Effect of Volume Expansion on Sodium Excretion in the Presence and Absence of Increased Delivery from Superficial Proximal Tubules

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ABSTRACT The role of the proximal tubule in the natriuresis after volume expansion was investigated by evaluating sodium excretion both in the presence and absence of increased delivery from the proximal tubule. Proximal delivery was calculated from fractional reabsorption in superficial proximal tubules determined by micropuncture and glomerular filtration rate of the micropunctured kidney. Infusion of Ringer's solution in six dogs increased delivery from the proximal tubule 4.7±1 ml/min ($P < 0.01$) and increased fractional sodium excretion 3.6±1.1% ($P < 0.025$). Infusion of hyperoncotic albumin into the renal artery during sustained volume expansion decreased delivery from the proximal tubule 6.5±0.9 ml/min ($P < 0.01$). Although proximal delivery was restored to below control levels, fractional sodium excretion was significantly increased 2.5±0.5% ($P < 0.01$) as compared with the hydropenic control period. Fractional phosphate excretion was increased 15.5± 3.7% ($P < 0.01$) after Ringer's infusion and was decreased 10.5±1.6% ($P < 0.005$) after intrarenal albumin infusion, suggesting that changes in superficial nephron reabsorption were paralleled by changes in reabsorption in deeper nephrons. Similar results were found in six additional dogs in which other factors known to affect phosphate reabsorption were controlled; however, these studies cannot completely eliminate a role for deep nephrons in the natriuresis after intrarenal albumin infusion. Since 70% of the natriuresis after volume expansion was present without increased delivery from superficial proximal tubules, it is likely that increased delivery from the proximal tubule contributes a relatively minor fraction to the natriuresis of volume expansion.

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INTRODUCTION

Expansion of the extracellular fluid volume is accompanied by a marked decrease in fractional sodium reabsorption by the proximal tubule of the dog (1). The resulting increased delivery from the proximal tubule was more than sufficient to account for the ensuing natriuresis and consequently was considered responsible for the natriuresis. However, Howards, Davis, Knox, Wright, and Berliner (2) have shown that preferential expansion of the plasma volume results in a marked decrease in proximal sodium reabsorption, accompanied by only a small increase in sodium excretion, compared with the large increase in sodium excretion after extracellular fluid volume expansion. They inferred a relative decrease in sodium reabsorption in some distal segment of the nephron after extracellular fluid volume expansion; however, it is possible that differences in delivery from the proximal tubule between the two groups of dogs accounted for the differences in sodium excretion. In the present studies, oncotic pressure in the peritubule microcirculation was elevated to increase reabsorption by the proximal tubule during sustained volume expansion. Thus, the role of alterations in sodium reabsorption by the proximal tubule after volume expansion was investigated by evaluating sodium excretion both in the presence and absence of increased delivery from the proximal tubule in the same dog.

METHODS

Three groups of dogs were examined with the following protocols, each consisting of three phases. In the first group of six dogs, measurements were made during continued

hydropenia, after extracellular fluid volume expansion with Ringer's solution, and during infusion of hyperoncotic albumin solution into the renal artery during sustained volume expansion. Dogs were prepared for micropuncture and clearance measurements as previously described (3). A 20-gauge curved needle was inserted against the flow of blood into the left renal artery for infusion of albumin solution. After control periods, modified dog Ringer's solution[1] was infused at 1 ml/min/kg of body weight for 20 min and then infused at 0.5 ml/min/kg of body weight for 40 min. At this time the urine flow rate and the rate of infusion were matched. The infusion rate was then kept constant for the duration of the experiment. Measurements were repeated after volume expansion. Albumin solution,[2] containing inulin and para-aminohippuric acid (PAH)[3] in concentration approximating those in systemic plasma, was then infused into the renal artery at 0.45 ml/min/kg of body weight and the measurements were repeated. Collections of tubule fluid were obtained from late segments of superficial proximal tubules for determination of fractional sodium reabsorption. Phosphate clearances were obtained to provide a qualitative index of proximal sodium reabsorption for nephron populations inaccessible to micropuncture in the dog.

The second group of three dogs served as a control for the triple-collection experimental design. The protocol was the same as for the first group with the exception that the third phase of the experiment was performed during sustained volume expansion without albumin infusion. Fig. 1 illustrates the constancy of fractional sodium reabsorption by the proximal tubule during sustained volume expansion.

The third group of six dogs followed a protocol similar to the first group, with the additional control of variables important in the regulation of phosphate reabsorption. To control for changes in the release of parathyroid hormone, these animals were acutely thyroparathyroidectomized and parathyroid hormone levels were maintained constant by the infusion of 0.01 U/kg/min bovine parathyroid hormone.[4] The constancy of hormone levels was verified by measurement of unchanged phosphate clearances in additional control experiments of similar duration in thyroparathyroidectomized dogs receiving hormone infusion. To avoid changes in plasma calcium which could have a direct effect on phosphate reabsorption, the ionized calcium concentration in the hyperoncotic albumin solution was adjusted to normal plasma concentrations by addition of $CaCl_2$. Whereas before Ca addition, the ionized calcium concentration in salt-poor hyperoncotic albumin solution was undetectable, after Ca addition the ionized Ca concentration was 4.45 ±0.9 mg/100 ml. This value was not significantly different from that in systemic plasma of six dogs, 4.44±0.2 mg/100 ml. To achieve this ionized Ca concentration, the total calcium concentration was increased from 9.7±0.1 mg/100 ml to 38.5± 3.6 mg/100 ml in the hyperoncotic albumin solution. Estimates of the protein concentration in efferent arterioles were calculated from the protein concentration in the renal vein and from the filtration fraction. Oncotic pressure was determined from its empiric relationship to protein concentration.

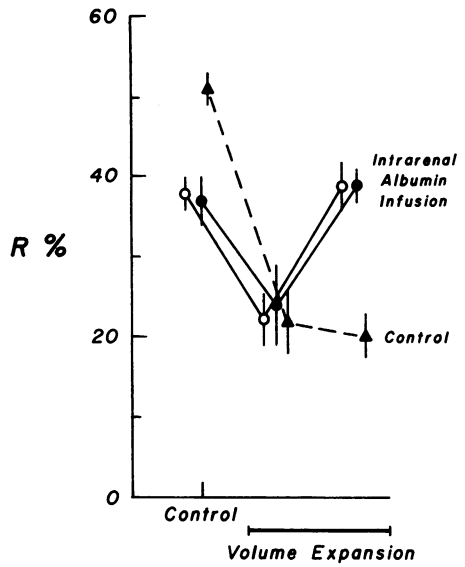

FIGURE 1 The effects of volume expansion and intrarenal albumin infusion are depicted for intact dogs by open circles (31 tubules), and for thyroparathyroidectomized dogs given a constant parathyroid hormone infusion by closed circles (7 tubules). R is the reabsorption by superficial proximal tubues. The effect of sustained volume expansion without intrarenal albumin infusion is depicted by solid triangles and a dashed line (15 tubules). Intrarenal albumin infusion restored fractional sodium reabsorption by superficial proximal tubules to control levels during sustained volume expansion.

RESULTS

The effects of volume expansion and subsequent albumin infusion on fractional reabsorption by the proximal tubule for both intact dogs and thyroparathyroidectomized dogs are shown in Fig. 1. In intact dogs, infusion of Ringer's solution decreased calculated oncotic pressure in efferent arterioles 10.5±0.8 mm Hg ($P < 0.001$)[5] and decreased fractional reabsorption by the proximal tubule 0.16±0.02 ($P < 0.001$). Infusion of albumin solution into the renal artery during continued volume expansion increased calculated oncotic pressure in efferent arterioles 17.5±3.3 mm Hg ($P < 0.005$) and increased fractional reabsorption by the proximal tubule 0.18±0.02 ($P < 0.001$). Single nephron filtration rates were not significantly changed, averaging 103±11, 108± 6, and 105±9 nl/min in each phase of the experiment, respectively. Consequently, absolute reabsorption by proximal tubules decreased 15.7±4.1 nl/min ($P < 0.025$) after Ringer's infusion and increased 13.5±2.4 nl/min ($P < 0.005$) after infusion of albumin solution.

[1] Sodium 138 meq/liter, potassium 3.7 meq/liter, chloride 123 meq/liter, bicarbonate 25 mm/liter, phosphate 6 mg/100 ml, calcium 6 mg/100 ml, magnesium 2.2 mg/100 ml, dextrose 100 mg/100 ml, and bubbled with 6% CO_2–94% O_2 gas mixture.

[2] Salt-poor hyperoncotic human albumin, Parke, Davis & Co., Detroit, Mich.

[3] *Abbreviations used in this paper:* GFR, glomerular filtration rate; PAH, para-aminohippuric acid.

[4] Kindly provided by Dr. Claude Arnaud.

[5] Mean difference ±1 SE, paired t statistic.

TABLE I

Effect of Volume Expansion and Albumin Infusion on Delivery from the Proximal Tubule and on Sodium Excretion

Dog	Proximal delivery*			Phosphate clearance/ inulin clearance‡			Fractional sodium excretion		
	H§	R	A	H	R	A	H	R	A
	ml/min				%			%	
1	15	17	13	15	29	18	1.36	7.57	4.76
2	19	22	16	19	27	17	0.57	3.59	2.83
3	12	17	10	16	47	30	0.50	3.84	3.48
4	12	15	9	39	49	38	0.22	7.50	4.35
5	11	18	12	12	33	28	0.10	0.54	0.51
6	17	25	15	24	33	24	0.46	2.00	2.37
Mean	14	19	13	21	37	26	0.54	4.17	3.05
SE	1	2	1	4	4	3	0.18	1.17	0.63

* Proximal delivery = GFR × (P/TF) inulin concentration.
‡ Index of proximal delivery (see text).
§ H,R,A = Hydropenia, Ringer's infusion, and albumin infusion.

Glomerular filtration rate was not significantly changed after Ringer's infusion, + 0.2±1.0 ml/min, and slightly decreased after albumin infusion, − 3.8±0.9 ml/min, $P <$ 0.01. Delivery from the proximal tubule, as calculated from fractional reabsorption and glomerular filtration rate (GFR), was significantly increased 4.7±1 ml/min ($P < 0.01$) after Ringer's infusion (Table I). Infusion of albumin solution into the renal artery decreased delivery from the proximal tubule 6.5±0.8 ml/min ($P <$ 0.01). Delivery from the proximal tubule was significantly less in the expansion-plus-albumin-infusion phase of the experiment when compared to the hydropenic control phase. The mean difference in delivery was − 1.8± 0.6 ml/min ($P < 0.025$).

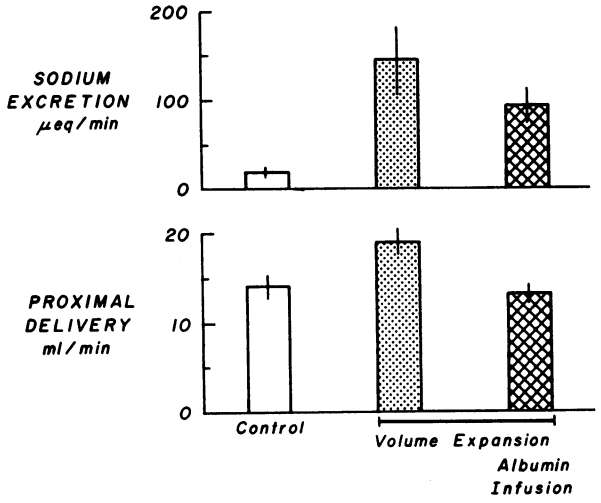

FIGURE 2 Effect of volume expansion on sodium excretion in the presence and absence of increased delivery from proximal tubules. The relationship between delivery from the proximal tubule and sodium excretion is depicted before and after expansion of the extracellular fluid volume. Proximal delivery was restored to control values during continued volume expansion by infusion of albumin into the renal artery.

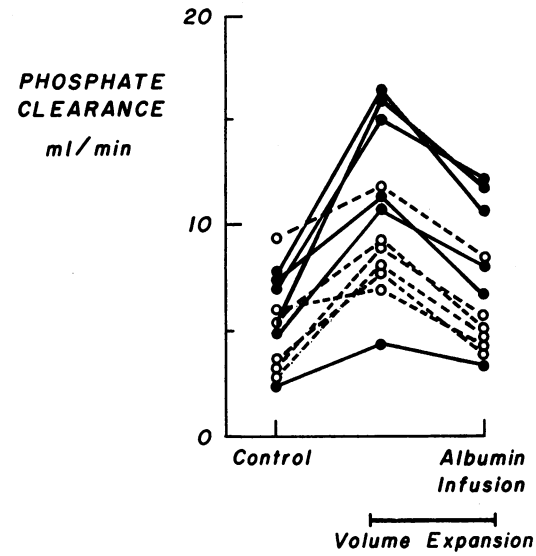

FIGURE 3 The effects of volume expansion and intrarenal albumin infusion on phosphate clearance are depicted for intact dogs by open circles (6 dogs) and for thyroparathyroidectomized dogs given a constant parathyroid hormone infusion by closed circles (6 dogs).

Fractional sodium clearance increased 3.64±1.08 ml/min/100 ml GFR ($P < 0.025$) after infusion of Ringer's solution (Table I). During infusion of albumin solution the mean change in fractional sodium clearance was − 1.22±0.61 ml/min/100 ml GFR. Fractional sodium clearance in this third period was significantly increased 2.52±0.53 ml/min/100 ml GFR ($P < 0.01$) when compared to the hydropenic control period. Absolute sodium excretions were similarly changed (Fig. 2). There were no significant differences in phosphate clearances between intact dogs and thyroparathyroidectomized dogs with a constant parathyroid hormone infusion. The results are shown together in Fig. 3. Infusion of Ringer's solution significantly increased phosphate clearance 5.1±0.8 ml /min, $P < 0.001$, and intrarenal infusion of albumin solution significantly decreased phosphate clearance 3.5± 0.3 ml/min, $P < 0.001$. Fractional phosphate excretion increased 15.5±3.7 ml/min/100 ml GFR ($P < 0.01$) after Ringer's infusion, and decreased 10.5±1.6 ml/min/ 100 ml GFR ($P < 0.005$) after albumin infusion (Table I). Filtration fraction and blood pressure were unchanged from control values.

DISCUSSION

The objective of the present study was to evaluate the role of delivery from the proximal tubule in the natriu-

resis of extracellular fluid volume expansion. Thus, comparisons were made of the natriuretic effect of volume expansion both with and without increased delivery from superficial proximal tubules. It is important to note that the calculation of delivery of tubule fluid from the proximal tubules of the kidney as a whole is based on the premise that changes in fractional reabsorption measured in superficial nephrons are representative of changes in reabsorption in deep nephrons.

Whereas the intrarenal infusion of albumin solution restored reabsorption by superficial tubules to control values, reabsorption by deep proximal tubules might not be similarly restored. An uneven distribution of the intrarenal infusion of albumin solution could have prevented restoration of proximal sodium reabsorption by some nephrons. In this regard, the infusion rate into the renal artery is an important consideration. The infusion rate of approximately 10% of the renal plasma flow used in this study has been shown by Bland and Cohen to result in uniform distribution of isotopes throughout the kidney (4). To evaluate this point further, Lissamine green dye was infused at the same rate as albumin solution at the conclusion of the group 3 experiments. Uniform distribution of the dye was observed in five of the six experiments. In the sixth experiment, dye reached all parts of the kidney but the density was greater in some parts than in others.

Phosphate clearances were measured as an index of the role of nephrons which are inaccessible to micropuncture. This use of phosphate clearance is based primarily on the premise that phosphate reabsorption is predominantly confined to the proximal tubule. Although Amiel, Kuntziger, and Richet (5) have concluded that there is some distal reabsorption of phosphate, recent evidence from the microinjection studies of Staum, Hamburger, and Goldberg (6) indicates that over 95% of the reabsorption of radiophosphate occurs in the proximal tubule and that there was no detectable phosphate reabsorption in the distal convoluted tubule or collecting duct. Furthermore, the proximal reabsorption of phosphate has been shown to be closely related to proximal sodium transport. Puschett, Agus, Senesky, and Goldberg (7) and Schneider, Strandhoy, Willis, and Knox (8) have shown by direct micropuncture correlation that changes in proximal sodium reabsorption are accompanied by corresponding changes in fractional phosphate excretion for a variety of experimental circumstances. However, it must be emphasized that this correlation is a qualitative rather than quantitative relationship. For example, increases in parathyroid hormone activity result in a phosphaturia out of proportion to the decrease in sodium reabsorption by the proximal tubule (9, 10). For this reason, attempts were made to minimize changes in parathyroid hormone activity by including calcium and magnesium in the solution used to expand the extracellular fluid volume of group 1 dogs, and by parathyroidectomy with constant parathyroid hormone infusion in group 3 dogs. Further, changes in plasma calcium concentration may have a direct effect on phosphate reabsorption by the proximal tubule. This possibility is important in group 1 dogs because the salt-poor hyperoncotic albumin would be expected to bind calcium and lower ionized calcium concentrations in the infused kidney. Although Eisenberg has reported that prolonged infusions of calcium solutions in hypoparathyroid patients result in decreases in phosphate reabsorption (11), several studies of the acute effect of altered calcium concentration indicate that hypocalcemia results in decreased phosphate reabsorption (12–14). To control for possible effects of plasma calcium on phosphate reabsorption, an attempt was made to minimize changes in plasma calcium by increasing ionized calcium concentrations in the albumin solution to normal plasma levels in group 3 experiments. Since fractional phosphate clearance for the whole kidney paralleled the changes in fractional sodium reabsorption measured for superficial tubules, it is likely, but not definitely proven, that sodium reabsorption in deep nephrons responded similarly to that of the superficial nephrons.

Restoration of sodium reabsorption by superficial proximal tubules to control levels did not abolish the natriuretic effect of volume expansion. Although a contribution to the natriuresis by deep nephrons cannot be eliminated, the alternative, that the increased proximal delivery accompanying acute volume expansion does not entirely account for the natriuresis, has additional support from experiments both in dogs and rats. In addition to the studies by Howards and associates (2), a dissociation between changes in delivery of sodium from proximal tubules and sodium excretion in dogs has been demonstrated for a number of experimental circumstances: in the presence of furosemide diuresis (15), in chronic sodium retention due to mineralocorticoid excess (16), in experimental low-output heart failure (17), and in experimental high-output heart failure (18). In each of these studies it was concluded that a major component of regulation of sodium reabsorption after volume expansion occurred beyond the proximal tubule. Like the findings in the dog, studies in rats by Davidman, Alexander, LaLone, and Levinsky showed that expansion with albumin produced a delivery to the distal nephron equivalent to that after saline expansion, yet a marked natriuresis was observed only with saline expansion (19). Morgan and Berliner found from microperfusion studies in rats that saline expansion did not alter sodium reabsorption by Henle's loop or the distal tubule, and advanced the hypothesis of a decrease in sodium reabsorption in the collecting system after volume expansion

(20). Stein, Osgood, and Ferris (21) extended these studies with measurements of the sodium load to the collecting system and simultaneous urinary sodium excretion. After expansion of the extracellular fluid volume, less than 50% of the sodium delivered to the collecting duct was reabsorbed, whereas in those animals with preferential expansion of the plasma volume, approximately 90% of the sodium delivered to the collecting duct was reabsorbed. Sonnenberg has also shown by comparison of end-distal sodium load and simultaneous sodium excretions that volume expansion decreases net sodium reabsorption in the collecting system of rats (22). Our experiments provide additional support for the thesis that the increase in sodium excretion after expansion of the extracellular fluid volume is dependent in large part on alterations in sodium reabsorption beyond the proximal tubule.

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