Proximal Tubule Reabsorption After Hyperoncotic Albumin Infusion

ROLE OF PARATHYROID HORMONE AND DISSOCIATION FROM PLASMA VOLUME

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ABSTRACT Preferential expansion of the plasma volume by infusion of salt-poor hyperoncotic albumin solution decreases sodium reabsorption by the proximal tubule. The present micropuncture studies test the thesis that albumin infusion depresses proximal reabsorption by an effect unrelated to expansion of the plasma volume, perhaps due to an effect of parathyroid hormone (PTH) on proximal sodium reabsorption. Infusion of salt-poor hyperoncotic albumin significantly decreased plasma ionized calcium, increased immunoreactive PTH (iPTH) in plasma, decreased sodium reabsorption by the proximal tubule, and increased phosphate clearance. In contrast, infusions of albumin, in which the ionized calcium was restored to normal plasma levels, had no significant effect on ionized calcium, iPTH, proximal reabsorption, or phosphate clearance in intact dogs. Similarly, in parathyroidectomized animals given a constant replacement infusion of PTH, albumin infusion had no significant effect on proximal reabsorption or phosphate clearance. Plasma volume was markedly expanded following albumin infusion in all groups of dogs. These findings (*a*) indicate that PTH plays a significant role in the decrease in sodium reabsorption by the renal proximal tubule after salt-poor hyperoncotic albumin infusion, and (*b*) dissociate preferential expansion of the plasma volume from decreases in sodium reabsorption by the proximal tubule.

This work was presented in part at the Fall meetings of the American Physiological Society (1973. *Physiologist.* **16**: 365).

Dr. Knox is a recipient of Research Career Development Award HL-18518 from the National Heart and Lung Institute. E. G. Schneider is an Established Investigator of the American Heart Association, Doctors Ott, Willis, and Strandhoy are NIH postdoctoral fellows and Dr. Cuche is a Mayo Research Fellow.

Received for publication 22 May 1973 and in revised form 10 September 1973.

INTRODUCTION

Dirks, Cirksena, and Berliner (1) and Watson (2) showed in independent micropuncture studies that expansion of the extracellular fluid volume by intravenous saline infusion in the dog resulted in marked decreases in sodium reabsorption by the proximal tubule. Subsequently, Howards, Davis, Knox, Wright, and Berliner infused hyperoncotic albumin solution to expand the plasma volume at the expense of the interstitial fluid volume so that the effect of preferential expansion of the plasma volume could be determined on proximal sodium reabsorption (3). Infusion of hyperoncotic albumin solution significantly increased the plasma volume and decreased proximal sodium reabsorption. Thus, these results were consistent with an inverse relationship between plasma volume and proximal sodium reabsorption.

In recent studies we observed that the clearances of sodium and phosphate as well as changes in proximal sodium reabsorption were similar after infusions of albumin or parathyroid hormone (PTH)[1] (4). Decreases in proximal sodium reabsorption following in-

[1] *Abbreviations used in this paper:* bPTH, bovine PTH; GFR, glomerular filtration rate; iPTH, immunoreactive PTH; PAH, *p*-aminohippuric acid; P_{In}, plasma inulin concentration; PTH, parathyroid hormone; *TF*, tubule fluid; V_c, volume collected per minute; V_o, single nephron filtration rate.

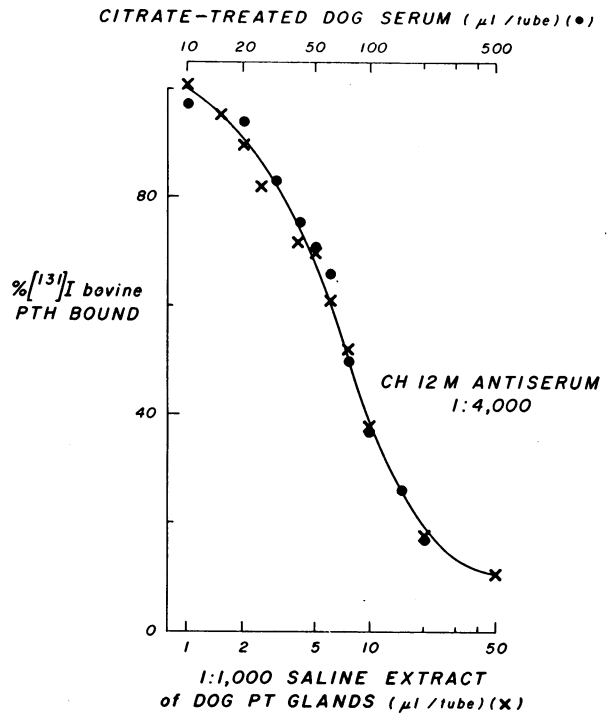

FIGURE 1 Standard PTH radioimmunoassay curves obtained with 1:1,000 dilution of a saline extract of normal dog parathyroid glands (×) and serum from a dog after several hours of intravenous citrate-induced hypocalcemia (●). [^{131}I]bovine PTH employed as radioactive labeled species and CH 12M antiserum, 1:4,000 dilution in incubation mixtures with 500 μl total volume. Note superimposition of standard curves.

fusion of parathyroid hormone in the dog have been previously reported (5–7). Based on these observations, we hypothesized that the effects of intravenously infused hyperoncotic albumin solution could be mediated by parathyroid hormone. The salt-poor hyperoncotic albumin solution used in the previous studies was found to have sufficient binding sites for calcium to significantly lower plasma ionized calcium. The present studies indicate that albumin infusion resulted in consistent decreases in plasma ionized calcium and increases in immunoreactive parathyroid hormone (iPTH) levels in dogs. In additional micropuncture studies in which plasma PTH was controlled, infusion of albumin solution and subsequent plasma volume expansion did not significantly decrease sodium reabsorption by the proximal tubule.

METHODS

Three groups of experiments were performed: group 1, albumin infusion experiments in which parathyroid hormone was not controlled; group 2, albumin infusion experiments in which plasma ionized calcium was maintained at normal plasma levels by the addition of calcium chloride to the salt-poor hyperoncotic albumin solution; and group 3, albumin infusion experiments in which parathyroid hormone was controlled by surgical removal of the parathyroid glands and a constant infusion of bovine parathyroid hormone.

Dogs were fed a standard pellet diet providing approximately 30 meq of sodium per day. They were allowed free access to water, and food was withheld on the day of the experiment. They were anesthetized with pentobarbital and a tracheotomy was performed. Cannulas were placed in jugular veins for infusions, in a femoral vein and a left renal vein (through the ovarian or testicular vein) for blood sampling, and in the right femoral artery for blood pressure recordings.

In group 1 experiments, after completion of three successive clearance periods of 15 min each in continued hydropenia, 6.5 ml/kg body weight of hyperoncotic albumin solution was infused over a 20-min interval.[2] Three additional clearance periods were performed 30 min after completion of the infusion of albumin solution. In group 2 experiments the ionized calcium concentration in the hyperoncotic albumin solution was adjusted to normal plasma concentrations by addition of calcium chloride; whereas before calcium addition the ionized calcium concentration in salt-poor hyperoncotic albumin solution was undetectable, after calcium addition the ionized calcium concentration was 4.45±0.6 mg/100 ml. This value is not significantly different from that in systemic plasma of 11 dogs of 4.24±0.3 mg/100 ml. To achieve this ionized calcium concentration the total calcium concentration in the hyperoncotic albumin solution was increased from 9.7±0.1 mg/100 ml to 38.5 ±3.6 mg/100 ml. In group 3, to control for changes in the release of parathyroid hormone, animals were thyroparathyroidectomized during the preparation for micropuncture, and parathyroid hormone levels were maintained constant by the infusion of 0.01 U/kg per min bovine parathyroid hormone. This rate of hormone infusion was chosen so that phosphate clearances would approximate values in intact animals. The constancy of hormone levels was inferred from measurements of unchanged phosphate clearance in additional control experiments of similar duration in thyroparathyroidectomized dogs receiving the same hormone infusion.

In groups 1 and 2, arterial blood samples were drawn before, during, and after albumin infusion for ionized calcium and iPTH determinations. Plasma calcium was measured by atomic absorption spectroscopy. Blood for ionized calcium was collected into a syringe and then injected into 5-ml Vacutainers (Becton-Dickinson & Co., Rutherford, N. J.) containing 143 U of heparin. Plasma was withdrawn anaerobically into a tuberculin syringe through the rubber stopper of the Vacutainer after centrifugation at 3,000 rpm for 10 min. For measurements of ionized calcium in plasma, the Orion[3] flow-through calcium activity electrode (8, 9) was used with the following modifications. (*a*) Standards were prepared in Vacutainers containing the same amount of heparin as did samples, (*b*) no trypsin or triethanolamide was added to the standards, which were prepared weekly, and (*c*) the membrane was primed by pooled normal plasma before the daily standard curve was run. Plasma pH was measured by a microelectrode and a standard pH meter.

The techniques of measurement of serum iPTH were the same as those previously reported (10). The antiserum

[2] Salt-poor hyperoncotic (25%) human albumin, Parke, Davis & Co., Detroit, Mich.

[3] Orion Research, Inc., Cambridge, Mass.

used in the present studies, CH 12M (chicken antibovine PTH), was described in the previous report. In the present studies, a saline extract of pooled normal dog parathyroid glands was used as a standard in all radioimmunoassays. A 1 : 100 dilution of this extract was assigned an arbitrary value of 1,000 μl-eq/ml and immunoassay curves produced with this standard superimposed on curves produced with multiple dilutions of a serum obtained from a dog made chronically hypocalcemic with citrate infusions (Fig. 1). [^{131}I]bovine PTH (bPTH) was used in assays as the labeled hormone species. Antiserum CH 12M reacts with synthetic bovine PTH 1-34 (Beckman Bioproducts) almost as well as with bovine PTH 1-84 and therefore recognizes the biologically active region of the PTH molecule. Sera from normal dogs consistently decreased the ratio of antibody bound to free [^{131}I]bPTH (B/F ratio) by 30–50% whereas sera from hypoparathyroid dogs did not alter this ratio significantly, indicating the iPTH was being measured and not some nonspecific effect of serum on the immune system. In order that this potential problem could be circumvented, we used hypoparathyroid dog serum in assays as a blank and made corrections for small nonspecific changes in the B/F ratio as has been described (10). All measurements of serum iPTH in the present study were done in duplicate in three different serum dilutions. Intra-assay and interassay variations were 12 and 15%, respectively.

Additional dogs were prepared for micropuncture as previously described (11). Micropuncture measurements of fractional and absolute reabsorption by the proximal tubule, clearance determinations of glomerular filtration rate and renal plasma flow, and measurements of electrolyte excretion were made during continued hydropenia. Surface segments of proximal tubules were micropunctured using the recollection micropuncture technique as previously described (11). The volume of the tubule fluid sample was measured with a micropipette which was calibrated with a radioactive tracer. The single nephron filtration rate (V_o) was calculated from the expression $V_o = V_c \times TF/P_{In}$ where V_c is the volume collected per minute and the TF/P_{In} is the ratio of inulin concentration in tubule fluid to that in plasma. The concentration of inulin in tubule fluid samples was determined in duplicate by the microfluorometric method (12).

Blood samples for clearance determinations were collected at the midpoint of 15-min urine collections. Inulin concentration in plasma and urine was measured by the method of Fjeldbo and Stamey (13). *P*-aminohippuric acid (PAH) concentration in plasma and urine was measured by the method of Harvey and Brothers (14). Renal plasma flow was calculated from the clearance and extraction of PAH. Changes in plasma volume were calculated from changes in hematocrit. Urine and plasma concentrations of sodium were measured by flame photometry and that of phos-

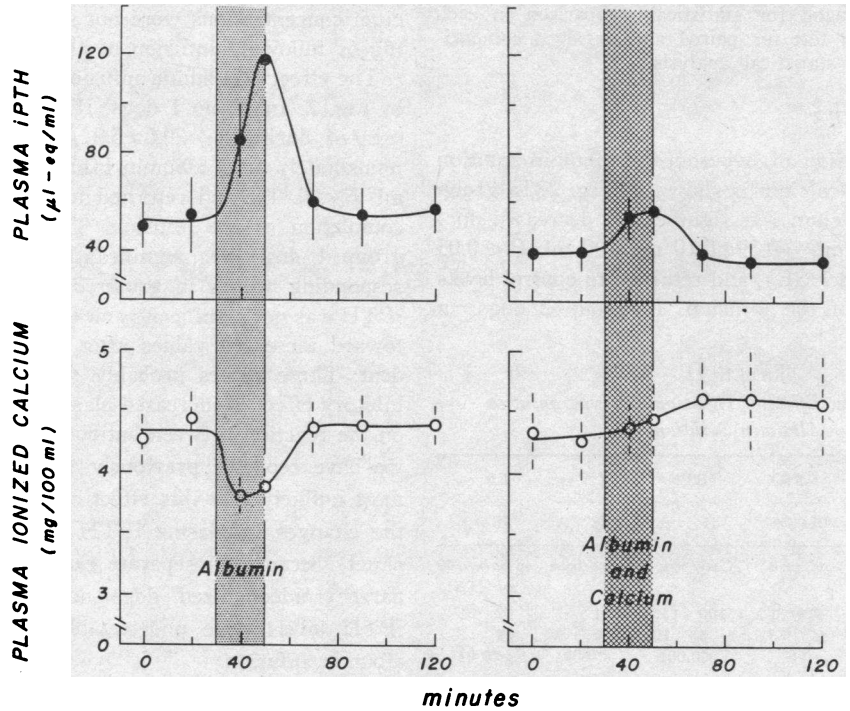

FIGURE 2 The effects of infusion of salt-poor hyperoncotic albumin solution on plasma ionized calcium and iPTH in four intact dogs (group 1 protocol) are depicted in the left panel. Plasma ionized calcium was significantly decreased and iPTH was significantly increased during and immediately after albumin infusion. The effects of infusion of albumin solution, in which ionized calcium concentration was elevated to normal plasma levels, on plasma ionized calcium and iPTH in four intact dogs (group 2 protocol) are depicted in the right panel. There were no significant changes in either variable. The tendency toward increased iPTH values in this group is probably a nonspecific effect of increased plasma protein concentration (see text). Mean values ±1 SE are shown.

TABLE I

Systemic Effects of Infusion of Hyperoncotic Albumin Solution

Group, *n* dogs	Hct			Total calcium			Protein			BP		
	H	A	%Δ PV	H	A	Δ	H	A	Δ	H	A	Δ
				mg/100 ml			*g/100 ml*			*mm Hg*		
1. PTH uncontrolled (12)	48*	38	54	9.5	9.9	0.48	5.7	6.2	0.49	127	123	4
	±1	1	5	0.1	0.2	0.12	0.1	0.1	0.05	6	4	5
P	<0.001			<0.01			<0.001			NS		
2. PTH chemically controlled (7)	47	37	49	9.7	10.4	0.66	7.4	7.9	0.53	119	123	4
	±2	2	4	0.2	0.2	0.11	0.3	0.3	0.11	4	2	5
P	<0.001			<0.001			<0.01			NS		
3. PTH surgically controlled (12)	47	38	42	9.4	8.6	−0.87	6.4	6.9	0.54	110	120	10
	±2	2	6	0.2	0.3	0.11	0.2	0.2	0.07	5	6	3
P	<0.001			<0.001			<0.001			<0.005		

H, hydropenia; A, after infusion of hyperoncotic albumin solution; %ΔPV, percent change in plasma volume; Hct, hematocrit; BP, mean arterial blood pressure.
* Mean ±1 SE.

phorus by the method of Young (15). The data for each variable were averaged for statistical comparison in each dog. The Student *t* test for paired and unpaired comparisons was used for statistical analysis.

RESULTS

The effect of infusion of hyperoncotic albumin solution on plasma ionized calcium is shown in Fig. 2. In group 1 dogs, ionized calcium was significantly decreased during albumin infusion, −0.39±0.10 mg/100 ml, $P < 0.05$ (mean difference ±1 SE), and returned to control levels after completion of the infusion. In group 2 dogs, in which calcium was added to the albumin, ionized calcium concentrations were not significantly changed during or following infusion of albumin solution (Fig. 2).

The effect of albumin infusion on iPTH is also shown in Fig. 2. In group 1 dogs, iPTH was significantly increased during (+29.8±5.8 μl-eq/ml, $P < 0.025$) and immediately after albumin infusion (+61.5±10.5 μl-eq/ml, $P < 0.01$), and returned to control levels following completion of the infusion. The increase in iPTH in group 1 dogs was significantly greater than the corresponding values in group 2 dogs. In group 2 dogs, iPTH was not significantly elevated; however, a tendency toward increased values after albumin infusion is evident. These values probably reflect the nonspecific inhibitory effect of increased plasma protein concentrations on the reaction between antibody and [^{131}I]bPTH which we have reported previously (10, 16). However, it is most unlikely that this effect contributed importantly to the changes in plasma iPTH shown in Fig. 2 (right panel) because, in separate experiments, in four thyroparathyroidectomized dogs not given PTH infusion, iPTH levels were undetectable both before and after albumin infusion.

The systemic effects of hyperoncotic albumin infusion in the micropuncture experiments are detailed in Table I. Plasma volume was markedly and similarly increased in all three groups of dogs. Plasma calcium concentration was significantly increased in groups 1 and 2 and was significantly decreased in group 3. Plasma protein concentration was significantly increased in all three groups. Mean arterial blood pressure was not significantly

TABLE II

Clearance Data, Effect of Infusion of Hyperoncotic Albumin Solution

	GFR		RPF		UV_{Na}		FE_{PO_4}	
	H	A	H	A	H	A	H	A
	ml/min		*ml/min*		*μeq/min*		*ml/min/100 ml GFR*	
1. PTH uncontrolled (12)	28*	31	100	153	21	57	12	21
	±2	3	8	10	6	9	2	2
P	NS		<0.001		<0.001		<0.01	
2. PTH chemically controlled (7)	21	23	77	94	17	33	18	17
	±2	2	8	11	6	10	4	5
P	NS		<0.025		NS		NS	
3. PTH surgically controlled (12)	20	23	69	87	10	30	15	18
	±3	3	10	14	4	12	4	5
P	NS		<0.05		<0.05		NS	

H, hydropenia; A, after infusion of hyperoncotic albumin solution; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; UV_{Na}, urinary sodium excretion; FE_{PO_4} fractional excretion of phosphate.
* Mean ±1 SE.

changed in groups 1 and 2 and was significantly increased (10±3 mm Hg, $P < 0.005$) in group 3.

Clearance data are summarized in Table II. Sodium excretion was significantly increased in group 1 (35.5± 5.9 μeq/min, $P < 0.001$) and in group 3 (19.8±8.4 μeq/min, $P < 0.05$) but not in group 2 (15.6±12.3 μeq/min). The failure to achieve statistical significance in group 2 may be related to the smaller number of animals in this group and to inclusion of one experiment with a marked fall in sodium excretion. Sodium excretion was significantly increased when all experiments with control of PTH are considered together. Fractional phosphate excretion was significantly increased in group 1 dogs (9.2±2.8 ml/min per 100 ml glomerular filtration [GFR], $P < 0.01$) but not in group 2 dogs (−1.2±2.5 ml/min per 100 ml GFR) or in group 3 dogs (3.1± 2.5 ml/min per 100 ml GFR).

The effects of hyperoncotic albumin infusion on sodium reabsorption by superficial proximal tubules are detailed in Table III and in Fig. 3. Fractional reabsorption was significantly decreased in group 1 (−9.2±1.7%, $P <$ 0.001) but not in group 2 (−1.3±3.2%) or in group 3 dogs (−0.2±1.5%). Changes in proximal reabsorption were significantly different between group 1 and groups 2 and 3, $P < 0.01$. Similarly, absolute reabsorption was decreased in group 1, $P < 0.025$, but not in group 2 or 3. Single nephron filtration rate was not significantly changed in any group.

TABLE III

Effect of Infusion of Hyperoncotic Albumin Solution on Sodium Reabsorption by the Proximal Tubule

	R			V_o		V_R	
	H	A	Δ	H	A	H	A
	%	%	%	*nl/min*		*nl/min*	
1. PTH uncontrolled	38.6*	29.3	−9.2	82	88	32	26
(12)	±2.0	3.4	1.7	6	7	3	3
P		<0.001		NS		<0.025	
2. PTH chemically	34.2	32.8	−1.3	79	81	30	30
controlled (7)	±2.8	3.8	3.2	9	9	4	4
P		NS		NS		NS	
3. PTH surgically	35.9	35.8	−0.2	69	71	25	25
controlled (12)	±3.6	4.2	1.5	7	8	3	4
P		NS		NS		NS	

H, hydropenia; A, after infusion of hyperoncotic albumin solution; R fractional reabsorption by the proximal tubule; V_o, single nephron filtration rate; V_R, absolute reabsorption by single nephrons.
* Mean ±1 SE.

DISCUSSION

The present studies indicate an important role for parathyroid hormone in the mediation of the decreased sodium reabsorption by the proximal tubule after infusion of hyperoncotic albumin solution. The release of parathyroid hormone after infusion of albumin solution is probably a consequence of the low concentration of calcium per gram of protein in the salt-poor hyperoncotic albumin solution. Thus, the infused albumin binds calcium, decreases plasma ionized calcium, and provides a stimulus for the release of parathyroid hormone. The subsequent release of parathyroid hormone was documented by the demonstration of increased iPTH concentrations in plasma during and immediately after the infusion of the albumin

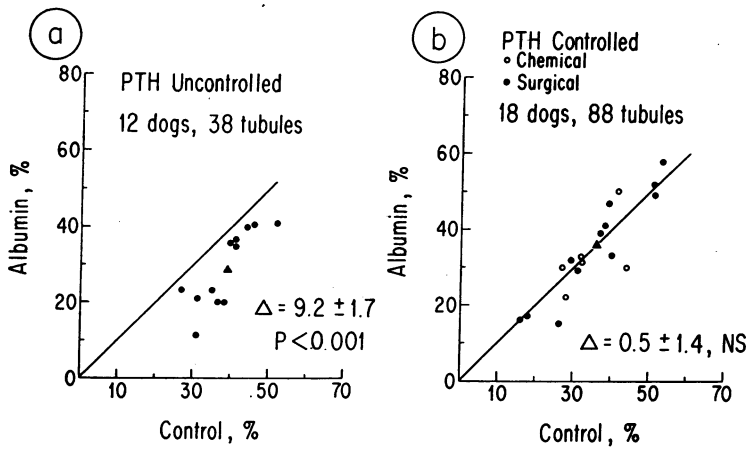

FIGURE 3 Fractional sodium reabsorption by the proximal tubule is depicted before and after the infusion of hyperoncotic albumin solution. Each point represents the mean value for one dog. Triangles represent the mean value for all dogs. Panel *a* depicts results from experiments with release of PTH. Panel *b* depicts results from experiments in which PTH was controlled by restoration of ionized calcium in the albumin solution to normal plasma levels (chemical control, group 2 protocol) and experiments in which PTH was controlled by parathyroidectomy with constant infusion of PTH (surgical control, group 3 protocol).

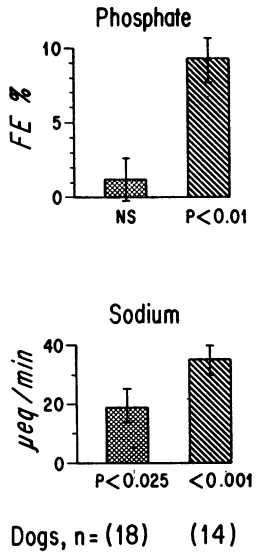

FIGURE 4 Changes in urinary electrolyte excretion after albumin infusion are depicted. Fractional phosphate excretion was increased only in experiments with release of PTH. Sodium excretion was significantly increased in both groups, lower panel.

solution. Parathyroid hormone in turn resulted in a return in plasma ionized calcium concentrations to control levels with a subsequent increase in total calcium. The restoration of plasma ionized calcium concentration to control levels presumably resulted in a return in serum iPTH values to control levels. The significant phosphaturia in the time period 30 min after completion of the albumin infusion (Fig. 4) indicates that the renal effects of PTH persisted for some time beyond the period of peak serum iPTH levels. Similarly, sodium reabsorption by the proximal tubule was consistently decreased in association with the phosphaturia. In contrast, albumin infusion had no significant effect on plasma ionized calcium, iPTH, sodium reabsorption by the proximal tubule, or phosphate clearance when the ionized calcium concentration in the hyperoncotic albumin solution was elevated to normal plasma levels. Further, in animals which were parathyroidectomized and maintained with a constant replacement infusion of parathyroid hormone there were no changes in sodium reabsorption by the proximal tubule or phosphate clearance after albumin infusion.

The plasma volumes were markedly expanded after infusion of albumin solution in dogs with or without control of parathyroid hormone (Fig. 5). These findings clearly dissociate changes in preferential expansion of the plasma volume from changes in sodium reabsorption by the proximal tubule. Howards and co-workers (3) had noted that additional factors must have been operative in their experiments since 30–90 min after infusion of hyperoncotic dextran, plasma volume was significantly expanded whereas fractional sodium reabsorption by the proximal tubule was not depressed. It should be noted that in the interval 90–180 min after infusion of dextran solution, decreases in sodium reabsorption by the proximal tubule were observed (3). The findings in the present study make it likely that the delayed effect after infusion of dextran solution is due to an effect other than preferential expansion of the plasma volume.

It should be emphasized that the plasma volume was expanded at the expense of the interstitial fluid volume in the present experiments. In contrast, vascular volume expansion with isotonic solutions or blood significantly expand the interstitial fluid volume as well as the plasma volume and may thereby decrease reabsorption. In previous micropuncture studies in dogs, only 60% of infused blood volume remained within the vascular space (17). In addition, although expansion of the vascular volume with isotonic solutions or blood in the rat produces a fall in fractional reabsorption by the proximal tubule, this fall can be attributed to increased filtration rate without inhibition of absolute reabsorption (18, 19).

In general, the systemic effects of albumin infusion were similar in all three groups; however, a small but significant increase in blood pressure occurred in group 3. Larger increases in blood pressure have been associated with decreases in proximal sodium reabsorption

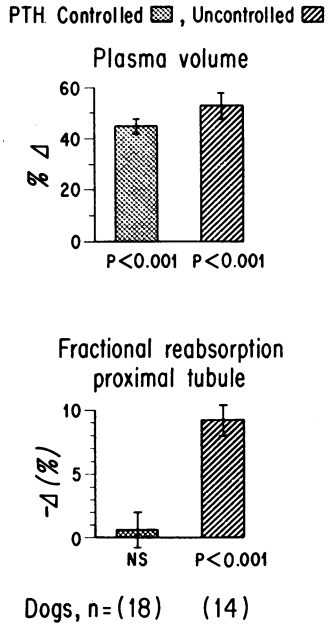

FIGURE 5 Changes in plasma volume and proximal reabsorption after albumin infusion are depicted. Whereas plasma volumes as determined from changes in hematocrit were markedly increased in both groups with and without control of PTH, fractional reabsorption by the proximal tubule was significantly changed only in experiments with release of PTH.

(20, 21); however, since there was no change in proximal reabsorption in group 3, it is likely that the small increase in blood pressure was inconsequential. Efferent arteriolar protein concentration, as calculated from the filtration fraction and plasma protein concentration, was not significantly changed in any of the groups of experiments. It is unlikely, therefore, that differences in proximal reabsorption were mediated by altered peritubule capillary Starling forces. In this regard, in previous studies we were unable to account for changes in proximal reabsorption based on altered peritubule Starling forces after infusion of albumin solution (22).

Preferential expansion of the plasma volume was associated with increased sodium excretion both in experiments with and without control of parathyroid hormone (Fig. 4). The increases in sodium excretion were smaller in the groups in which there were no detectable changes in proximal reabsorption. Although undetectable differences in proximal reabsorption could account for the modest natriuresis, these results are compatible with a distal inhibition of sodium reabsorption after vascular volume expansion (23).

ACKNOWLEDGMENTS

The technical assistance of John Haas, Theresa Berndt, Julie Quast, JoAnn Caron, Diane Brinck, and Julianna Gilkinson and secretarial assistance of Carma Jean Fink are gratefully acknowledged.

This investigation was supported in part by Public Health Service Grants HL-14133 and AM 12302 and grants from the Mayo Foundation.

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