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Response of the Isolated Kidney to Acute Volume Expansion with Equilibrated Blood[1] (38401)

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We have previously reported that volume expansion with equilibrated blood of a chronically salt and DOCA loaded dog elicits a natriuretic response in an isolated dog kidney perfused with blood from the volume expanded dog (1). Since the natriuresis in the isolated kidney could not be related to changes in renal hemodynamics or physical factors, we postulated that a humoral mechanism other than mineralocorticosteroids or vasopressin mediated the increase in sodium excretion. In view of the magnitude of the natriuresis it seems possible that this humoral mechanism could play a physiological role in the renal regulation of sodium balance. The question arises, however, whether this mechanism participates in the daily regulation of sodium balance or whether it represents an adaptative response to chronic extracellular volume expansion that attends the chronic administration of mineralocorticosteroids.

To evaluate if a similar mechanism might participate in the natriuretic response to acute volume expansion we performed a second series of experiments identical to that previously reported (1) except the dog was not chronically loaded with DOCA and salt.

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. All animals were fed a standard kennel ration; the perfusion dog received 15 mg desoxycorticosterone acetate (DOCA) in oil given intramuscularly on the morning of the study. The animals were anesthetized with sodium pentobarbital 30 mg/kg, given intravenously with supplemental doses as required to maintain light anesthesia. An endotracheal tube was inserted and respirations were regulated with a Harvard respirator adjusted to maintain arterial pH between 7.35 and 7.45.

The preparation of the isolated kidney was similar to that previously described (1). In all experiments the reservoir was filled with 5% bovine albumin in 0.9% saline in an amount equal to 35 ml/kg body wt plus a basal volume of 200 ml. After perfusion of the isolated kidney was established, the perfusion animals received a priming dose of inulin followed by a constant infusion of inulin calculated to achieve a plasma inulin concentration of 20 mg/100 ml. Aqueous pitressin was added to the infusion to deliver 0.5 milliunits/kg/min. A minimum of 60 min was allowed for the solutions to equilibrate and renal function to stabilize before urine samples were collected.

Group I consisted of 13 experiments. Following the collection of two 15-min control urine and midpoint blood samples the dog was expanded with equilibrated blood (35 ml/kg) from the reservoir over a 30-min period and the volume expansion stimulus was maintained by replacing urine output with equal volumes of 0.12 *M* NaCl. Sixty minutes following the start of volume expansion two 15-min experimental urine and midpoint blood samples were collected. Throughout the study renal arterial pressure (P_{RA}) was maintained constant at approximately 104 mmHg and renal venous pressure was maintained at 0 mmHg.

Group II consisted of nine experiments which were identical to group I except the dog was not expanded between the control and experimental urine samples. These experiments served as a time-function control for the group I experiments.

Group III consisted of experiments previously published describing the natriuretic response to volume expansion with equilibrated blood in dogs chronically loaded with DOCA and salt (1).

Blood pressure monitoring and recording,

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urine collection and blood sampling were carried out as previously described (1). All blood and urine samples were analyzed for sodium and inulin; packed cell volume (PCV) and plasma protein concentration were determined on all blood samples. Sodium was measured with an Instrumentation Laboratories flame photometer. Inulin was determined by the method of Fuhr *et al.* (2) and plasma protein concentration by refractometry (Goldberg refractometer, American Optical Company, Buffalo, NY). PCV was determined using a microhematocrit centrifuge. Renal blood flow (RBF) was measured directly by timing the flow from the renal vein into a graduated cylinder.

The data in the text and table are expressed as the mean ± SE. Student's *t* test for paired and nonpaired data was used for statistical analysis.

Results. The data from groups I and II are summarized in Fig. 1 and Table I. Similar to our previous study we observed a significant increase in absolute ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) in group I following volume expansion with equilibrated blood. $U_{Na}V$ increased from 93 ± 20 to 131 ± 24 μEq/min ($P < 0.05$) and FE_{Na} rose from 2.0 ± 0.4 to 3.0 ± 0.5 ($P < 0.025$). The increase in sodium excretion was accompanied by a slight increase in urine flow from 0.8 ± 0.2 to 1.2 ± 0.3 ml/min ($P < 0.05$). In the absence of volume expansion (group II) sodium excretion and urine volume remained unchanged. C_{IN} remained constant in both groups, whereas RBF fell to the same extent in both groups due to an increase in renal vascular resistance similar to that observed previously in this preparation. P_{RA} remained constant in both groups. Although a slight but significant fall in PCV and plasma protein concentration occurred between the control and experimental periods, the magnitude of change was similar in both groups.

Discussion. The present experiments confirm the results of our previous study (1) that volume expansion of a dog with equilibrated blood effects a significant increase in sodium excretion from an isolated kidney perfused with blood from the expanded animal. The natriuresis occurred in the face of a constant renal perfusion pressure and glomerular filtration rate and despite a significant fall in RBF. Although decreases in PCV and plasma protein concentration have been demonstrated to depress tubular sodium reabsorption (3–5), it is unlikely that the

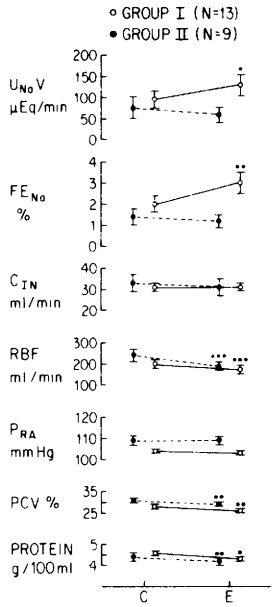

FIG. 1. Summary of data from group I (volume expansion) and group II (no volume expansion) during the control (C) and experimental (E) periods. $U_{Na}V$ = absolute sodium excretion, FE_{Na} = fractional sodium excretion, C_{IN} = inulin clearance, RBF, = renal blood flow, P_{RA} = renal arterial pressure, PCV = packed cell volume. Asterisks indicate significant change from control. * = $P < 0.05$, ** = $P < 0.025$, *** = $P < 0.01$.

slight changes in these variables observed in the present experiments can account for the natriuresis. The fact that similar changes in PCV and plasma protein concentration were noted in the control group but without an increase in urinary sodium excretion argues against this possibility. Indeed since there were no significant differences between the two groups with respect to changes in renal hemodynamics or physical factors, it seems reasonable to postulate that volume expansion of the dog activated release of a humoral factor which depressed sodium reabsorption in the isolated kidney.

Subsequent studies suggest that this factor most likely exerts a direct effect on tubule sodium transport rather than indirectly through a change in intrarenal distribution of blood flow or glomerular filtration rate (6). The identity of this factor remains unknown. However, organ ablation studies argue against the adrenal, parathyroid, thyroid or pituitary gland being the source (7). Whether this factor plays a physiological role in regulating the renal han-

TABLE I. Summary of Data From the Isolated Kidney of Groups I, II and III During the Control (C) and Experimental (E) Periods.[a]

	$U_{Na}V$ (μEq/min)		FE_{Na} (%)		C_{IN} (ml/min)		RBF (ml/min)		P_{RA} (mmHg)		PCV (%)		Plasma protein (g/100 ml)	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Group I (N = 13) Acute volume expansion in the absence of chronic DOCA and NaCl loading														
Mean	93	131[b]	2.0	3.0[b]	31	31	200	159	104	103	28	26	4.6	4.3
± SE	± 20	± 24	±.4	±.5	± 2	± 3	± 18	± 13	± 1	± 1	± 1	± 1	±.1	±.1
P	< 0.05		< 0.025		NS		< 0.01		< 0.01		< 0.025		< 0.05	
Group II (N = 9) No volume expansion														
Mean	77	62	1.4	1.2	33	31	243	189	110	110	31	29	4.4	4.2
± SE	± 28	± 18	±.4	±.3	± 4	± 4	± 29	± 18	± 3	± 3	± 1	± 1	±.2	±.2
P	NS		NS		NS		< 0.025		NS		< 0.01		< 0.01	
Group III (N = 14) Acute volume expansion superimposed on chronic DOCA and NaCl loading														
Mean	154	346	3.4	8.1[c]	31	29	239	189	114	109	26	27	4.6	4.5
± SE	± 28	± 58	±.6	±1.2	± 2	± 2	± 19	± 17	± 1	± 1	± 1	± 1	±.1	±.2
P	< 0.001		< 0.01		< 0.01		< 0.001		< 0.001		NS		NS	

[a] $U_{Na}V$, absolute sodium excretion; FE_{Na}, fractional sodium excretion; C_{IN}, inulin clearance; RBF, renal blood flow; P_{RA}, renal arterial pressure; PCV, packed cell volume.

[b] Group I significantly different from group II, $P < 0.05$;

[c] Group III significantly different from group I, $P < 0.01$.

dling of sodium will be answered only if and when the factor is identified and its action characterized. The present studies suggest that in response to acute volume expansion the humoral factor may promote excretion of at least 1% of the filtered sodium load and thus warrant consideration of the possibility that this mechanism may participate in the daily modulation of urinary sodium excretion.

The magnitude of the natriuresis observed in the present experiments ($\Delta U_{Na}V = 39 \pm 11$ μEq/min: $\Delta FE_{Na} = 1.0 \pm 0.3\%$) was significantly less ($P < 0.01$) than that noted in our previous study ($\Delta U_{Na}V = 192 \pm 36$ μEq/min; $\Delta FE_{Na} = 4.7 \pm 0.7\%$). The major difference in experimental design between the two studies was that in the present experiments the perfusion dog was not chronically loaded with DOCA and salt whereas in our previous study we had pretreated the perfusion dog with DOCA and NaCl for eleven days and most likely had induced the DOCA-escape phenomenon in these animals. It should be emphasized, however, that in all experiments the isolated kidney was obtained from a non-DOCA loaded dog and that changes in renal hemodynamics and physical factors were quite similar in all groups. Accordingly, the difference in sodium excretion by the isolated kidney of the various groups reflects primarily the influence of the milieu, *i.e.*, the perfusate, on renal function. Thus, it is possible that the greater natriuresis seen following volume expansion of DOCA-loaded animals indicates an adaptative increase in the activity of this humoral mechanism in response to chronic extracellular volume expansion. Buckalew and Lancaster (8) have reported finding a humoral inhibitor of sodium transport in the plasma of dogs injected with DOCA similar to that found in dogs expanded with saline. Whether the level of this factor is higher following acute volume expansion in DOCA-loaded dogs compared to normal animals is not clear from their study.

If expansion of the extracellular volume activates a humoral natriuretic mechanism, it might be expected that the circulating level of this factor would be elevated in the DOCA treated animals even during the control period since these animals presumably were in a state of chronic volume expansion. Consequently, control sodium excretion of isolated kidneys perfused with blood from DOCA-loaded animals should be higher than that of kidneys perfused with blood from non-DOCA loaded animals. Indeed inspection of Table I reveals that the control absolute and fractional sodium excretion tended to be higher in group III than in group I; however, the difference did not reach statistical significance ($0.05 < P < 0.1$). Development of a more sensitive assay may provide an answer to this interesting question.

Summary. The effect of acute volume expansion of a dog with equilibrated blood on sodium excretion in an isolated kidney perfused with blood from the volume expanded animal was examined. A significant increase in absolute and fractional sodium excretion was observed in the isolated kidney that could not be explained on the basis of changes in renal hemodynamics or physical factors and thus suggests that a humoral mechanism may have mediated the natriuretic response. The magnitude of the natriuresis was less than that previously reported when the isolated kidney was perfused with blood from a chronically DOCA and NaCl loaded animal. It is suggested that a humoral natriuretic mechanism may participate in the renal regulation of sodium balance and that the activity of this mechanism may undergo an adaptive increase in response to chronic extracellular volume expansion

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