Relative Roles of Cardiac and Arterial Baroreceptors in Vasopressin Regulation During Hemorrhage in Conscious Dogs


To determine the relative roles of cardiac and sinoaortic baroreceptors in mediation of arginine vasopressin (AVP) release, hemorrhage was performed in five groups of conscious splenectomized dogs: 1) all nerves intact; 2) either chronic surgical or acute pharmacological (intrapericardial lidocaine) cardiac denervation (CD); 3) chronic sinoaortic denervation (SAD); 4) combined chronic sinoaortic denervation plus either chronic or acute cardiac denervation (SAD+CD); and 5) all nerves intact, but with ganglionic blockade. Hemorrhage (0.5 ml/min/kg) reduced mean arterial pressure similarly in the intact and CD groups. Decreases in mean arterial pressure were augmented in SAD, SAD+CD, and ganglion-blocked groups compared with responses in intact and CD groups. There were no differences in responses of plasma AVP to hemorrhage in the intact and CD groups, but the AVP response was significantly blunted in the SAD+CD group as compared with SAD alone. When compared during the early stage of hemorrhage, at the same reduction in mean arterial pressure, the rise in AVP was greater in the ganglion-blocked group than in the SAD+CD group, but was less than in the intact group. With a protocol to reduce mean arterial pressure by 20 mm Hg over the same period (42±0.6 minutes) in four of the groups, the blood volume required to reduce mean arterial pressure by 20 mm Hg was similar in the intact (20±1 ml/kg) and CD (21±1 ml/kg) groups, but was less in the SAD (12±1 ml/kg) and SAD+CD (12±1 ml/kg) groups. Again, similar increases were observed in AVP between the intact (50±9 pg/ml) and CD (51±9 pg/ml) groups, whereas increases in AVP were diminished in the SAD (11±3 pg/ml) and SAD+CD (7±2 pg/ml) groups. In the presence of an AVP antagonist, decreases in mean arterial pressure and increases in total peripheral resistance with hemorrhage were affected similarly in both the intact and CD groups, whereas hemodynamic impairment by AVP blockade was less marked in the SAD and SAD+CD groups. These results indicate that cardiac receptors are not the major regulators of AVP release during progressive hemorrhage in conscious dogs. However, when the complicating influences of sinoaortic reflexes were eliminated, a modest role for cardiac receptors was uncovered. (Circulation Research 1991;68:1422-1436)

One of the major compensatory adjustments to hemorrhage involves the release of plasma arginine vasopressin (AVP), stimulated by the hypotension and contracted blood volume. This hormone, by conserving fluid and constricting peripheral blood vessels, helps defend arterial pressure against the consequences of blood loss. While both sinoaortic and cardiac reflexes may be important in regulation of AVP release, several recent studies suggest a predominant role for cardiac receptors, particularly those with vagal afferents.1-4 In contrast to the results of these studies, recent circumstantial evidence obtained from our experiments in conscious dogs suggested that cardiac receptors are not the major regulators of AVP release during hemorrhage.5 In that study decreases in arterial pressure and increases in total peripheral resistance in response to graded hemorrhage were remarkably similar in dogs with intact nerves and dogs with total cardiac denervation. If elimination of cardiac nerves reduced AVP release substantially, then arterial hypotension should have been more severe and in-

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creases in total peripheral resistance less in the dogs with cardiac denervation, since AVP is one of the most potent vasoconstrictors.6–8 It has also been shown that hypophysectomized dogs, unable to release AVP, exhibit a far greater fall of arterial pressure than do normal dogs in response to equivalent levels of hemorrhage.9

The present investigation was designed to address directly the role of AVP regulation in hemorrhage. This was accomplished by examining responses to graded hemorrhage in five groups of conscious dogs: 1) all nerves intact; 2) total cardiac denervation leaving sinoaortic baroreceptors intact; 3) sinoaortic denervation leaving cardiac receptors intact; 4) combined sinoaortic and cardiac denervation; and 5) all nerves intact, but with ganglionic blockade. In addition, two other subgroups of dogs were studied: 1) those with intact nerves but an intrapericardial catheter to infuse lidocaine and induce acute cardiac denervation, and 2) those with chronic sinoaortic denervation and the intrapericardial catheter to induce acute cardiac denervation. These latter groups were studied to address the question of whether under chronic conditions other adjustments occurred that obscured the acute loss of control by cardiac reflexes. In the current experiments AVP levels were assessed in two types of protocols. In one, AVP was determined at each 5 ml/kg of blood loss, while hemodynamics were measured continuously. In the other, AVP was determined when mean arterial pressure (rather than volume) was reduced by a set amount over the same time period. Finally, to determine if AVP release during hemorrhage was of physiological significance in any specific group, the effects of an AVP antagonist on hemodynamic responses to hemorrhage were also examined.

Materials and Methods

Seventy-three mongrel dogs of either sex, weighing 16–26 kg, were studied. All dogs were sedated with xylazine (10 mg/kg i.m.). General anesthesia was induced with thiamylal (10–20 mg/kg i.v.) and maintained with halothane (0.5–1.5 vol%) or sodium pentobarbital (30 mg/kg i.v.). Sterile technique was used to make an incision through the left fourth intercostal space, and Tygon catheters (Norton Plastics, Akron, Ohio) were implanted in the descending thoracic aorta and right atrium for measurements of aortic and right atrial pressures. An electromagnetic flow probe (Zepeida Instruments, Seattle) or Transonic flow probe (Transonic Systems, Inc., Ithaca, N.Y.) was implanted around the ascending aorta. All wires and catheters were tunneled subcutaneously to the interscapular region. The chest was closed and negative pressure was restored; the incision was closed in layers. To eliminate the variability in the changes of blood volume as a result of contraction of the spleen during hemorrhage, all dogs were splenectomy through a midline abdominal incision 1–3 weeks after the chest surgery.

The control group consisted of 17 dogs with all nerves intact. Total chronic cardiac denervation was accomplished in 14 dogs (CD group) by using the intrapericardial denervation technique as previously described.10 This procedure was performed at the time of instrumentation by stripping the adventitia and nerve fibers from the main pulmonary artery, left superior pulmonary vein, and right pulmonary artery; sectioning the left ventrolateral cardiac nerve and the pericardial reflections in the transverse sinus and around the superior vena cava; and ligating the azygos vein. Further dissection around the pericardial reflection at the bifurcation of the right pulmonary artery completed this procedure. Completeness of the denervation was tested at surgery by confirming that the electrocardiographic responses to bilateral stellate ganglion stimulation (10 Hz, 5 msec, 3–5 V) or bilateral vagal stimulation (20 Hz, 5 msec, 3–5 V) had been eliminated. Completeness of the denervation was again tested after the dogs were killed with an overdose of sodium pentobarbital (>50 mg/kg) by measuring tissue catecholamine content in the left ventricle. Tissue catecholamine levels in the CD group were essentially depleted (10±4 pg/g) as compared with the intact group (426±60 pg/g).

In 11 dogs sinoaortic baroreceptor denervation was performed at the time of instrumentation (SAD group). In this procedure the aorta was stripped of all nerve fibers and connective tissue from the aortic root to the second intercostal artery. The brachiocephalic and the subclavian arteries were also stripped from the aorta cranially to the second set of branches. Phenol (2%) was carefully applied to the stripped surfaces with a small cotton-tipped applicator. Carotid sinus denervation was performed through a midline incision in the ventral neck region; the right and left common carotid arteries were isolated and stripped of nerve fibers and connective tissue 2–3 cm past the bifurcation of the internal and external carotid arteries. Phenol (2%) was sparingly applied to the stripped surfaces of the vessel. In 11 dogs both the sinoaortic and cardiac denervation procedures were combined (SAD+CD group). In five of 17 intact dogs and five additional dogs with sinoaortic denervation, a catheter was implanted in the pericardial space to induce acute reversible cardiac denervation.11,12 In a subset of five additional intact dogs, the effects of hemorrhage were examined in the presence of ganglionic blockade with hexamethonium bromide (30 mg/kg i.v.) and methyl atropine bromide (0.1 mg/kg i.v.). Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (DHHS publication No. [NIH] 85-23, revised 1985).

The experiments were conducted 2–3 weeks after surgery when the animals were healthy, exhibited normal hematocrit and body temperature, and were trained to lie in the right lateral position. The efficacy of chronic arterial baroreceptor denervation was...
confirmed by the elimination of the reflex heart rate changes in response to alterations in blood pressure by injections of phenylephrine (5 \( \mu \)g/kg i.v.) and nitroglycerin (10 \( \mu \)g/kg i.v.), whereas the response to left atrial injection of veratrine alkaloid (5 \( \mu \)g/kg) remained intact. To verify chronic cardiac denervation, reflex responses to veratrine alkaloid as well as to phenylephrine and nitroglycerin were shown to be abolished (Table 1). In the intact dogs and chronic arterial baroreceptor denervated dogs with implanted intrapericardial catheters, experiments were conducted in the presence and absence of acute cardiac denervation induced by an injection of lidocaine (5 mg/kg) into the pericardial space every 20 minutes throughout the hemorrhage. The average time from injection of lidocaine to starting hemorrhage was approximately 30 minutes. Experiments were randomized between the acute CD and control groups in which saline was injected into the intrapericardial catheter. Additional control experiments with intrapericardial lidocaine, but without subsequent hemorrhage, demonstrated no effects on arterial pressure, although heart rate rose. The efficacy of denervation in these experiments was also tested by injection of phenylephrine (5 \( \mu \)g/kg i.v.), nitroglycerin (10 \( \mu \)g/kg i.v.), and veratrine alkaloid (8 \( \mu \)g/kg i.v.). Reflex responses to phenylephrine, nitroglycerin, and veratrine alkaloid were abolished in the presence of acute cardiac denervation. In the dogs with chronic arterial baroreceptor denervation, before injection of lidocaine to induce acute cardiac denervation, the response to veratrine alkaloid remained intact (Table 1). The efficacy of ganglionic blockade was confirmed at the end of the experiment by the absence of reflex heart rate responses to phenylephrine (5 \( \mu \)g/kg i.v.) and nitroglycerin (10 \( \mu \)g/kg i.v.). In addition, the efficacy of chronic or acute denervation or ganglionic blockade was again confirmed by the observation of lack of increasing heart rate in response to hemorrhage as compared with responses observed in intact dogs (Table 2).

Hemorrhage was performed in all dogs by withdrawing venous blood through a catheter inserted in the inferior vena cava and using a calibrated syringe pump (Harvard Apparatus, South Natick, Mass.). After the dog was given sodium heparin (500 units/kg i.v.), blood was removed at a constant rate (0.5 ml/kg/min) until a total blood loss of 30 ml/kg was achieved. If mean arterial pressure fell below 40 mm Hg, the hemorrhage was discontinued. The shed blood was collected in sterile bags and reinfused after cessation of hemorrhage. To compare the AVP responses to equivalent reductions in mean arterial pressure, hemorrhage was also induced by withdrawing venous blood until a reduction in mean arterial pressure of 20 mm Hg was achieved. It is important to note that in this protocol, blood was removed at a rate by which the same period of time elapsed for denervated dogs and intact dogs. To accomplish this and mimic the time course of hemorrhage in intact dogs, it was necessary to reduce the hemorrhage rate in SAD or SAD+CD dogs, but not in CD dogs. In this protocol, the average duration over which hemorrhage occurred was 42±0.6 minutes.

<table>
<thead>
<tr>
<th>Mean arterial pressure (%)</th>
<th>Phenylephrine</th>
<th>Nitroglycerin</th>
<th>Veratrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (n=12)</td>
<td>+28±3.9</td>
<td>−24±2.3</td>
<td>−48±3.0</td>
</tr>
<tr>
<td>Chronic CD (n=14)</td>
<td>+55±3.3</td>
<td>−44±2.9</td>
<td>−4±2.2</td>
</tr>
<tr>
<td>SAD (n=11)</td>
<td>+56±6.7</td>
<td>−46±3.8</td>
<td>−48±3.5</td>
</tr>
<tr>
<td>SAD+chronic CD (n=11)</td>
<td>+47±4.4</td>
<td>−45±3.2</td>
<td>−3±1.2</td>
</tr>
<tr>
<td>Heart rate (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (n=12)</td>
<td>−37±4.5</td>
<td>+58±4.8</td>
<td>−38±3.3</td>
</tr>
<tr>
<td>Chronic CD (n=14)</td>
<td>+3±1.1</td>
<td>+1±1.0</td>
<td>−1±0.5</td>
</tr>
<tr>
<td>SAD (n=11)</td>
<td>0±1.5</td>
<td>+1±0.6</td>
<td>−51±3.8</td>
</tr>
<tr>
<td>SAD+chronic CD (n=11)</td>
<td>+2±1.5</td>
<td>+1±0.4</td>
<td>−1±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CD, cardiac denervation; SAD, sinoaortic denervation.
The same hemorrhage protocol was performed in subgroups of the dogs in the absence and presence of AVP antagonist on separate days. Studies were carried out in seven conscious dogs with intact nerves, five CD dogs, six SAD dogs, and two SAD+CD dogs. The experiments with and without the AVP antagonist were conducted 3–5 days apart, and the order of this protocol was randomized. The AVP antagonist, [β-Mercapto-β,β-cyclopentamethylenepropionyl]1-O-Me-Tyr2,Arg决心AVP, was injected (10 μg/kg i.v.) before hemorrhage, which blocks the response to AVP. The efficacy of AVP blockade was confirmed at the end of hemorrhage by injection of AVP. AVP (20–25 ng/kg i.v.) increased mean arterial pressure by 50±4% in the absence of blockade but failed to increase arterial pressure after AVP blockade. In a final series of experiments, AVP (1 ng/kg/min) was infused intravenously for 60 minutes in six dogs with intact nerves and two CD dogs.

Hemodynamic measurements were continuously recorded on a multichannel tape recorder (Honeywell Inc., Denver) and played back on a direct writing oscillograph (Gould-Brush, Cleveland, Ohio). Fluid-filled catheters in the aorta and right atrium were connected to strain gauge manometers (Statham Instruments, Oxnard, Calif.) to measure arterial and atrial pressures. A square-wave electromagnetic flowmeter (Benton Instruments, Cupertino, Calif.) or a Transonic flowmeter was used to measure cardiac output. Mean aortic pressure was assessed using RC filters with 2.5-second time constants. A cardiogalvanometer, triggered by the pressure pulse, provided instantaneous and continuous records of heart rate.

Total peripheral resistance was calculated as mean aortic pressure divided by cardiac output. Blood volume was measured on a separate day in a subset of the dogs by using the Evans blue technique. In brief, blood samples were taken before and after injection of a known amount of dye. The total plasma volume was determined based on a standard curve by using spectrogrographic techniques. Plasma osmolality was measured with an osmometer (Precision System Inc., Natick, Mass.)

Arterial blood samples for determination of plasma levels of AVP were taken before hemorrhage (baseline) and at each 5 ml/kg level of blood loss or at the end of protocol that involved hemorrhage over the same time period to reduce mean arterial pressure by 20 mm Hg. In the AVP infusion protocol, arterial blood samples were taken before infusion and at the end of infusion. The blood samples were collected in EDTA tubes. The arterial blood samples for determination of plasma osmolality were also taken before and at the end of hemorrhage. All blood samples were kept in an ice bath until centrifuged at 4°C for plasma separation after each experiment. The plasma was stored at −70°C for analysis. Plasma osmolality was measured without freezing the samples. Plasma AVP was determined by a specific and sensitive radioimmunoassay procedure developed by Cowley et al. Briefly, the assay consisted of lyophilizing a reduced volume of the sample for later reconstitution with AVP antisem. 125I-AVP was added to the antisera.

After an incubation period of 48 hours the precipitant was counted. Simultaneous determination of saline standard curves was used as an internal control for the
TABLE 3. Blood Volume in Conscious Dogs With All Nerves Intact, Cardiac Denervation, Sinoaortic Denervation, and Sinoaortic Denervation Plus Cardiac Denervation

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Blood volume (ml/kg)</th>
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</thead>
<tbody>
<tr>
<td>Intact (n=9)</td>
<td>21.3±0.7</td>
</tr>
<tr>
<td>CD (n=6)</td>
<td>21.6±0.7</td>
</tr>
<tr>
<td>SAD (n=4)</td>
<td>20.5±1.0</td>
</tr>
<tr>
<td>SAD+CD (n=3)</td>
<td>19.6±1.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CD, cardiac denervation; SAD, sinoaortic denervation.

assay. The percent of "nonspecific plasma binding" to 125I-AVP was subtracted from the percent labeled AVP bound to the antibody of plasma unknowns and used for final determination of AVP concentration.

All data were stored on an IBM-AC computer. The hemodynamic comparison among groups was tested by one-way analysis of variance with the least significant difference (LSD) test.14 When nonhomogeneity of variance was noted, the data for AVP were transformed logarithmically before statistical analysis. The comparison of these data among the groups was performed by Student's group t test with one tail of probability, since the only question was whether denervation reduced the AVP responses. The regression lines for the response of AVP and either blood loss or reduction in mean arterial pressure were compared using BMDP1R program (BMDP Statistical Software, Inc., Los Angeles). Statistical significance was accepted at a level of p<0.05. All values are expressed as mean±SEM.

Results

Baseline Values

Baseline values of mean arterial pressure in the intact group (92±2 mm Hg) were slightly, but significantly, lower (p<0.05) than in the CD (101±2 mm Hg) and SAD+CD (104±2 mm Hg) groups (Table 2). Baseline heart rates in the intact group (86±2 beats/min) were lower (p<0.05) than in the CD (111±3 beats/min), SAD (111±4 beats/min), SAD+CD (110±2 beats/min), and ganglion-blocked (128±4 beats/min) groups. There were no differences in right atrial pressure among the five groups. Baseline values of blood volumes were similar in the intact, CD, SAD, and SAD+CD groups (Table 3). There were also no differences in plasma osmolality before and after hemorrhage among the different groups (Table 4). Baseline values of AVP among the five groups were also similar (Table 5).

Hemodynamic Responses to Progressive Hemorrhage

The absolute values during baseline and the changes from baseline at each 5 ml/kg level of blood loss for mean arterial pressure, right atrial pressure, and heart rate in conscious dogs with all nerves intact, cardiac denervation, sinoaortic denervation, combined sinoaortic and cardiac denervation, and all nerves intact, but with ganglionic blockade are compared in Table 2. During hemorrhage, mean arterial pressure in the intact group was well maintained through 15 ml/kg of blood loss and then fell by 17±2.8 mm Hg. The responses of mean arterial pressure to hemorrhage were similar in the CD group, except that at 10 ml/kg of blood loss mean arterial pressure was reduced more (p<0.05) than in the intact group. The greater decrease in arterial pressure most likely was the result of the loss of the reflex chronotropic responses to hemorrhage, since peripheral vasoconstrictor responses to hemorrhage were not attenuated in CD dogs.5 However, in the SAD group, mean arterial pressure decreased progressively with blood loss, and the decreases were significantly greater than those observed in the intact and CD groups at each 5 ml/kg level of blood loss. The decline in mean arterial pressure in the SAD+CD and ganglion-blocked groups were almost identical with that observed in the SAD group. Right atrial pressure fell progressively with blood loss in all groups. Heart rate in the intact group increased gradually during hemorrhage, while heart rate did not rise in the other four groups; these responses were significantly different from those observed in the intact group.

AVP Responses to Progressive Hemorrhage

The absolute values of AVP at baseline and at each 5 ml/kg level of blood loss are shown in Table 5. The data for the CD and SAD+CD groups include both chronic CD and acute CD dogs. The increases in AVP during hemorrhage in either chronic or acute CD groups were similar to those observed in the intact group (Figure 1). At 15 ml/kg of blood loss in the chronic CD group, the appearance of AVP in the blood appeared to be depressed, but these differences were not significant. With lesser or greater amounts of hemorrhage these minor differences were less apparent. The data for chronic sinoaortic denervation combined with either acute or chronic cardiac denervation are compared with data for sinoaortic denervation alone in Figure 2. In contrast to the comparison of the effects between CD and intact groups, the AVP response to hemorrhage was significantly blunted with either acute or chronic cardiac
denervation in the SAD group as compared with sinoaortic denervation alone.

When the data for AVP appearance in blood in response to hemorrhage were normalized for similar reductions in mean arterial pressure, the slopes of the regression relations between increases in AVP and reductions in arterial pressure were not significantly different between the intact and CD groups (Figure 3). However, under the same conditions the responses of AVP in the SAD group were markedly

**TABLE 5. Arginine Vasopressin Responses (pg/ml) to Hemorrhage in Conscious Dogs With All Nerves Intact, Cardiac Denervation, Sinoaortic Denervation, Sinoaortic Denervation Plus Cardiac Denervation, and Ganglionic Blockade**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Change from baseline at each 5 ml/kg of blood loss</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Intact (n=17)</td>
<td>2.2±0.4</td>
<td>2.4±0.4</td>
</tr>
<tr>
<td>CD (n=19)</td>
<td>2.8±0.4</td>
<td>4.7±1.1</td>
</tr>
<tr>
<td>SAD (n=16)</td>
<td>2.6±0.4</td>
<td>3.4±0.7</td>
</tr>
<tr>
<td>SAD+CD (n=16)</td>
<td>2.8±0.3</td>
<td>5.2±1.5</td>
</tr>
<tr>
<td>Ganglionic (n=5)</td>
<td>4.4±1.7</td>
<td>19.0±7.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CD, cardiac denervation; SAD, sinoaortic denervation.
*Vasopressin levels lower than in SAD group, p<0.05.
†Vasopressin levels lower than in intact group, p<0.05.

**FIGURE 1.** The plasma levels of arginine vasopressin (right panels) and percent changes in mean arterial pressure (left panels) in response to hemorrhage are shown for each 5 ml/kg level of blood loss in conscious dogs with all nerves intact and chronic cardiac denervation (CD) (top) and in conscious dogs with all nerves intact and acute CD induced by intrapericardial lidocaine (bottom). There were no significant differences in the response of arginine vasopressin and arterial pressure to hemorrhage either in the intact and chronic CD groups or in the intact and acute CD groups. The arginine vasopressin responses to hemorrhage of up to 15 ml/kg are expanded and shown in the corner insets. With 15 ml/kg of hemorrhage, arginine vasopressin tended to be higher in the intact group, but not significantly. These differences were even less prominent with more severe hemorrhage. C, control.
depressed \((p<0.05)\). In the SAD+CD group the rise in AVP with hemorrhage was impaired even further, when mean arterial pressure fell by more than 40 mm Hg (Figure 3). In the ganglion-blocked group, when compared for similar reductions in mean arterial pressure during early hemorrhage, the rise in AVP was diminished \((p<0.05)\) as compared with the intact group (Figure 4). When compared with the SAD+CD group, the responses of AVP in the ganglion-blocked group were greater \((p<0.05)\) (Figure 4) even though the reductions in mean arterial pressure were similar (Table 2).

It might be noted that there was a tendency toward less AVP appearance in the CD group as compared with the intact group at 10 mm Hg reduction in arterial pressure (Figure 3, inset). However, it must be kept in mind that at these low levels of arterial pressure reduction, a considerably greater volume of blood was withdrawn from the intact dogs than the CD dogs. It was for this reason that the next protocol was examined.

**AVP Responses to Hemorrhage for Equivalent Reductions in Mean Arterial Pressure Induced Over Equivalent Time Periods**

The plasma AVP levels, right atrial pressure, heart rate, and amounts of blood loss in response to hemorrhage are compared for dogs with all nerves intact, cardiac denervation, sinoaortic denervation, and combined sinoaortic and cardiac denervation at baseline and after sufficient hemorrhage to reduce mean arterial pressure by 20 mm Hg over a similar period of time (Table 6). The volume of blood required to reduce mean arterial pressure by 20 mm Hg was similar in the intact (20.1±0.8 ml/kg) and CD (20.9±0.7 ml/kg) groups and also in the SAD...
(11.7±1.2 ml/kg) and SAD+CD (12.0±1.2 ml/kg) groups but was significantly depressed (p<0.05) in the presence of SAD as compared with the intact or CD groups. Therefore, in this protocol, the rate of hemorrhage was slower in the SAD and SAD+CD groups. The reductions in right atrial pressure were also less in the SAD (-1.8±0.4 mm Hg) and SAD+CD (-1.9±0.4 mm Hg) groups than those observed in the intact (-2.8±0.4 mm Hg) and CD (-3.3±0.3 mm Hg) groups, but these differences were not statistically significant. In the intact group, heart rate rose by 17±6.4 beats/min from a baseline of 90±3.3 beats/min. Heart rate did not change in the other three groups. Similar increases in plasma AVP were observed in the intact (+46.4±8.6 pg/ml) and CD (+47.5±8.6 pg/ml) groups. The increases in AVP in both the SAD and combined SAD+CD groups were less (p<0.05) than in the intact group. In the SAD+CD group, the increase in AVP (+4.6±1.3 pg/ml) was diminished compared with the SAD group (+9.1±2.7 pg/ml), but the difference was not statistically significant (p=0.15).

**Effects of AVP Blockade on Hemodynamic Responses to Hemorrhage**

Hemodynamic responses in conscious dogs with all nerves intact, cardiac denervation, sinoaortic denervation, and combined sinoaortic and cardiac denervation were examined in response to progressive hemorrhage before and after AVP blockade (Figures 5–7). During early hemorrhage, mean arterial pressure was maintained, cardiac output fell, and total peripheral resistance gradually rose in the intact and CD groups in the absence of AVP blockade. However, in the SAD group alone or the SAD+CD group, mean arterial pressure and cardiac output decreased progressively with blood loss, and total peripheral resistance failed to rise in response to hemorrhage. After AVP blockade, the baseline values for mean arterial pressure in the intact (100±3

**FIGURE 3.** Hemorrhage is compared for similar reductions in mean arterial pressure for conscious dogs with all nerves intact, cardiac denervation (CD), sinoaortic denervation (SAD), and combined sinoaortic and cardiac denervation (SAD+CD). The arginine vasopressin (AVP) responses to reductions in mean arterial pressure of up to 30 mm Hg are expanded and shown in the inset. During early hemorrhage (up to 30 mm Hg decrease in mean arterial pressure), the slope of the response of AVP was similar in the intact and CD groups and also in the SAD and SAD+CD groups (p>0.05). However, the slopes of the responses in both SAD and SAD+CD groups were less (p<0.05) than those in the intact and CD groups. Furthermore, the slope of response of AVP in the SAD+CD group was more depressed (p<0.05) than that in the SAD group for reductions of mean arterial pressure above 30 mm Hg.

**FIGURE 4.** The effects of hemorrhage on arginine vasopressin (AVP) release is compared for reductions in mean arterial pressure for conscious dogs with all nerves intact, combined sinoaortic and cardiac denervated dogs (SAD+CD), and intact dogs with ganglionic blockade. The AVP responses to reductions in arterial pressure of up to 30 mm Hg are expanded and shown in the inset. During early hemorrhage (up to 30 mm Hg reduction in mean arterial pressure), the slope of the response of AVP in the ganglion-blocked group was less (p<0.05) than that in the intact group but was greater (p<0.05) than that in the SAD+CD group. When the reductions in mean arterial pressure were above 30 mm Hg, the slope of the response was not significantly different in the intact and ganglion-blocked groups (p>0.05).
TABLE 6. Hemodynamic and Arginine Vasopressin Responses to Hemorrhage at Reduction in Mean Arterial Pressure of 20 mm Hg in Conscious Dogs With All Nerves Intact, Cardiac Denervation, Sinoaortic Denervation, and Sinoaortic Plus Cardiac Denervation

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Change from baseline</th>
</tr>
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<tbody>
<tr>
<td>AVP (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (n=10)</td>
<td>3.2±1.0</td>
<td>+46.4±8.6</td>
</tr>
<tr>
<td>CD (n=11)</td>
<td>3.5±0.5</td>
<td>+47.5±8.6</td>
</tr>
<tr>
<td>SAD (n=7)</td>
<td>2.1±0.3</td>
<td>+9.1±2.7*</td>
</tr>
<tr>
<td>SAD+CD (n=8)</td>
<td>2.2±0.4</td>
<td>+4.6±1.3*</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (n=9)</td>
<td>2.0±0.3</td>
<td>-2.8±0.4</td>
</tr>
<tr>
<td>CD (n=8)</td>
<td>1.9±0.2</td>
<td>-3.3±0.3</td>
</tr>
<tr>
<td>SAD (n=7)</td>
<td>1.0±0.4</td>
<td>-1.8±0.4</td>
</tr>
<tr>
<td>SAD+CD (n=8)</td>
<td>1.7±0.3</td>
<td>-1.9±0.4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (n=10)</td>
<td>90±3.3</td>
<td>+17±6.4</td>
</tr>
<tr>
<td>CD (n=11)</td>
<td>109±3.5*</td>
<td>+1±1.6*</td>
</tr>
<tr>
<td>SAD (n=7)</td>
<td>115±7.5*</td>
<td>-8±3.3*</td>
</tr>
<tr>
<td>SAD+CD (n=8)</td>
<td>107±4.6*</td>
<td>-2±1.8*</td>
</tr>
<tr>
<td>Blood loss (ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (n=10)</td>
<td>...</td>
<td>-20.1±0.8</td>
</tr>
<tr>
<td>CD (n=11)</td>
<td>...</td>
<td>-20.9±0.7</td>
</tr>
<tr>
<td>SAD (n=7)</td>
<td>...</td>
<td>-11.7±1.2*</td>
</tr>
<tr>
<td>SAD+CD (n=8)</td>
<td>...</td>
<td>-12.0±1.2*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CD, cardiac denervation; SAD, sinoaortic denervation.
*Different from intact, p<0.05.

mm Hg), CD (101±5 mm Hg), SAD (97±6 mm Hg), and SAD+CD (93±9 mm Hg) groups were similar to those observed in the intact (90±2 mm Hg), CD (104±4 mm Hg), SAD (98±5 mm Hg), and SAD+CD (109±3 mm Hg) groups without AVP blockade. Hemorrhage induced a greater fall in mean arterial pressure in the intact and CD groups as compared with those responses observed without AVP blockade. The gradual rise in total peripheral resistance with hemorrhage was abolished in the intact and CD groups. In the SAD group, mean arterial pressure and total peripheral resistance tended to decrease more in the presence of AVP blockade, but these differences were not as great as those observed in either the intact or CD groups. In the SAD+CD group, before and after AVP blockade a mean arterial pressure and total peripheral resistance responses to hemorrhage were similar. There were no differences in reductions in cardiac output before and after AVP blockade in all groups (Figure 7).

Effects of Infusion of AVP on Hemodynamics

In six conscious dogs with all nerves intact and two conscious CD dogs, the effects of infusion of AVP (1 ng/kg/min i.v. for 60 minutes) were examined. In intact dogs, infusion of AVP increased mean arterial pressure by 18±3 mm Hg from a baseline of 94±4 mm Hg and total peripheral resistance by 57±8% from a baseline of 41±3 mm Hg/l/min. The plasma AVP level was elevated to 38±2.5 pg/ml from a baseline of 1.6±0.2 pg/ml. In CD dogs, infusion of AVP increased mean arterial pressure by 23 mm Hg from a baseline of 112 mm Hg and total peripheral resistance by 65% from a baseline of 46 mm Hg/l/min. The plasma AVP level was elevated to 50 pg/ml from a baseline of 2.4 pg/ml.

Discussion

The results of the present investigation in conscious dogs indicate that cardiac receptors are not the major regulators of AVP release in response to progressive hemorrhage. Two different procedures for cardiac denervation were used, that is, acute administration of intrapericardial lidocaine and chronic surgical denervation. All animals were tested for completeness of denervation in several different ways, and there were no differences in plasma osmolality or baseline blood volume. Neither acute nor chronic cardiac denervation altered the response of AVP to hemorrhage. However, when the complicating influences of sinoaortic reflexes were eliminated, both acute and chronic cardiac denervation caused further inhibition of AVP release in response to hemorrhage. Thus, the expression of the role of cardiac receptors in mediating AVP release in response to hemorrhage was obscured by the presence of intact sinoaortic baroreceptors.

Since decreases in intracardiac and intravascular pressures as well as decreases in intracardiac and intravascular volumes may all be important stimuli for AVP release in response to hemorrhage, we compared data at both equivalent volumes of hemorrhage and also at equivalent reductions in mean arterial pressure. When the latter method was used, we still did not observe a significant difference in AVP responses in the intact and CD groups but observed clear depression of AVP levels in response to hemorrhage in the SAD group and further depression in the SAD+CD group.

One other concern was also addressed, that is, when comparing the intact and SAD groups at equivalent volumes of hemorrhage, the decreases in arterial pressure were not equivalent. Conversely, when comparing these groups at equivalent reductions in arterial pressure, the volumes of hemorrhage were significantly different. Obviously, this concern was not as applicable to the comparison of the intact and CD groups or to the comparison of the SAD and SAD+CD groups. To address this concern, a different protocol was devised. This protocol was designed to reduce mean arterial pressure by the same amount (20 mm Hg) over the same time period. Under these conditions, dogs with cardiac denervation again demonstrated similar increases in AVP, as did intact dogs, while responses to AVP in the SAD dogs were depressed markedly and responses of AVP in SAD+CD dogs were depressed only slightly further. These experiments suggest an important role for arterial baroreceptors in mediating release of
AVP but again failed to elucidate a major role for cardiac receptors in the regulation of AVP.

A role for cardiac receptors during hemorrhage was identified in the presence of sinoaortic denervation, where AVP responses to hemorrhage were blunted further after either acute or chronic cardiac denervation. However, it was unexpected that this contribution by cardiac receptors would occur at relatively large volumes of hemorrhage with relatively larger reductions in arterial pressure. One might have predicted that the role of cardiac receptors would have been identified earlier, with the first 10 ml/kg of blood loss; at this time atrial pressure had fallen, but arterial pressure was relatively unchanged. Earlier studies reported that the increases in AVP release during the nonhypotensive hemorrhage were related to the low-pressure system, suggesting that atrial “volume-sensitive” receptors contribute an important role in regulation of AVP release.15–18 Other studies of humans observed that lower body negative pressure resulted in reflex vasoconstriction during low levels of venous pooling, without changes in arterial pressure.19,20 However, studies of humans also demonstrated that during venous pooling induced by lower body negative pressure the plasma level of AVP was unchanged, indicating that cardiopulmonary receptors do not exert a role in mediation of AVP release, but AVP rose when hypotension caused unloading of arterial baroreceptors.21,22 In the present study at low levels of hemorrhage for a given decrease in volume, the increases in AVP tended to be slightly greater in CD dogs as compared with those observed in intact dogs (Table 5), whereas the reverse was observed for a given decrease in arterial pressure (Figure 3). It was for this reason we included an additional protocol examining similar volumes of hemorrhage withdrawn to reduce arterial pressure similarly over an equivalent period of time.

FIGURE 5. Comparisons of percent changes in mean arterial pressure in response to hemorrhage in the absence and presence of arginine vasopressin (AVP) blockade are shown for each 5 ml/kg of blood loss in conscious dogs with all nerves intact (top left panel), cardiac denervation (CD) (top right panel), sinoaortic denervation (SAD) (bottom left panel), and combined sinoaortic and cardiac denervation (SAD+CD) (bottom right panel). AVP blockade resulted in similar impairment in arterial pressure responses to hemorrhage in the intact and CD groups but less of an effect in the SAD group. C, control.
These experiments demonstrated similar increases in AVP in intact and CD groups, suggesting a minimal role for cardiac receptors in AVP regulation in response to hemorrhage, when arterial baroreceptors are intact. Interestingly, Ledsome et al.\textsuperscript{23} observed that plasma AVP rose more at lower levels of atrial pressure, at a time when atrial neural activity was not changing substantially, a finding that is also consistent with the concept that atrial neural reflexes are not the major regulators of AVP release in response to hemorrhage. Our data in the presence of sinoaortic denervation suggest a role for cardiac receptors in mediating AVP release at the greater volumes of blood loss and a nonclassical population of cardiac afferents, which play a role only with massive stimulation.

The major difference between the results of the present and prior studies\textsuperscript{1–4,15,17,24–32} involves the importance of cardiac receptors in regulating AVP release. One reason for this difference is that in some of the prior studies activation of atrial receptors was induced by volume expansion, aortic constriction, or inflation of an intra-atrial balloon, all of which act to increase atrial stretch and inhibit AVP release.\textsuperscript{24–29,31} Clearly, an inhibitory role of cardiac receptors induced by increasing atrial pressure and volume might be completely different from the role played in hemorrhage for which atrial pressure and volume decline, and AVP release should be stimulated.

It is more difficult to reconcile the differences between the current study and several prior studies in which effects of hemorrhage\textsuperscript{1,2,4,15,17,30,32} or constriction of the inferior vena cava\textsuperscript{3} were examined. In part, differences may be attributed to the presence or absence of general anesthesia, which exerts profound effects on AVP regulation\textsuperscript{33} and which was used in

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**FIGURE 6.** Comparisons of percent changes in total peripheral resistance in response to hemorrhage in the absence and presence of arginine vasopressin (AVP) blockade are shown for each 5 ml/kg of blood loss in conscious dogs with all nerves intact (top left panel), cardiac denervation (CD) (top right panel), sinoaortic denervation (SAD) (bottom left panel), and combined sinoaortic and cardiac denervation (SAD+CD) (bottom right panel). Vasoconstrictor responses to hemorrhage were abolished by AVP blockade in both intact and CD groups. In SAD and SAD+CD groups, AVP blockade exerted a lesser effect on hemodynamic responses to hemorrhage. C, control.
FIGURE 7. Comparisons of percent changes in cardiac output in response to hemorrhage in the absence and presence of arginine vasopressin (AVP) blockade are shown for each 5 ml/kg of blood loss in conscious dogs with all nerves intact (top left panel), cardiac denervation (CD) (top right panel), sinoaortic denervation (SAD) (bottom left panel), and combined sinoaortic and cardiac denervation (SAD+CD) (bottom right panel). In the presence and absence of vasopressin blockade, hemorrhage resulted in similar reductions in cardiac output in all groups studied. There were no differences before and after blockade in all groups. C, control.

Our data with some of the previous studies. Many prior studies were conducted in dogs with intact spleens, which constitutes a variable blood volume reservoir and which is hard to assess during hemorrhage. Furthermore, in those studies arterial pressure was not regulated carefully, with the resultant effects of smaller reductions in arterial pressure with hemorrhage or constriction of the inferior vena cava in dogs with chronic cardiac denervation as compared with the reduction in arterial pressure in intact dogs. These important differences in hemodynamics could be responsible for the differences between our results and those in prior studies. In the current investigation, the unpredictable role of the spleen was not a factor, because it was removed, and blood volumes were measured and found similar in the different groups. Most important, there were no hemodynamic differences observed between the ins
tact and CD groups, or between the SAD and SAD+CD groups. Another preliminary report, also conducted in conscious dogs, failed to observe a prominent role for cardiac receptors in regulation of AVP during hemorrhage.34

Earlier findings from anesthetized dogs17,30 and cats39 demonstrate that the increased release of AVP in response to hemorrhage was markedly attenuated by acute vagotomy but not by carotid sinus denervation. Furthermore, in the vagotomized animals in the absence of functional carotid sinuses, the AVP responses to hemorrhage were abolished17,30 or reduced,35 indicating that the rise in AVP during hemorrhage is controlled by both sets of receptors, but primarily the vagal afferents. The data from the current investigation also suggest that both sets of receptors regulate AVP release during hemorrhage, but with an emphasis on the opposite set of baroreflexes. It is important to note that in the studies by Share17,30 acute surgical denervation of the vagi or carotid sinuses resulted in substantial increases in baseline levels of AVP before hemorrhage. Therefore, it is questionable whether under these conditions hemorrhage could induce a much greater release of AVP.

Another major difference between the current data and the concepts proposed by Share17,30 is that the AVP response to hemorrhage was blunted, but not abolished, by combined cardiac and sinoaortic denervation. It appears that other sites may be more important than previously considered in release of AVP during hemorrhage. These sites include noncardiac vagal afferents as well as central nervous system receptors.36–43 The rise of AVP in the presence of combined sinoaortic and cardiac denervation occurred in the presence of marked hypotension, which could have induced cerebral ischemia with concomitant AVP release.36,40,42–44 In addition, a recent study by Wood et al45 demonstrated that in fetal sheep AVP responses to hemorrhage were related to changes in arterial pH, also suggesting that AVP release could be mediated by chemoreceptors. In that study, there were also no differences in the AVP responses to hemorrhage in the intact and vagotomized sheep.45 Furthermore, it has been proposed that nicotinic synapses in the central nervous system have an important role in mediating AVP release in response to hypotension.46,47 Because these pathways can be inhibited by ganglionic blockade,46,47 we examined a subset of intact dogs with ganglionic blockade in response to hemorrhage. Comparing these data with those obtained in the intact and SAD+CD dogs, that is, in the latter group for which hypotensive responses to hemorrhage were similar, we identified a role of nicotinic receptors, but these mechanisms were not nearly as important as afferents from arterial baroreceptors and cardiac receptors (Figure 4).

The hemodynamic consequences of AVP release also must be considered. AVP is a potent vasoconstrictor, which should increase total peripheral resistance and help maintain arterial pressure in the face of hemorrhage. To verify this, two sets of experiments were conducted. First, in experiments in which vasopressin was infused, plasma AVP rose more than 20-fold and total peripheral resistance increased by 57±8%. Therefore, if there was a major difference in release of AVP in the intact and CD dogs in response to hemorrhage, then there should have been a major difference in arterial pressure control and the ability to increase total peripheral resistance. Indeed, a prior study from this laboratory5 failed to observe hemodynamic differences to hemorrhage in the intact and CD dogs, which is consistent with the current observation that there were no differences in AVP release in the two groups. Surprisingly, prior studies demonstrating major differences in AVP levels in the presence and absence of cardiac nerves failed to detect hemodynamic impairment in the CD dogs.1,4,32 These earlier studies, therefore, appear internally inconsistent, as well as inconsistent with both the present observations and those of Montani et al,48 which demonstrated important pressor and constrictor responses with plasma AVP levels of approximately 60 pg/ml.

In the current investigation, one final series of experiments was conducted to substantiate the physiological role of cardiac receptors as regulators of AVP release in response to hemorrhage. In these experiments the same subgroups of dogs were studied on separate days in the presence and absence of an AVP antagonist. The working hypothesis was that if increases in plasma AVP were similar in the subgroups of dogs with and without either cardiac or arterial baroreceptor denervation, then blockade of AVP would induce similar hemodynamic impairment in both subgroups. In these experiments there were major differences in hemodynamic responses to hemorrhage in the presence and absence of AVP blockade. However, responses were similar in the intact and CD dogs, and lesser hemodynamic impairment was observed in SAD dogs after AVP blockade, supporting the concept that sinoaortic baroreceptors are important in mediating AVP release.

Our data demonstrate an important role for AVP in mediating compensatory peripheral vasoconstriction during hemorrhage in dogs with intact reflexes. The magnitude of this response was unexpected and points to a potential interaction among AVP, renin-angiotensin, and sympathoadrenal systems, which all play a role in mediating compensatory peripheral vasoconstriction during hemorrhage. One study in which AVP antagonists were used failed to support the concept of AVP-mediated vasoconstriction during hemorrhage in rats.49 Another study suggested that AVP became important only when blood volume had been reduced by 30–35%.50 In contrast, in our study a significant role for AVP in regulating mean arterial pressure and total peripheral resistance was noted by 10 ml/kg of blood loss (Figures 5 and 6). The differences between the present investigation, which is consistent with the study of conscious dogs by Schwartz and Reid,8 and those studies49,50 could
be due to differences in species, that is, dogs versus rats or rabbits, or differences in the rate of hemorrhage used. In regard to the latter point, in our protocol, blood was withdrawn at a rate of 0.5 ml/kg/min. In those studies demonstrating a less important role of AVP, the rate of hemorrhage was approximately 1.5 ml/kg/min, perhaps too rapid to allow for AVP release and vasoconstrictor actions.

In summary, the present investigation conducted in conscious, splenectomized dogs demonstrated an important role for AVP in mediating hemodynamic responses to hemorrhage. Neither hemodynamic responses to hemorrhage in the presence or absence of AVP blockade nor appearance of AVP in blood with hemorrhage differed between intact dogs and dogs with acute or chronic cardiac denervation. After elimination of sinoaortic baroreceptors, a role for AVP control by cardiac reflexes was also uncovered. However, the latter set of reflexes are clearly not preeminent in the regulation of AVP release during hemorrhage in conscious dogs.

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**KEY WORDS** • cardiac receptors • reflex control • hypotension • arginine vasopressin • ganglionic blockade • blood pressure
Relative roles of cardiac and arterial baroreceptors in vasopressin regulation during hemorrhage in conscious dogs.

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