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Effect of Hemorrhage on Plasma Atriopeptin Levels in Conscious Dogs (42672)

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Abstract. An increase in atrial pressure has been shown to cause an increase in the concentration of atrial peptides (atriopeptin) in plasma. We therefore hypothesized that a reduction in atrial pressure would decrease the concentration of atriopeptin in plasma. In formulating this hypothesis we assumed that changes in the concentration of other circulating hormones or changes in cardiac nerve activity during hemorrhage would not affect the secretion of atriopeptin. To test the hypothesis, we bled sham-operated conscious dogs at a rate of 0.8 $ml \cdot kg^{-1} \cdot min^{-1}$ to decrease right and left atrial pressures. Hemorrhage was continued until a total of 30 ml of blood per kilogram body weight had been removed. Identical experiments were performed on conscious cardiac-denervated dogs. The concentration of plasma atriopeptin was decreased in each group of dogs after 10 ml of blood per kilogram of body weight had been removed, but the decrease achieved statistical significance only in the cardiac-denervated dogs. Further hemorrhage, however, produced no further decreases in circulating atriopeptin in either group even though atrial pressures continued to decline as more blood was removed. A comparison of the atriopeptin response to hemorrhage revealed no significant difference between the sham-operated and cardiac-denervated dogs, thus providing no evidence for a specific effect of cardiac nerves on atriopeptin secretion during hemorrhage. Our results demonstrate that the relationship between atrial pressure and plasma atriopeptin that has been observed repeatedly during atrial stretch is not evident during relatively slow, prolonged hemorrhage. There is, however, a small decline in circulating atriopeptin during the initial stage of hemorrhage that could be of biological significance.

Although factors that regulate the secretion of atrial peptides (atriopeptin) have not been completely elucidated, several reports have indicated that atrial distension increases the concentration of atriopeptin in plasma. For example, Dietz (1) raised the height of a venous reservoir in a rat heart–lung preparation to increase filling pressure and reported that the perfusate collected during cardiac distension caused an increase in urine flow and sodium excretion in anesthetized rats used for bioassay. Subsequently, radioimmunoassay measurements confirmed that a significant elevation in the plasma concentration of atriopeptin was elicited by atrial distension in experimental animals (2–4). A comparable effect of atrial distension also has been demonstrated indirectly in humans (5–8).

Because an increase in atrial pressure elicits a rise in the circulating level of atriopeptin, we hypothesized that a reduction in atrial pressure would be accompanied by a decrease in the circulating level of this peptide. To test our hypothesis, we withdrew blood slowly from normal conscious dogs and measured atrial pressures and the concentrations of atriopeptin and a number of other circulating hormones that have been reported to affect atriopeptin secretion (9–12). In addition, identical experiments were performed on conscious dogs with surgically denervated hearts to investigate whether the absence of cardiac nerves affected the atriopeptin response during hemorrhage.

Throughout this paper we will use the term "atriopeptin" when referring to the radioimmunoassayable polypeptide that is secreted from atrial myocytes. This term avoids any implication regarding the primary physiological role of the atrial peptides.

Materials and Methods. *Surgical preparation.* Fourteen female mongrel dogs (17.8 ± 0.4 kg) were anesthetized by intravenous injection of pentobarbital sodium (25–30 mg/kg); supplements were given as needed during the operation. A thoracotomy was performed aseptically through the fourth intercostal space on the left side. Catheters

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were placed in the descending aorta, right and left atria, and pulmonary artery; an electromagnetic flow probe was placed around the ascending aorta to obtain an estimate of cardiac output. In the cardiac-denervated group (n = 7), the heart was denervated by the intrapericardial method of Randall *et al.* (13) as modified by Fater *et al.* (14). In the sham-operated group (n = 7), the pericardium was opened, but the heart was not denervated. The chest was closed, and all catheters and the flow probe cable were tunneled subcutaneously to the back and passed through the skin. A vinyl catheter was inserted into the pleural cavity and connected to a vacuum pump to remove residual air and fluid from the chest. The catheter was sealed after 2–3 hr and left in place for 3–4 days to allow daily removal of accumulated fluid. Penicillin (500,000 U, im) was given postoperatively for 3 days. On alternate days the catheters were flushed with saline and filled with a dilute heparin solution to prevent clotting. The animals were allowed at least 2 weeks to recover from surgery before experiments were performed.

Tests for cardiac denervation. The initial test for completeness of cardiac denervation was performed during surgery by electrical stimulation of the ansae subclavia and the thoracic vagi as described by Randall *et al.* (13). After recovery from the operation, confirmatory tests for denervation (14) included the absence of reflex changes in heart rate following intravenous administration of methoxamine (50 μg/kg) and nitroglycerin (24 μg/kg) to raise and lower arterial pressure, respectively. The absence of heart rate changes (±3 beats/min) indicated that the efferent nerves to the heart had been effectively eliminated. In addition, veratridine (25 μg) was injected into the left atrium of the cardiac-denervated dogs. Although the absence of bradycardia following veratridine administration could be attributed to the elimination of either afferent or efferent cardiac neural pathways, the absence of changes in arterial blood pressure indicated that afferent cardiac pathways responsible for eliciting the Bezold–Jarisch reflex had been effectively eliminated.

Experimental procedures. Experiments were performed while each dog was fully conscious and resting quietly in a commercial dog sling. Immediately prior to blood removal, each animal was given 3,000 U of sodium heparin iv to prevent clotting. The experiment consisted of a 30-min control period followed by slow, continuous hemorrhage in which blood was withdrawn by a pump (Sage Instruments, Model 375A) at a rate of 0.8 $ml \cdot kg^{-1} \cdot min^{-1}$ from the catheter in the pulmonary artery. Hemorrhage was continued until 30 ml/kg of blood, or approximately 37.5% of the estimated blood volume, had been removed. Intravascular pressures and cardiac output were recorded continuously and analyzed by computer as described previously (14). Briefly, each analog signal was sampled by computer at 10-msec intervals and averaged each minute. The minute averages were plotted on a video screen and stored for later analysis. Blood (42 ml) for determination of hematocrit, plasma osmolality, and plasma levels of atriopeptin, vasopressin, renin, aldosterone, epinephrine, norepinephrine, sodium, and potassium was obtained from the pulmonary artery 5 min before the start of hemorrhage and after 10, 20, and 30 ml of blood per kilogram body weight had been removed. The initial blood sample was replaced with an equal volume of 6% dextran in isotonic saline; the three remaining blood samples were obtained as part of the hemorrhage and were not replaced. After completion of hemorrhage, the shed blood was filtered and reinfused into the animal. In addition, the packed cells from each blood sample were suspended in isotonic saline and reinfused.

Radioimmunoassay of plasma atriopeptin. Blood samples were collected in plastic syringes containing disodium ethylenediaminetetraacetic acid (EDTA, 1 mg/ml). Plasma was separated by centrifugation and stored at −20°C. Prior to extraction, plasma samples (1 ml) were thawed and acidified with 3 ml of 4% acetic acid. Sep Pak C_{18} cartridges (Waters Associates) were primed with 5 ml of 86% ethanol in 4% acetic acid and rinsed with 10 ml of distilled water. The acidified plasma was applied to individual Sep Pak cartridges and evacuated with mild vacuum at a rate of 2 ml/min. The cartridges were rinsed again with 10 ml of distilled water, and the adsorbed peptide was eluted

with 3.5 ml of 86% ethanol in 4% acetic acid. The eluates were evaporated to dryness in a vortex evaporator (Buchler) and stored at −20°C. This extraction method yielded a recovery of 66% radiolabeled α-human atrial natriuretic peptide and 63% unlabeled peptide that had been added to plasma.

Prior to assay, plasma extracts were thawed and reconstituted in 1 ml assay buffer containing 19 m*M* monobasic and 81 m*M* dibasic sodium phosphate (pH 7.4), 0.05 *M* sodium chloride, 0.1% BSA, 0.1% Triton X-100, and 0.01% sodium azide. Radioimmunoassay standard (α-human atrial natriuretic polypeptide, Peninsula Laboratories) was diluted in assay buffer to concentrations ranging from 15 to 1000 pg/ml. Lyophilized rabbit anti-α-atrial natriuretic polypeptide serum (Peninsula Laboratories) was diluted 1:100 with 0.1% Triton X-100. This dilution was sufficient to bind 30–40% of radiolabeled α-human atrial natriuretic peptide in the absence of unlabeled peptide. The radioimmunoassay was begun by combining 0.1 ml antiserum with either 0.1 ml standard (1.5–100 pg) or 0.1 ml plasma extract in polystyrene tubes and incubating the contents for 24 hr at 4°C. Then 0.1 ml assay buffer containing approximately 10,000 cpm of 3-[^{125}I]iodotyrosyl^{28} α-human atrial natriuretic peptide (Amersham) was added to each tube, and the reaction mixture was incubated at 4°C for an additional 24 hr. Antigen–antibody complexes were precipitated by adding 0.1 ml each of pretitered goat anti-rabbit IgG serum and normal rabbit serum (Peninsula Laboratories) to each tube. The reaction continued for 2 hr at room temperature before adding 0.5 ml assay buffer. The tubes were centrifuged at 3500 rpm for 30 min, and the supernatant was aspirated immediately. The pellets were counted for 5 min in a gamma radiation counter (Beckman). All determinations were performed in triplicate. The minimal detectable amount of atriopeptin was 3 pg/tube. The concentrations of atriopeptin were not corrected for extraction loss.

Other chemical analyses. Arginine vasopressin was extracted from plasma with Sep Pak C_{18} cartridges and measured by radioimmunoassay as described previously (15). Plasma renin activity was determined using antiserum and standards provided by a kit (Clinical Assays). Plasma aldosterone was measured with a solid-phase radioimmunoassay kit (Diagnostic Products). The concentrations of epinephrine and norepinephrine in plasma were determined by the single isotope radioenzymatic method (Cat-A-Kit, Upjohn). Plasma osmolality was measured by freezing point depression (Advanced Instruments, Model 3R). Plasma sodium and potassium concentrations were measured by internal standard flame photometry (Instrumentation Laboratories, Model 143). Hematocrit was measured in triplicate by a microcapillary method.

Statistical analysis. Grouped data were expressed as means ± SE. Hemodynamic data were averaged over consecutive 4-min periods and compared with mean values representing the final 4 min of the control period. The effect of hemorrhage and state of cardiac innervation (intact or denervated) on measured parameters were assessed by two-way analysis of variance for repeated measures. When significant differences between groups were detected, a series of completely randomized *F* tests were employed to detect the level of hemorrhage at which the two groups differed (16). Data within each group were evaluated by Dunnett's test to determine which values differed from control values and by Newman–Keuls' test to determine the difference between all possible means. A *P* value of less than 0.05 was considered to be statistically significant.

Results. The hemodynamic responses to slow continuous hemorrhage are shown in Fig. 1. Heart rate in the sham-operated control dogs tended to increase during the first half of hemorrhage (NS) and subsequently returned toward control values. Heart rate in the cardiac-denervated dogs remained constant during the initial stages of hemorrhage and then increased significantly. Hemorrhage elicited increases in total peripheral resistance and decreases in stroke volume, cardiac output, aortic pressure, and right and left atrial pressures in both groups of dogs. During hemorrhage right atrial pressure fell progressively from 2.5 to 0.0 mm Hg in the control dogs and from 1.5 to −1.7 mm Hg in the cardiac-denervated dogs. Right atrial pressure was significantly lower in the car-

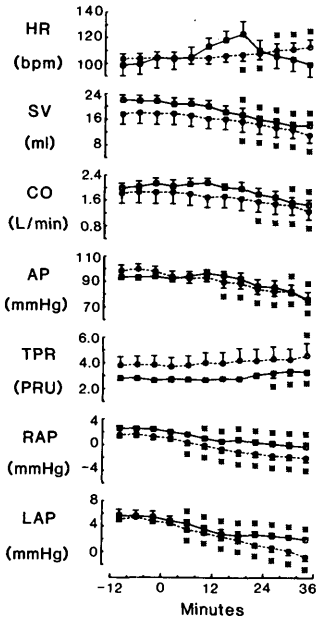

FIG. 1. Effects of continuous hemorrhage (0.8 $ml \cdot kg^{-1} \cdot min^{-1}$) on hemodynamic variables in conscious sham-operated (solid lines) and cardiac-denervated (dashed lines) dogs. Hemorrhage began at zero time. Each variable was averaged during consecutive 4-min periods. HR, heart rate; SV, stroke volume; CO, cardiac output; AP, arterial pressure; TPR, total peripheral resistance; RAP, right atrial pressure; LAP, left atrial pressure. The only significant difference between groups occurred during the final 20 min of hemorrhage (last five plotted values) when right atrial pressure was significantly lower in the cardiac-denervated group (not indicated in figure). For within-group comparisons, * indicates a difference from the mean of the final 4 min of the control period ($P < 0.05$).

diac-denervated dog group than the sham-operated group during the final 20 min of hemorrhage. Left atrial pressure fell progressively during hemorrhage from 5.5 to 2.0 mm Hg in the control dogs and from 4.8 to −0.8 mm Hg in the denervated dogs. There was no significant difference in left atrial pressure between the two groups of dogs throughout the entire experiment.

Circulating levels of atriopeptin showed a tendency to decrease only during the initial phase of hemorrhage (Fig. 2). In the sham-operated dogs, absolute atriopeptin concentration fell from a control level of 115 ± 16 pg/ml to 95 ± 10, 94 ± 11, and 104 ± 9 pg/ml after removal of 10, 20, and 30 ml of blood per kilogram, respectively, but these changes were not statistically different from control values. In the cardiac-denervated dogs, hemorrhage produced slightly greater reductions in plasma atriopeptin levels; atriopeptin concentration fell from a control level of 126 ± 20 pg/ml to 89 ± 10, 86 ± 11, and 84 ± 11 pg/ml after 10, 20, and 30 ml/kg of blood had been removed, respectively (all $P < 0.05$ compared to control). It is noteworthy that circulating atriopeptin concentration failed to decline further in either group of dogs after the initial 10 ml/kg of blood had been removed even though atrial pressures continued to decline. When atriopeptin levels from sham-operated and cardiac-denervated dogs were compared with each other, no significant differences between the two groups of dogs were found either before or during hemorrhage. Plasma atriopeptin values were somewhat higher in this study than values usually obtained from conscious dogs in this laboratory. It is conceivable that the administration of heparin to the dogs

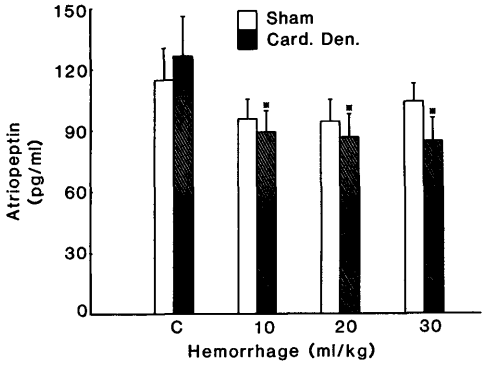

FIG. 2. Effects of continuous hemorrhage on plasma atriopeptin concentration in conscious sham-operated and cardiac-denervated dogs. Although absolute levels of circulating atriopeptin declined in each group of dogs, the decrease was statistically significant only in the cardiac-denervated group. A comparison of the plasma atriopeptin concentration in the two groups of dogs revealed that there was no significant difference between the sham-operated and cardiac-denervated groups either in the control period or in the measurements made during hemorrhage. For within-group comparisons, * indicates a difference from the mean control value ($P < 0.05$).

prior to blood sampling may somehow have augmented the circulating level of atriopeptin, but we know of no data to substantiate this speculation. Heparin has been shown to complex with atriopeptin in plasma and reduce its biological activity (17); however, it does not interfere with the radioimmunoassay of atriopeptin in plasma (17).

Vasopressin, renin, aldosterone, epinephrine, and norepinephrine concentrations increased significantly from control values during hemorrhage (Table I). As shown in a previous study (15), removal of 20 and 30 ml/kg of blood caused an increase in vasopressin concentration that was significantly greater in the sham-operated control dogs than in the cardiac-denervated dogs. Epinephrine concentration was greater in the control dogs after removal of 30 ml/kg of blood.

The effects of hemorrhage on plasma sodium, potassium, osmolality, and hematocrit are shown in Table II. Hemorrhage did not change plasma sodium; plasma potassium remained stable throughout most of the hemorrhage, but a small significant decrease was detected in the sham-operated dogs after 30 ml/kg of blood had been removed. Osmolality and hematocrit increased significantly near the end of hemorrhage in each group of dogs. The increase in plasma osmolality may have been due to the mobilization of glucose by high levels of epinephrine (18). The increase in hematocrit may have been caused by the release of stored red blood cells into the bloodstream following splenic contraction during hemorrhage.

Discussion. These experiments demonstrated that an acute loss of over one-third of the estimated blood volume in conscious sham-operated and cardiac-denervated dogs was accompanied by a progressive decrease in right and left atrial pressures and a modest initial decrease in circulating atriopeptin concentration that achieved statistical significance only in the cardiac-denervated group. Although the absolute decrease in plasma atriopeptin during hemorrhage did not achieve statistical significance in the sham-operated dogs, a two-way analysis of variance revealed that the plasma concentration of atriopeptin in this group of dogs did not differ significantly from that in cardiac-denervated dogs either before or during hemorrhage. Consequently, these data provide no evidence for a specific effect of cardiac nerves

TABLE I. EFFECTS OF CONTINUOUS HEMORRHAGE ON THE PLASMA CONCENTRATIONS OF VARIOUS HORMONES IN SHAM-OPERATED AND CARDIAC-DENERVATED DOGS

		Cumulative hemorrhage (ml/kg)		
	Control	10	20	30
AVP (pg/ml)				
Sham	2.4 ± 0.3	6.2 ± 1.7	200.0 ± 65.4*	991.3 ± 220.9*
Card. den.	1.5 ± 0.6	2.0 ± 0.5	7.4 ± 1.3*†	133.2 ± 42.0*†
PRA ($ngAl \cdot ml^{-1} \cdot hr^{-1}$)				
Sham	1.4 ± 0.4	2.8 ± 0.7	7.0 ± 1.6*	7.5 ± 1.5*
Card. den.	1.4 ± 0.3	3.7 ± 0.9	10.5 ± 1.7*	16.6 ± 1.9*†
Aldo (ng/dl)				
Sham	1.8 ± 0.6	3.2 ± 0.9	9.9 ± 2.9	31.6 ± 5.5*
Card. den.	2.9 ± 1.3	5.2 ± 2.2	12.8 ± 3.5*	27.4 ± 5.4*
E (pg/ml)				
Sham	88 ± 27	176 ± 30	687 ± 177	1,865 ± 409*
Card. den.	68 ± 33	110 ± 15	203 ± 62	758 ± 283*†
NE (pg/ml)				
Sham	170 ± 11	319 ± 34*	400 ± 49*	513 ± 83*
Card. den.	148 ± 30	289 ± 27*	373 ± 33*	494 ± 58*

Note. Values are means ± SE. AVP, arginine vasopressin; PRA, plasma renin activity; Aldo, aldosterone; E, epinephrine; NE, norepinephrine.

* Difference from control value ($P < 0.05$).

† Difference between groups ($P < 0.05$).

TABLE II. EFFECTS OF CONTINUOUS HEMORRHAGE ON PLASMA ELECTROLYTES, OSMOLALITY, AND HEMATOCRIT IN SHAM-OPERATED AND CARDIAC-DENERVATED DOGS

		Cumulative hemorrhage (ml/kg)		
	Control	10	20	30
P_{Na} (meq/liter)				
Sham	142.3 ± 0.3	143.1 ± 0.3	142.6 ± 0.4	142.7 ± 1.0
Card. den.	141.9 ± 0.9	142.7 ± 0.8	142.8 ± 0.9	142.6 ± 0.9
P_K (meq/liter)				
Sham	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	3.9 ± 0.1*
Card. den.	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
P_{osm} (mosmole/kg H_2O)				
Sham	290.3 ± 1.6	293.0 ± 1.7	292.8 ± 1.6	295.2 ± 1.3*
Card. den.	290.1 ± 2.6	290.8 ± 2.6	291.7 ± 2.6*	292.0 ± 2.6*
Hct (%)				
Sham	34.3 ± 0.9	33.4 ± 1.0	36.0 ± 2.0	37.3 ± 2.0*
Card. den.	36.0 ± 1.3	36.1 ± 0.7	37.1 ± 0.6	38.6 ± 1.4*

Note. Values are means ± SE. P_{Na}, plasma sodium concentration; P_K, plasma potassium concentration; P_{osm}, plasma osmolality; Hct, hematocrit.

* Difference from control value ($P < 0.05$).

on atriopeptin secretion. This interpretation is consistent with data from an earlier study from our laboratory in which an increase in left atrial pressure elicited increases in the concentration of atriopeptin in plasma that were similar in both cardiac-denervated and sham-operated conscious dogs. Those results indicated that cardiac nerves are not required to increase circulating atrial peptide levels during left atrial distension (4). Similarly, Ledsome *et al.* (19) observed that mitral obstruction produced an elevation in circulating atrial peptide in anesthetized intact dogs that did not differ significantly from peptide levels achieved in a group of anesthetized dogs after vagotomy and β-adrenergic receptor blockade. Supporting data also have been obtained from experiments with rats. Kihara *et al.* (20) infused saline into guanethidine-treated rats and control rats and found comparable increases in the levels of atrial peptide in the two groups. Haass *et al.* (21) reported that acute blockade of the parasympathetic nervous system and autonomic ganglia did not affect levels of atriopeptin following volume expansion. The present study, as well as those cited above, also demonstrated that basal levels of atriopeptin in animals lacking cardiac reflexes do not differ from levels in animals with intact cardiac reflexes. Thus considerable evidence indicates that cardiac nerves do not exert an appreciable effect on the secretion of atrial peptides from atrial myocytes under several different experimental conditions.

Our finding that there was no correlation between atrial pressures and plasma levels of atriopeptin during the latter two-thirds of hemorrhage does not necessarily mean that the secretory rate of atriopeptin did not decrease in response to the decrease in atrial pressures. It is possible that atriopeptin secretion did decline and was accompanied by a decrease in the metabolic clearance of the peptide, possibly in response to the decline in cardiac output during blood removal. In other words, a reduced metabolic clearance may have prevented further decreases in the plasma concentration of atriopeptin during the latter stages of hemorrhage. Alternately, it is conceivable that the secretion of atriopeptin may have decreased to a basal level that could not be suppressed further by further decreases in atrial pressures. Data consistent with this possibility were reported by Ogawa *et al.* (22); these investigators found that plasma atriopeptin levels did not fall during head-up tilt in humans and suggested that atriopeptin secretion may be tonic and therefore not decrease when atrial pressure is lowered by upright posture. It also is possible

that the aforementioned increases in other circulating hormones may have acted to offset any inhibitory effect of a further decline in atrial pressures on atriopeptin secretion. Evidence from several studies implies that increased levels of circulating hormones may potentiate atriopeptin release. Sonnenberg and Veress (9) reported that both epinephrine and vasopressin potentiate the secretion of atriopeptin from isolated rat atria, and Currie and Newman (10) reported that norepinephrine stimulates the release of atriopeptin from isolated rat hearts. Evidence from Garcia *et al.* (11) suggested that mineralocorticoids may have a permissive effect on the regulatory role of glucocorticoids on atrial peptide synthesis. It also has been reported that intracellular messengers may affect atrial peptide release (12), an observation consistent with the possibility that circulating hormones may influence atriopeptin secretion. However, in the present study substantial elevations of plasma norepinephrine, epinephrine, vasopressin, and aldosterone that occurred during hemorrhage were unable to increase the plasma concentration of atriopeptin in conscious dogs. One may conclude, therefore, that any possible stimulatory effects of these hormones on circulating atriopeptin were relatively minor in these experiments.

In summary, the initial stage of hemorrhage (up to 10 ml/kg) resulted in a decline in plasma atriopeptin that achieved statistical significance only in cardiac-denervated dogs. Further hemorrhage produced no further decreases in circulating atriopeptin concentration in either group of dogs even though atrial pressures declined progressively during blood removal. Consequently, we conclude that the direct relationship between atrial pressures and circulating atriopeptin that has been demonstrated during increases in atrial pressure is absent when moderate to severe hemorrhage occurs slowly in conscious dogs. Several possible reasons for the lack of continuous decreases in atriopeptin during hemorrhage in these experiments have been discussed. Finally, statistical analysis revealed no difference in the atriopeptin levels of cardiac-denervated and sham-operated dogs either before or during hemorrhage, thus providing further evidence that cardiac nerves do not exert appreciable effects on the secretion of atriopeptin during slow hemorrhage.

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