious explanation for the effect of these drugs on the emetic response, and they do not cause other behavioral disturbances.

Perspectives

The results obtained in this study indicate that unloading arterial baroreceptors can account for the exponential increase in plasma AVP during progressive hemorrhage. The implication of this conclusion is that the mechanism involved in the control of AVP secretion in response to graded hypovolemia in dogs is the same mechanism controlling AVP secretion in human and nonhuman primate responses to hypovolemia. There is no clear and convincing evidence that atrial receptors reflexly lead to increases in plasma AVP in any of these species. In contrast, there is good evidence that stimulating atrial receptors can inhibit AVP secretion in dogs and also human subjects. Therefore, there is no compelling reason to consider volume control of AVP secretion as fundamentally different among canine, primate, and human species.

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