Evidence that the Brain Participates in the Humoral Natriuretic Mechanism of Blood Volume Expansion in the Dog

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ABSTRACT We examined the role of the central nervous system in the activation of the humoral natriuretic mechanism elicited by blood volume expansion. Studies were performed in anesthetized dogs pretreated with deoxycorticosterone acetate (15 mg/day) and sodium chloride for 12 days. An isolated dog kidney perfused with blood from the femoral artery of the volume expanded dog served as the bioassay system for the humoral natriuretic factor. In group I volume expansion of intact dogs (n = 14) with equilibrated blood promoted an increase in fractional sodium excretion (FE_{Na}) from a control level of 2.6±0.5 to 13.6±1.6%, $P < 0.001$. In the isolated kidney FE_{Na} increased from 3.6±0.8 to 6.8±1.1%, $P < 0.01$. The natriuresis from the isolated kidney occurred in the absence of significant changes in renal arterial pressure, glomerular filtration rate, plasma protein concentration, or packed cell volume, whereas renal blood flow decreased slightly. In group II (n = 20) the dogs were decapitated by means of a specially designed neck vise. In 10 dogs blood pressure was supported by a constant infusion of dopamine (3.8±0.7 μg/min per kg body weight). Despite the fact that in response to the same volume stimulus, decapitated dogs manifested an increase in blood volume and cardiac output similar in magnitude to that of intact dogs whereas the rise in mean arterial pressure of decapitated dogs exceeded that of intact dogs, the natriuretic response of decapitated dogs was significantly less than that of intact dogs. FE_{Na} in decapitated dogs increased 4.7±1.1 compared to 11.1±1.4% in intact dogs ($p < 0.01$). Furthermore, volume expansion of decapitated dogs failed to elicit a natriuretic response from the isolated kidney. FE_{Na} in the isolated kidney measured 2.6±0.4 before and 2.6±0.4% after blood volume expansion. These data indicate that decapitation inhibits activation of the humoral natriuretic mechanism elicited by blood volume expansion and are consistent with the interpretation that the brain is the source of the natriuretic factor or that the brain participates in the activation of the humoral natriuretic mechanism at some other site in the body.

INTRODUCTION

Expansion of the extracellular fluid volume elicits an increase in urinary sodium excretion. A large body of evidence implicates the participation of a humoral natriuretic factor in this response (1, 2). Thus far, however, studies have failed to establish the identity and source of this factor, its mechanism of action, and its physiological role in the renal regulation of sodium balance.

Studies in our laboratory also support the hypothesis that a humoral mechanism, unrelated to mineralocorticosteroid or vasopressin, participates in the natriuretic response elicited by expansion of the blood volume (3–5). We have used a cross-circulation technique involving an isolated dog kidney connected to the circulation of an intact dog and have observed that infusing equilibrated blood into the dog promotes a significant increase in sodium excretion in the isolated kidney. Because the natriuresis could not be explained by changes in renal hemodynamics or physical factors, we postulated that expansion of the dog's blood volume stimulated release of a natriuretic factor, which promoted an increase in sodium excretion by the isolated kidney. In a previous study (5) we sought to identify the source of this factor by examining the effect of selected organ ablation upon the natriuretic response of the isolated kidney during blood volume expansion and

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were able to exclude the adrenal glands, the thyroparathyroid glands, and the hypophysis as the source of this factor. The purpose of the present investigation was to evaluate the possibility that the postulated natriuretic factor might derive from some site in the brain other than the hypophysis or that the brain might play a role in activating the humoral natriuretic mechanism. We examined the effect of decapitation on the natriuretic response of the isolated kidney during blood volume expansion. The results demonstrate that decapitation abolishes the natriuretic response of the isolated kidney during blood volume expansion and, thus, suggest that the brain is either the source of the natriuretic factor or that the brain plays a critical role in activating the humoral natriuretic mechanism at some other site in the body.

METHODS

Experiments were performed on mongrel dogs that weighed 18–24 kg. A kidney, removed from a donor dog, was connected to the circulation of the experimental animal and served as a bioassay organ for monitoring the activation of the humoral mechanism in response to blood volume expansion. All dogs were fed a standard kennel ration which provided ≅ 65 meq of sodium per day. The diet of dogs scheduled for blood volume expansion was supplemented with 150 meq of NaCl and 80 meq of KCl/day. In addition these dogs were injected intramuscularly with 15 mg of deoxycorticosterone acetate in oil each day for an average of 12 days (range 9–16 days). The dogs were anesthetized with pentobarbital sodium (30 mg/kg), given intravenously, with supplemental doses as required to maintain adequate anesthesia. A cuffed endotracheal tube was inserted and the dogs were ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.) adjusted to maintain arterial pH between 7.38 and 7.45. In group II the endotracheal tube was inserted through a tracheostomy located just above the sternal notch.

Group I consisted of 14 experiments in which intact dogs were subjected to blood volume expansion. These experiments served as a control for the group II experiments (n = 20) in which a separate group of dogs were decapitated before blood volume expansion. Particular care was taken to ensure that the animals used in these experiments were well anesthetized and unresponsive to painful stimuli.

Decapitation was accomplished with a specially designed neck vise, powered by a 4-ton hydraulic jack, which crushed the tissues of the neck including the vertebral bodies and cervical spinal cord. To ensure complete functional decapitation the blood vessels and spinal cord cephalad to the neck vise were surgically severed. To block sympathetic reflexes and obviate the severe, albeit transient, hypertension stimulated by this method of decapitation, tetracaine hydrochloride (10 mg in a volume of 1 ml) was injected into the fourth ventricle via the foramen magnum. This procedure usually limited the transient rise in mean arterial blood pressure to less than 25 mm Hg. Mean arterial blood pressure always fell after decapitation. In many instances blood pressure declined to less than 70 mm Hg in which case it was supported by infusing dopamine. The infusion of dopamine always was begun before the isolated kidney was connected to the dog's circulation and, once started, it was maintained constant throughout the study. However, concern that the use of dopamine might complicate the interpretation of the data prompted us to pursue additional studies in which dopamine was not required. Moreover, to draw meaningful comparisons between the responses to volume expansion of decapitated and intact dogs, we deemed it essential that the decapitated dogs should mimic the intact dogs as closely as possible in terms of circulatory hemodynamics before and after blood volume expansion. Thus, criteria for inclusion in the study included stable blood pressure and glomerular filtration rate within the normal range during the control period and a sustained rise in mean arterial blood pressure to at least 130 mm Hg in response to blood volume expansion. A total of 71 experiments was attempted of which 20 fulfilled these criteria. In 10 of the 20 experiments blood pressure was supported by infusion of dopamine (mean infusion rate 3.8±0.7 μg/min per kg body weight; range 1.2–6.2 μg/min per kg body weight). The high failure rate in these experiments primarily reflected the inability of the decapitated dog to generate or sustain the expected rise in blood pressure during blood volume expansion.

A minimum of 75 min was allowed for the dog to stabilize after decapitation before connecting the isolated kidney to the dog's circulation. Preparation of the isolated kidney was similar to that previously described (3). In brief, a kidney was removed from a donor dog and placed in a receptacle filled with 0.9% NaCl maintained at 38°C. The kidney was perfused with blood from the femoral artery of the experimental dog. Renal venous blood flowed by gravity into a reservoir from which it was pumped to the experimental dog's femoral vein. The dog rested on an adjustable platform and, by raising or lowering the platform, the hydrostatic pressure between the femoral artery and the isolated kidney could be altered to maintain the desired renal arterial pressure. The reservoir was filled with bovine serum albumin, 50 g/liter, in 0.9% NaCl to a volume equal to 40 ml/kg plus 200 ml and allowed to equilibrate with the dog's blood. The latter volume represents the basal volume maintained in the reservoir after volume expansion. After blood flow to the isolated kidney was established, the perfusion dog received a priming dose of inulin followed by a constant infusion of inulin in 0.9% NaCl at 1.0 ml/min to achieve a plasma inulin concentration of 20 mg/dl. Aqueous pitressin was added to the infusion to deliver 0.1 mU/kg per min. This dose was sufficient to prevent the appearance of diabetes insipidus. A minimum of 45 min was allowed for stabilization of renal function as judged by a constant urine and blood flow. After the collection of two 10-min control urine samples with midpoint blood samples, the dog was expanded with 40 ml/kg of equilibrated blood from the reservoir over a 30-min period. The volume expansion stimulus was maintained by replacing urine losses with hypotonic saline (120 meq/liter) which approximated the urinary NaCl concentration. 60 min after initiating volume expansion, two 10-min experimental urine samples were collected.

Cardiac output was measured in 16 dogs (8 in each group) by a dye dilution technique. Indocyanine green, 2.5 mg in a volume of 0.5 ml, was injected through a venous catheter advanced to the right ventricle. Aortic blood was withdrawn at a constant rate through a catheter connected to the flow-through cell of a densitometer. The signal from the densitometer was fed into an integrator-computer which corrected automatically for dye recirculation (Cardiac Output System 140, Gilford Instrument Laboratories Inc., Oberlin, Ohio). Cardiac output was determined as the mean of three cardiac output measurements obtained immediately before the first control and experimental periods.

In nine dogs in group I and eight dogs in group II blood volume was determined from the dilution of ^{125}I-albumin. Control blood volume was measured after the dog's blood volume had equilibrated with the albumin solution in the

reservoir but before connecting the isolated kidney to the dog's circulation. A second blood volume was determined at the end of the study. In both instances blood flow through the reservoir was stopped to obviate loss of ^{125}I-albumin in the extracorporeal circuit. A known quantity of ^{125}I-albumin was injected through a central venous line and precisely 15 min later a heparinized blood sample was withdrawn and assayed for radioactivity in a gamma spectrometer. In preliminary experiments it was established that equilibration of the label had occurred by 15 min and that the blood volume determined from the measurement of radioactivity in whole blood corrected for quenching was not significantly different from that determined by measurements of plasma volume and packed cell volume.

In all experiments arterial pressure of the isolated kidney was maintained constant throughout the study; renal venous pressure was maintained at 0 mm Hg. Blood pressure monitoring, urine collection, blood sampling, and the methods for monitoring pressures and for measuring glomerular filtration rate (GFR),[1] renal blood flow (RBF), inulin, electrolytes, plasma protein, and packed cell volume were the same as previously reported (3). Systemic vascular resistance (SVR) was calculated according to the formula $SVR = (\bar{P}aorta - \bar{P}cv) \div CO$ where $\bar{P}aorta$ equals mean aortic pressure, $\bar{P}cv$ equals mean central venous pressure, and CO equals cardiac output. Systemic vascular resistance was expressed in units of millimeters Hg per liter per minute.

Data in the text, tables, and figures are expressed as mean values ±SE. Student's *t* test was used for statistical analysis of paired data within each group and mean data between groups.

RESULTS

Table I summarizes the data before and after blood volume expansion in intact (group I) and decapitated (group II) dogs. Under group II the data from dogs infused with dopamine and those not receiving dopamine are presented separately. Group II dogs on dopamine tended to have a slightly higher cardiac output and lower systemic vascular resistance than did group II dogs not infused with dopamine. However, because of the small number of observations these differences did not achieve statistical significance. With respect to the other variables the two subgroups were very similar. Therefore, the data from all group II experiments were combined for statistical analysis of intra- and intergroup differences.

In the intact dogs (group I) volume expansion with equilibrated blood (40 ml/kg) was accompanied by a sharp rise in mean aortic blood pressure from 112±4 to 150±4 mm Hg ($P < 0.001$). The rise in blood pressure was accounted for primarily by the change in cardiac output which increased from 2.48±0.13 liters/min during the control period to 4.04±0.19 liters/min after volume expansion ($P < 0.001$). Systemic vascular resistance tended to decrease in response to blood volume expansion but the decline was not statistically significant. In the nine dogs in which blood volume was measured, it increased a mean of 35±5 ml/kg body weight ($P < 0.001$) after the infusion of equilibrated blood.

Volume expansion elicited a similar response pattern in the decapitated dogs (group II). Mean aortic blood pressure increased from 95±4 to 161±4 mm Hg ($P < 0.001$) which, as in group I, reflected a rise in cardiac output. In contrast to group I, there was no suggestion of a decline in systemic vascular resistance in response to blood volume expansion. The increase in blood volume of 32±5 ml/kg was not significantly different from that of group I ($P > 0.2$).

Comparison of systemic hemodynamic data for the control and volume expansion periods of the two groups revealed that mean aortic pressure during the control period of group II was significantly lower than the control blood pressure of group I, ($P < 0.01$). However, in response to blood volume expansion the mean rise in blood pressure (+66±3 mm Hg) in group II experiments was significantly greater ($P < 0.001$) than the mean rise in blood pressure (+38±3 mm Hg) of group I experiments. This difference persisted when the analysis was limited to the eight experiments from each group in which cardiac output was measured. The absolute change in cardiac output was the same in the two groups (+1.56±0.30 and +1.53±0.40 liters/min in groups I and II, respectively; $P > 0.4$). The explanation for the significantly different rise in blood pressure in the two groups during volume expansion is related to the finding that systemic vascular resistance tended to fall in group I. For example, if in group I systemic vascular resistance during volume expansion remained precisely the same as the control systemic vascular resistance, then the increase in cardiac output would have generated a rise in blood pressure of 60 mm Hg, a value similar to the rise of 66 mm Hg observed in group II experiments.

Plasma protein concentration and packed cell volume were similar in the two groups before and after volume expansion. There were no significant changes in these variables in either group in response to volume expansion.

Volume expansion elicited a significant diuresis and natriuresis in both groups (Table I). In the intact dog absolute sodium excretion ($U_{Na}V$) increased from 272±54 μeq/min during the control period to 1768 ±211 μeq/min during the volume expansion period ($P < 0.001$). Fractional sodium excretion (FE_{Na}) increased from 2.6±0.5 to 13.6±1.6%, ($P < 0.001$). The diuresis and natriuresis were associated with a significant rise in inulin clearance (C_{IN}). During the control period C_{IN} measured 71±6 ml/min and rose to 85±6 ml/min during volume expansion ($P < 0.001$). In decapitated dogs urine flow rate, $U_{Na}V$, and FE_{Na} during the control period were significantly lower

[1] *Abbreviations used in this paper*: C_{IN}, inulin clearance; FE_{Na}, fractional sodium excretion; GFR, glomerular filtration rate; RBF, renal blood flow; $U_{Na}V$, absolute sodium excretion.

TABLE I

*Summary of Data from Group I (Intact) and Group II (Decapitated) Dogs before (C) and after Volume Expansion (VE) with Equilibrated Blood**

	Body wt	$\dot{P}$aorta		CO		SVR		BV		Plasma protein		PCV		C_{IN}		V_U		$U_{Na}V$		FE_{Na}	
		C	VE	C	VE	C	VE	C	VE	C	VE	C	VE	C	VE	C	VE	C	VE	C	VE
	kg	*mm Hg*		*liters/min*		*mm Hg/ liter/min*		*liters*		*g/dl*		*%*		*ml/min*		*ml/min*		*μeq/min*		*%*	
Group I (intact) (*n*)	(14)	(14)		(8)		(8)		(9)		(14)		(14)		(14)		(14)		(14)		(14)	
Mean	20.5	112	150	2.48	4.04	40.3	33.4	2.47	3.16	4.9	4.9	22.7	22.4	71	85	2.1	14.5	272	1768	2.6	13.6
±SE	±0.6	±4	±4	±0.13	±0.19	±3.0	±1.8	±0.16	±0.14	±0.2	±0.2	±1.1	±1.0	±6	±6	±0.4	±1.7	±54	±211	±0.5	±1.6
P		<0.001		<0.001		NS		<0.001		NS		NS		<0.001		<0.001		<0.001		<0.001	
Group II (decapitated)																					
Dopamine (*n*)	(10)	(10)		(4)		(4)		(4)		(10)		(10)		(10)		(10)		(10)		(10)	
Mean	20.8	93	162	2.65	4.65	30.3	31.3	2.36	3.05	4.6	4.7	22.1	23.4	64	71	1.1	6.8	79	664	0.8	5.3
±SE	±0.7	±5	±6	±0.29	±0.70	±3.8	±4.2	±0.27	±0.36	±0.2	±0.2	±1.5	±1.7	±5	±6	±0.2	±2.3	±22	±245	±0.2	±1.6
P		<0.001		0.05 < *P* < 0.1		NS		<0.01		NS		NS		NS		<0.05		<0.05		<0.025	
No dopamine (*n*)	(10)	(10)		(4)		(4)		(4)		(10)		(10)		(10)		(10)		(10)		(10)	
Mean	21.6	97	161	1.92	3.13	45.3	46.3	2.19	2.90	4.8	4.9	22.7	24.0	60	60	1.4	6.7	79	502	0.9	5.6
±SE	±0.6	±5	±6	±0.33	±0.54	±6.2	±8.4	±0.22	±0.25	±0.1	±0.1	±1.7	±1.6	±3	±3	±0.3	±1.5	±19	±151	±0.2	±1.9
P		<0.001		<0.05		NS		<0.025		NS		NS		NS		<0.01		<0.025		<0.05	
Combined (*n*)	(20)	(20)		(8)		(8)		(8)		(20)		(20)		(20)		(20)		(20)		(20)	
Mean	21.2	95	161	2.28	3.89	37.8	38.8	2.27	2.97	4.7	4.8	22.4	23.7	62	65	1.2	6.8	79	581	0.8	5.5
±SE	±0.5	±4	±4	±0.25	±0.50	±4.4	±5.2	±0.16	±0.21	±0.1	±0.1	±1.0	±1.3	±3	±4	±0.2	±0.9	±14	±141	±0.1	±1.2
P		<0.001		<0.01		NS		<0.001		NS		NS		NS		<0.001		<0.01		<0.01	
Gr I vs. gr II																					
P	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	<0.025	<0.01	<0.001	<0.001	<0.01	<0.001

* $\dot{P}$aorta, mean aortic pressure; CO, cardiac output; SVR, systemic vascular resistance; BV, blood volume; PCV, packed cell volume; V_U, urine flow rate.

than the control urine flow rate and sodium excretion levels observed in intact dogs. Although urine flow rate, $U_{Na}V$, and FE_{Na} in decapitated dogs increased in response to blood volume expansion, the magnitude of the diuretic and natriuretic responses was impressively less than that of group I, ($P < 0.001$). Control C_{IN} was not statistically different in the two groups. However, in contrast to group I, no significant change in C_{IN} was observed in decapitated dogs in response to volume expansion. Fig. 1 illustrates the changes in $U_{Na}V$, FE_{Na}, and C_{IN} in group I and group II dogs.

Table II and Fig. 2 summarize the data from the isolated kidney in the two groups. In Table II data from group II experiments with and without dopamine infusion are presented separately. During the control period RBF per gram kidney weight was significantly greater ($P < 0.05$) in experiments in which dopamine was infused compared to experiments without dopamine (Table II). No other significant differences between the two subgroups of group II were evident. Thus, the data from the two subgroups were combined in the statistical analysis of intra- and intergroup differences.

The mean weight of the isolated kidney was similar in the two groups. There were no significant differences between the groups with respect to mean renal arterial pressure, C_{IN} per gram kidney weight, or RBF per gram kidney weight during either the control or volume expansion periods. In both groups mean renal arterial pressure was maintained constant throughout the study.

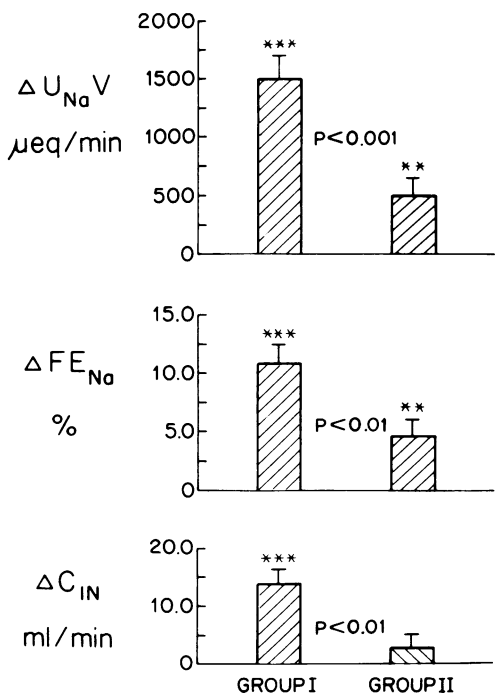

FIGURE 1 Comparison of the changes (Δ) in $U_{Na}V$, FE_{Na}, and C_{IN} in response to blood volume expansion of group I (intact) and group II (decapitated) dogs. Data are expressed as the mean±SE. **Signifies a significant change from control, $P < 0.01$. ***Signifies a significant change from control, $P < 0.001$.

No significant change in C_{IN} occurred in either group in response to volume expansion. RBF decreased to a similar extent in both groups; however, because of the greater variation of RBF in group I, the change was not statistically significant (Fig. 3). A significant diuresis and natriuresis was observed in the isolated kidney of group I experiments. $U_{Na}V$ increased from a control level of 148±34 to 284±47 μeq/min ($P < 0.01$), and FE_{Na} rose from 3.6±0.8 to 6.8±1.1% ($P < 0.01$). Control urine flow rate, $U_{Na}V$, and FE_{Na} in the isolated kidney of group II tended to be lower than that of group I. In contrast to group I experiments, no significant changes in urine flow rate, $U_{Na}V$, or FE_{Na} were observed in the isolated kidney of group II experiments after volume expansion. Fig. 3 illustrates the changes in $U_{Na}V$, FE_{Na}, C_{IN}, and RBF in the isolated kidney of group I and group II.

DISCUSSION

In the group I experiments volume expansion of the intact dog with equilibrated blood promoted a significant increase in sodium excretion by the isolated kidney perfused with blood from the femoral artery of the volume-expanded dog. The natriuresis from the isolated kidney occurred in the absence of changes in renal perfusion pressure, GFR, packed cell volume, or plasma protein concentration and in the face of a modest decline in blood flow. The fact that the isolated kidney was denervated excludes a neurogenic effector mechanism. Moreover, because the perfusion dog was preloaded with deoxycorticosterone and received a continuous infusion of vasopressin, decreases in the circulating levels of these hormones were excluded also as possible factors that influence the natriuretic response. These observations confirm our previous studies (3–5) and are consistent with the hypothesis that the increased sodium excretion from the isolated kidney is mediated by a humoral factor activated by expansion of the dog's blood volume.

In the group II experiments we sought to determine whether an intact central nervous system was essential for activating this humoral natriuretic mechanism. We resorted to functional decapitation by a technique similar to that used by Cevese and Guyton (6) except that we made no attempt to disrupt the spinal cord below the neck. This technique permitted complete interruption of neural and vascular connections to the head without appreciable blood loss. Despite the severe stress imposed by ablation of the central nervous

TABLE II

*Summary of Data from the Isolated Kidney of Group I and Group II Experiments before (C) and after Volume Expansion (VE) with Equilibrated Blood**

	n	Kidney wt	$\bar{P}_{RA}$		C_{IN}		RBF		V_U		$U_{Na}V$		FE_{Na}	
			C	VE	C	VE	C	VE	C	VE	C	VE	C	VE
		g	*mm Hg*		*ml/min/g*		*ml/min/g*		*ml/min*		*μeq/min*		*%*	
Group I (intact)														
Mean	14	46.3	111	110	0.60	0.60	6.0	5.3	1.4	3.3	148	284	3.6	6.8
±SE		±2.1	±1	±1	±0.04	±0.05	±0.4	±0.4	±0.3	±0.6	±34	±47	±0.8	±1.1
P			NS		NS		NS		<0.001		<0.01		<0.01	
Group II (decapitated)														
Dopamine														
Mean	10	40.1	113	113	0.60	0.54	6.9	5.8	0.7	0.8	78	73	2.1	2.2
±SE		±1.7	±1	±1	±0.06	±0.06	±0.7	±0.7	±0.2	±0.2	±16	±18	±0.5	±0.6
P			NS		<0.025		<0.001		NS		NS		NS	
No dopamine														
Mean	10	46.6	113	111	0.56	0.56	5.2	4.5	1.1	1.1	129	127	3.0	3.0
±SE		±2.3	±1	±1	±0.03	±0.02	±0.4	±0.3	±0.3	±0.3	±30	±32	±0.6	±0.7
P			NS		NS		<0.025		NS		NS		NS	
Combined														
Mean	20	43.3	113	112	0.57	0.55	6.1	5.2	0.9	0.9	103	100	2.6	2.6
±SE		±1.6	±1	±1	±0.03	±0.03	±0.4	±0.4	±0.2	±0.2	±17	±19	±0.4	±0.4
P			NS		NS		<0.01		NS		NS		NS	
Gr I vs. gr II														
P		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.001	NS	<0.001

* $\bar{P}_{RA}$, mean renal arterial pressure; V_U, urine flow rate.

system in this manner, it was possible, with the aid of a low infusion rate of dopamine in 10 experiments, to successfully complete 20 experiments in which the circulatory status of the decapitated dog closely approximated that of the intact dogs in group I.

Although control blood pressure was significantly lower in decapitated dogs compared to intact dogs, the two groups were remarkably similar with respect to control GFR, cardiac output, and blood volume. Furthermore, during volume expansion decapitated dogs exhibited increases in blood volume and cardiac output similar in magnitude to those observed during volume expansion of intact dogs, whereas the rise in blood pressure was significantly greater in decapitated dogs than that of intact dogs. Despite an equivalent degree of blood volume expansion and similar changes in systemic hemodynamics, the natriuretic response of decapitated dogs was significantly lower than that of intact dogs. Moreover, in contrast to group I experiments, volume expansion of decapitated dogs failed to elicit a natriuretic response by the isolated kidney. We interpret the absence of a natriuresis from the isolated kidney in group II as evidence that volume expansion of decapitated dogs failed to activate the humoral natriuretic mechanism observed during volume expansion of intact dogs.

Although the derivative of blood volume that elicits what may be presumed to be a volume receptor reflex remains unknown, it is unlikely that the failure to activate the humoral natriuretic mechanism in decapitated dogs reflected an inadequate volume stimulus. As noted above decapitated dogs demonstrated increases in blood volume and cardiac output similar to that of intact dogs and a rise in arterial blood pressure that exceeded the rise in pressure of intact dogs. Rather, the data are most consistent with the interpretation that decapitation interrupted a volume receptor reflex involving a humoral natriuretic mechanism. Thus, it is possible that the brain is the source of the natriuretic factor or, alternatively, that the brain is an essential link of a neurohumoral reflex which activates the humoral natriuretic mechanism at some site removed from the brain. The results of our experiments do not discriminate between these two possibilities.

Studies by other investigators have implicated the brain as the source of the natriuretic factor. Klahr and Rodriguez (1) reported that blood obtained from patients with head trauma who were natriuretic contained

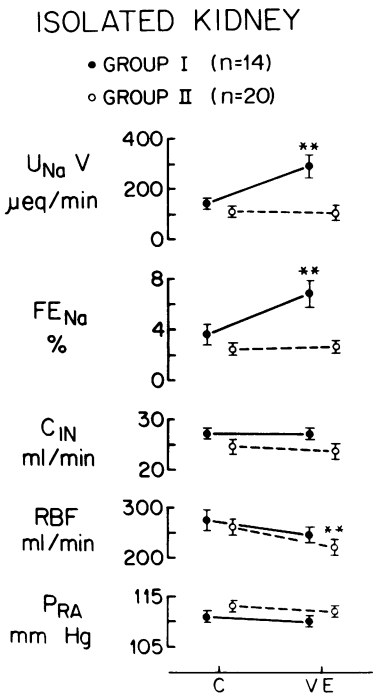

FIGURE 2 Summary of function in the isolated kidney before (C) and after volume expansion (VE) of intact (group I) and decapitated (group II) dogs. $\bar{P}_{RA}$, mean renal arterial pressure. **Signifies a significant change from C, $P < 0.01$.

a factor that inhibited sodium transport in the frog skin, whereas this factor was not present in the blood of patients with central nervous system lesions who were not natriuretic. Cort et al. (7) detected natriuretic activity in jugular venous blood but not in femoral venous blood. Buckalew et al. (8) reported that ultrafiltrates of jugular venous blood had a greater inhibitory effect on short circuit current in the toad bladder than did ultrafiltrates of femoral venous blood. Other investigators have suggested that the hypothalamus (9) or the hypophysis (10, 11) is the source of the factor. However, we have presented evidence in a previous paper which argues against the latter possibility (5). Although the above studies lend support to the concept that the brain is the source of the humoral natriuretic factor, more definitive studies are required to answer this question.

It should be noted that other investigators have examined the effect of decapitation on the natriuretic response to volume expansion and have reached conclusions opposite those of the present study. The observation that decapitated dogs still exhibited a modest natriuresis in response to saline loading led Levinsky to conclude that the postulated natriuretic hormone did not derive from the brain (12). Johnston and colleagues (13), with a cross-circulation technique, reported that decapitation of the donor dog did not abolish the natriuretic response to saline loading of the cross-circulated recipient dog. However, the fact that decapitation did not abolish natriuresis in either study does not negate the concept that the brain plays a critical role in the activation of natriuretic factor. It is now appreciated that changes in plasma composition and physical factors that attend saline loading could have mediated the natriuresis observed in the studies of Levinsky (12) and Johnston et al. (13).

Cevese and Guyton (6) circumvented the problem of changes in plasma composition and physical factors by infusing autologous blood. Dogs made areflexive by decapitation and destruction of the spinal cord exhibited a significantly greater diuresis and natriuresis than did intact dogs in response to isohemic blood

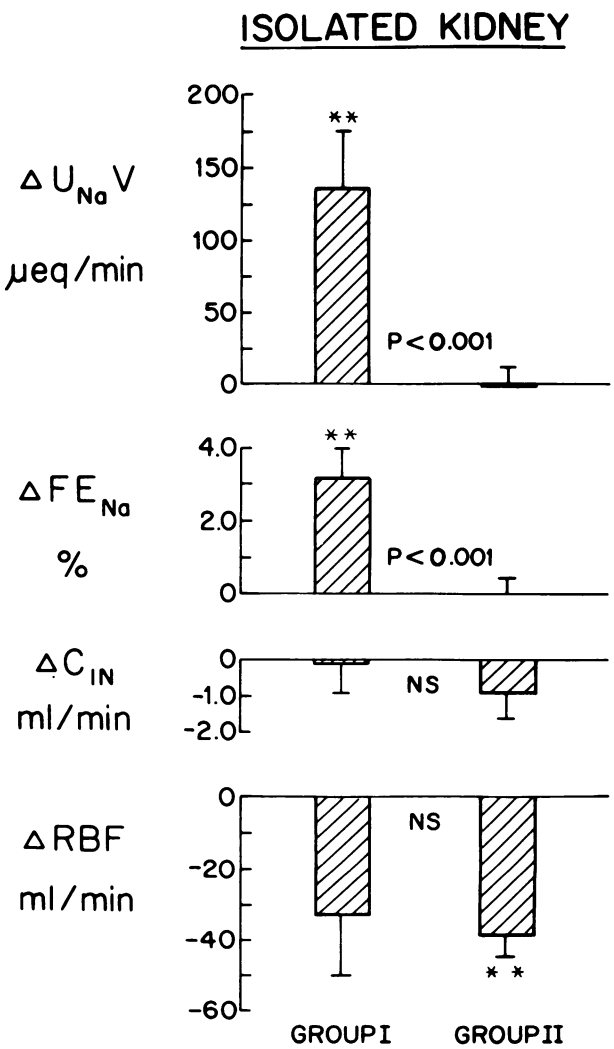

FIGURE 3 Comparison of the changes (Δ) in $U_{Na}V$, FE_{Na}, C_{IN}, and RBF in the isolated kidney in response to blood volume expansion of group I (intact) and group II (decapitated) dogs. **Signifies a significant change from control, $P < 0.01$; NS, not significant.

volume expansion. In fact no significant natriuresis was observed in intact dogs. The greater diuretic and natriuretic response of decapitated dogs was attributed to the marked rise in arterial pressure accompanied by increases in GFR and RBF. The authors concluded that their data provided no evidence for the elaboration of a natriuretic factor in response to blood volume expansion, which is true. However, it should be emphasized that their experiments were not designed to address this question, and thus, provide no evidence for or against the natriuretic hormone hypothesis.

The absence of a significant natriuresis during acute blood volume expansion of intact dogs in the study of Cevese and Guyton (6) stands in sharp contrast to the marked natriuresis observed in our experiments and underscores several major differences between the experimental designs of the two studies. Cevese's and Guyton's experiments focused on the circulatory adjustments to an acute volume expansion stimulus administered as a pulse over 5 min. In contrast, we employed a larger volume stimulus (40 compared to 25 ml/kg body weight) which was given over a 30-min period and the stimulus was sustained by the replacement of urinary losses. Moreover, the dogs in our study were preconditioned by chronic administration of deoxycorticosterone and salt to magnify the natriuretic response to blood volume expansion. Thus, under the conditions of our experiments FE_{Na} from intact dogs increased 11.0±1.5%, whereas in decapitated dogs the natriuretic response to blood volume expansion, although significant, was greatly attenuated (FE_{Na} increased 4.7±1.1%).

We do not infer that the attenuated natriuretic response of decapitated dogs was caused solely by the failure of blood volume expansion to activate the humoral natriuretic mechanism. Judging by the magnitude of the natriuresis of the isolated kidney in group I, it is unlikely that the humoral natriuretic factor augmented sodium excretion in intact dogs by much more than 3% of the filtered load. Because the difference in sodium excretion after expansion between intact and decapitated dogs was ≅6.3% of the filtered load, it may be presumed that decapitation impaired the activation or recruitment of other natriuretic mechanisms as well. Our experiments were not designed to examine this aspect of the problem. However, one obvious difference between the two groups was the absence of a significant rise in GFR in decapitated dogs. Although RBF was not measured in either group of dogs, we speculate that decapitation may have abrogated neurogenically mediated renal vasodilation (14, 15).

In conclusion, the results of our experiments provide additional evidence that a humoral factor, possibly a natriuretic hormone, participates in the natriuresis of blood volume expansion. We are impressed by the fact that of the four organ ablation studies performed in this experimental model (5), only the maneuver of decapitation has abolished the humoral natriuretic mechanism. This observation suggests that the brain is either the source of the factor or that the brain participates in the reflex activation of this factor from some other site.

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REFERENCES

1. Klahr, S., and H. J. Rodriguez. 1975. Natriuretic hormone. *Nephron.* **15:** 387–408.
2. Bricker, N. S., R. W. Schmidt, H. Favre, L. Fine, and J. J. Bourgoignie. 1975. On the biology of sodium excretion: the search for a natriuretic hormone. *Yale J. Biol. Med.* **48:** 293–303.
3. Kaloyanides, G. J., and M. Azer. 1971. Evidence for a humoral mechanism in volume expansion natriuresis. *J. Clin. Invest.* **50:** 1603–1612.
4. Kaloyanides, G. J., G. F. DiBona, and R. D. Bastron. 1975. Effect of blood volume expansion on tubule sodium transport in the isolated dog kidney. *Proc. Soc. Exp. Biol. Med.* **148:** 765–773.
5. Kaloyanides, G. J., L. Cohen, and G. F. DiBona. 1977. Failure of selected endocrine organ ablation to modify the natriuresis of blood volume expansion in the dog. *Clin. Sci. Mol. Med.* **52:** 351–356.
6. Cevese, A., and A. C. Guyton. 1976. Isohemic blood volume expansion in normal and areflexive dogs. *Am. J. Physiol.* **231:** 104–111.
7. Cort, J. H., T. Dousa, V. Pliska, B. Lichardus, J. Safarova, M. Vranesic, and J. Rudinger. 1968. Saluretic activity of blood during carotid occlusion in the cat. *Am. J. Physiol.* **215:** 921–927.
8. Buckalew, V. M. Jr., F. J. Martinez, and W. E. Green. 1970. The effect of dialysates and ultrafiltrates of plasma of saline-loaded dogs on toad bladder sodium transport. *J. Clin. Invest.* **49:** 926–935.
9. Clarkson, E. M., K. G. Koutsaimanis, M. Davidman, M. DuBois, W. P. Penn, and H. E. DeWardener. 1974. The effect of brain extracts on urinary sodium excretion of the rat and the intracellular sodium concentration of renal tubule fragments. *Clin. Sci. Mol. Med.* **47:** 201–213.
10. Lichardus, B., and J. Ponec. 1972. Effect of hypophysectomy on sodium excretion in rats without blood dilution during blood volume expansion. *Experientia (Basel).* **28:** 471–472.
11. Lichardus, B., and J. Ponec. 1972. Neurohypophyseal origin of a humoral factor restoring volume natriuresis in acutely hypophysectomized rats. *Experientia (Basel).* **28:** 1443–1445.
12. Levinsky, N. G. 1966. Nonaldosterone influences on renal sodium transport. *Ann. N. Y. Acad. Sci.* **139:** 295–303.
13. Johnston, C. I., J. O. Davis, S. S. Howards, and F. S. Wright. 1967. Cross-circulation experiments on the mechanism of the natriuresis during saline loading in the dog. *Circ. Res.* **20:** 1–10.
14. Pelletier, C. L., and J. T. Shepherd. 1973. Circulatory reflexes from mechanoreceptors in the cardio-aortic area. *Circ. Res.* **23:** 131–138.
15. Kahl, F. R., J. F. Flint, and J. P. Szidon. 1974. Influence of left atrial distention on renal vasomotor tone. *Am. J. Physiol.* **226:** 240–246.