Failure to Demonstrate a Humoral Mechanism in the Antinatriuresis of Acute Caval Constriction

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ABSTRACT Previous studies reported from this laboratory provided support for the hypothesis that the natriuresis of volume expansion is mediated in part by a humoral mechanism. In the present study we examined whether suppression of this factor participates in the antinatriuresis of acute constriction of the thoracic inferior vena cava. An isolated kidney was perfused by a second dog pretreated with deoxycorticosterone acetate. Expansion of the perfusion dog with equilibrated blood from a reservoir resulted in an increase in sodium excretion from 102±30 to 259±65 μEq/min, $P < 0.01$. Fractional sodium excretion increased from 2.3±0.6 to 6.2±1.2%, $P < 0.01$. Inulin clearance, plasma protein concentration, and packed cell volume remained constant; renal perfusion pressure and renal blood flow decreased. After the natriuresis was established, the thoracic inferior vena cava was constricted to decrease systemic arterial pressure in the perfusion dog 50 mm Hg. This maneuver suppressed urine output in the dog but did not significantly alter sodium excretion in the isolated kidney. During the period of caval constriction absolute sodium excretion in the isolated kidney measured 198±42 μEq/min and fractional sodium excretion measured 5.7±1.1%. Neither value is significantly different from that measured during volume expansion alone. The data suggest that the antinatriuresis of acute caval constriction probably does not require suppression of a humoral natriuretic factor and that other more rapidly acting mechanisms, presumably hemodynamic and neural, may be involved.

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INTRODUCTION

Constriction of the thoracic inferior vena cava in the dog is a potent stimulus for salt and water retention by the kidneys (1). The renal mechanisms involved in this response appear to involve such factors as reduction in perfusion pressure, alterations in renal blood flow, and increase in renal nerve stimulation (2–5). In addition, however, there is evidence which suggests that a humoral mechanism unrelated to mineralocorticoid activity may participate in the antinatriuresis (6, 7).

In a recent report (8) from this laboratory we presented phenomenological evidence in support of the hypothesis that the natriuresis associated with blood volume expansion is mediated in part by a humoral factor. The present investigation was designed to test the hypothesis that suppression of this factor contributes to the antinatriuresis of caval constriction.

METHODS

Experiments were performed on mongrel dogs weighing 15–30 kg. One dog served as the kidney donor; the second dog was used to perfuse the isolated kidney. The donor animals were fed a standard kennel ration whereas the perfusion dog was pretreated with 10 mg deoxycorticosterone acetate (DOCA, Organon Inc., West Orange, N. J.) in oil given intramuscularly each day for an average of 14 days including the morning of the study. In addition the daily diet of these animals was supplemented with 75–150 mEq of NaCl and 40–80 mEq of KCl. In nine animals about 1 wk before initiating DOCA [1] an inflatable cuff constrictor was placed around the thoracic inferior vena cava (TIVC) and exteriorized on the skin.

[1] *Abbreviations used in this paper:* C_{In}, inulin clearance; DOCA, deoxycorticosterone acetate; FE_{Na}, fractional sodium excretion; GFR, glomerular filtration rate; PCV, packed cell volume; P_{RA}, renal arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; TIVC, thoracic inferior vena cava; $U_{Na}V$, sodium excretion.

TABLE I

*Summary of Data from the Isolated Kidney in Group I**

Period	$U_{Na}V$	FE_{Na}	C_{In}	RBF	P_{RA}	RVR	FF	PCV	Plasma protein
	μEq/min	*%*	*ml/min*	*ml/min*	*mm Hg*	*PRU*		*%*	*g/100 ml*
Control	102±30	2.3±0.6	28.9±2.1	258±19	112±2	0.43±0.03	0.16±0.01	30±1	4.7±0.1
Volume expansion	259±65‡	6.2±1.2‡	27.1±2.5	210±18‡	108±2‡	0.53±0.05‡	0.18±0.01	28±2	4.7±0.1
Caval constriction	198±42§	5.7±1.1‡	24.3±2.2‡	172±19‡	109±2§	0.67±0.07‡	0.21±0.02§	31±3	4.5±0.1§

* $U_{Na}V$, sodium excretion; FE_{Na}, fractional sodium excretion; C_{In}, inulin clearance; RBF, renal blood flow; P_{RA}, renal arterial pressure; RVR, renal vascular resistance; PRU, peripheral resistance units; FF, filtration fraction; PCV, packed cell volume. Data represent mean ±SEM, n = 9.

‡ Significantly different from control, $P < 0.01$.

§ Significantly different from control, $P < 0.05$.

On the morning of the study the animals were anesthetized with sodium pentobarbital, 30 mg/kg, given intravenously and supplemental doses were given as required to maintain light anesthesia. An endotracheal tube was inserted and respirations were regulated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.) adjusted to maintain arterial pH between 7.35 and 7.45.

Preparation of the isolated kidney was performed as previously described (8). In brief, a kidney was removed from the donor dog and perfused with blood from the femoral artery of the perfusion dog. Renal venous blood flowed by gravity into a reservoir from which it was pumped (Holter roller pump, model RE161, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.) to the femoral vein of the perfusion dog at a rate adjusted to maintain the blood level in the reservoir constant. The perfusion animal rested on an adjustable platform and by raising or lowering the platform a hydrostatic pressure equal to the difference between the height of the animal and the isolated kidney could be added to or subtracted from the femoral arterial pressure supplied to the kidney. In this manner it was possible to regulate renal arterial pressure independent of changes in the perfusion dog's systemic arterial pressure.

In all experiments the reservoir was filled with 5% albumin in 0.9% saline in an amount equal to 35 ml/kg plus 200 ml. The latter volume represents the basal volume maintained in the reservoir after volume expansion. After perfusion of the isolated kidney was established, the perfusion animal received a priming dose of inulin followed by a constant infusion of inulin in 0.9% saline at 1.0 ml/min. Aqueous Pitressin (Parke, Davis & Co., Detroit, Mich.) was added to the infusion to deliver 0.5 mU/kg per min. A minimum of 60 min was allowed for equilibration between the perfusion animal's blood and the volume in the reservoir and for stabilization of renal function.

Group I consisted of nine experiments. After renal function had stabilized, two 15-min control urines were collected after which the perfusion animal was expanded with 35 ml/kg of equilibrated blood from the reservoir over a 30 min period. 60 min after initiating volume expansion two 15-min experimental urines were collected. Then the constrictor on the TIVC was inflated until systemic arterial pressure was reduced approximately 50 mm Hg. 60 min after the onset of TIVC constriction two 15-min urines were collected from the isolated kidney. During the volume expansion and caval constriction periods renal arterial pressure in the isolated kidney was maintained slightly below control levels.

Group II consisted of seven studies in which function of the isolated kidney was monitored over the same time interval as in group I, but in the absence of volume expansion or caval constriction.

The monitoring of pressures, analysis of samples, and calculation of data were performed as previously described (8).

Student's *t* test was used in the statistical analysis of paired data within each group. The data in the text and tables are expressed as the mean ±SEM.

TABLE II

*Summary of Data from the Isolated Kidney in Group II**

Period	$U_{Na}V$	FE_{Na}	C_{In}	RBF	P_{RA}	RVR	FF	PCV	Plasma protein
	μEq/min	*%*	*ml/min*	*ml/min*	*mm Hg*	PRU		*%*	*g/100 ml*
Control 1	114±31	2.9±0.5	24.8±4.1	198±30	110±2	0.59±0.07	0.18±0.01	32±2	4.7±0.2
Control 2	89±19	2.6±0.6	24.3±4.0	156±25‡	110±3	0.75±0.09‡	0.23±0.03§	32±2	4.4±0.3
Control 3	62±9§	1.9±0.3§	23.6±3.6	124±14‡	111±2	0.91±0.08‡	0.27±0.02§	32±2	4.5±0.1

* See Table I for explanation of abbreviations. Control periods 1, 2, and 3 correspond to the same time interval of the three periods in group I except that there was no volume expansion or caval constriction. Data represent mean ±SEM, n = 7.

‡ Significantly different from control, $P < 0.01$.

§ Significantly different from control, $P < 0.05$.

RESULTS

The data from the group I experiments are summarized in Table I. Similar to the results previously reported (8), volume expansion of the perfusion dog resulted in a significant increase in absolute ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) in the isolated kidney in the face of a constant C_{In} and a decrease in RBF and P_{RA}. Over the same time interval urine flow in the perfusion dog increased from 1.3±0.3 to 14.0±2.5 ml/min, $P < 0.001$. After constriction of the TIVC urine output in the perfusion dog was severely depressed. In four animals there was no detectable urine flow from the bladder; in the remaining animals urine output was markedly reduced below control levels to the extent that it was not possible to obtain adequate urine samples for analysis. We interpreted the suppression of urine output in the perfusion dog as evidence that the degree of caval constriction utilized constituted a potent antinatriuretic stimulus. In contrast, after 60 min of caval constriction $U_{Na}V$ and FE_{Na} in the isolated kidney remained significantly higher than control levels, and were not statistically lower than the levels achieved in the volume expansion period. C_{In} and RBF, however, decreased significantly during the period of caval constriction which might explain the fall in $U_{Na}V$. RVR and filtration fraction increased progressively during the course of the experiments. PCV did not change, whereas plasma protein concentration decreased slightly during the period of caval constriction.

The results of the group II experiments are summarized in Table II. In the absence of volume expansion and caval constriction a spontaneous decrease in $U_{Na}V$ and FE_{Na} was observed along with a fall in RBF and rise in RVR. C_{In} did not change significantly.

DISCUSSION

These studies were designed to test the hypothesis that the antinatriuresis induced by constricting the thoracic inferior vena cava might be mediated in part by suppression of a humoral natriuretic factor. The rationale for this hypothesis derives from the report of Davis, Holman, Carpenter, Urquhart, and Higgins (6) that dogs subjected to bilateral adrenalectomy and unilateral nephrectomy with the remaining kidney transplanted to the neck still exhibited sodium retention and ascites formation in response to constriction of the thoracic inferior vena cava. The authors were able to exclude such variables as glomerular filtration rate, renal plasma flow, renal nerve activity, and mineralocorticosteroids as factors mediating the antinatriuresis and therefore postulated an "extra-adrenal factor," presumably humoral, was necessary for sodium retention. More recently Schrier, Humphreys, and Ufferman (7) reported the results of their studies which indicate that the antinatriuresis of caval constriction cannot be explained entirely by changes in renal hemodynamics or renal nerve activity and thus also raise the possibility that a humoral factor unrelated to mineralocorticosteroid activity contributes to the antinatriuresis.

In a recent study (8) reported from this laboratory we presented evidence of a phenomenological nature in support of the hypothesis that the natriuresis of extracellular volume expansion was mediated in part by a humoral mechanism. In view of the above considerations it seemed reasonable that if extracellular volume expansion activated release of a natriuretic factor, then caval constriction might produce the opposite effect, namely suppress this factor, and thereby promote sodium retention.

The first part of this study involved demonstrating the presence of a humoral natriuretic mechanism. The observation that expanding the perfusion dog with equilibrated blood effected an increase in sodium excretion by the isolated kidney unrelated to changes in GFR, RBF, renal perfusion pressure, plasma colloid osmotic pressure, or packed cell volume supports such a mechanism. After the natriuresis was established, the thoracic inferior vena cava of the perfusion dog was constricted and although this maneuver suppressed urine output in the dog, it did not significantly alter sodium excretion in the isolated kidney. The persisting natriuresis in the isolated kidney suggests that either caval constriction did not suppress the release of the natriuretic factor activated by volume expansion or the biological half-life of this factor was sufficiently long that no change in activity could be detected during the period of experimental observation. If the latter explanation is correct, then the data would argue against an important role for this factor in modulating moment to moment changes in renal sodium excretion.

Regardless which explanation pertains, the data provide sufficient evidence to reject the proposed hypothesis. Since urine output in the perfusion dog was suppressed, acute constriction of the thoracic inferior vena cava must have activated a potent antinatriuretic mechanism(s) sufficient to antagonize the humoral natriuretic mechanism of volume expansion. The experimental design employed did not permit us to evaluate this mechanism. Nevertheless, in view of the marked decrease in systemic arterial pressure induced during caval constriction it seems likely that hemodynamic and neural factors must have played a role. In addition, however, the data do not exclude the possibility that TIVC constriction does activate a humoral antinatriuretic mechanism, but that this mechanism may be inadequate, by itself, to antagonize the humoral natriuretic mechanism of volume expansion.

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