Mode of Action of Parathyroid Hormone and Cyclic Adenosine 3′,5′-Monophosphate on Renal Tubular Phosphate Reabsorption in the Dog

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ABSTRACT To evaluate the effects of parathyroid hormone and cyclic adenosine monophosphate on proximal tubular sodium and phosphate reabsorption, micropuncture studies were performed on dogs that received a highly purified preparation of parathyroid hormone (PTH), dibutyryl cyclic 3′,5′-adenosine monophosphate (cyclic AMP), 5′-AMP, and saline. PTH resulted in a 30–40% inhibition of sodium and phosphate reabsorption in the proximal tubule unassociated with a rise in either total kidney or single nephron glomerular filtration rate (GFR). The bulk of the phosphate rejected proximally was excreted in the final urine while sodium excretion rose minimally despite the marked proximal inhibition, consistent with the presence of reabsorptive sites in the distal nephron for sodium but not phosphate. The infusion of dibutyryl cyclic AMP either systemically or directly into the renal artery inhibited proximal sodium and phosphate reabsorption in the absence of changes in either total kidney or single nephron GFR, resembling the effects of PTH quantitatively and qualitatively. In contrast, another adenine nucleotide, 5′-AMP, did not inhibit the reabsorption of either sodium or phosphate. These observations support the thesis that renal effects of PTH are mediated via stimulation of renal cortical adenyl cyclase. The infusion of a moderate saline load, 25 ml/kg, also produced a similar inhibition of proximal tubular fractional sodium and phosphate reabsorption with a marked phosphaturia but only minimal natriuresis. Thus, changes in sodium and phosphate reabsorption occur in parallel in the proximal tubule when sodium reabsorption is inhibited either with volume expansion or with administration of "specific" phosphaturic agents such as PTH or cyclic AMP. These data are consistent with the thesis that phosphate reabsorption is dependent upon proximal tubular sodium reabsorption wherein the phosphaturic effect of PTH might be the result of a primary inhibition of proximal tubular sodium reabsorption mediated by adenyl cyclase stimulation.

INTRODUCTION

The regulation of the renal excretion of phosphate is an incompletely understood subject. Stop-flow studies in the dog (1) and micropuncture studies in the rat (2) have indicated that the major site of phosphate reabsorption in the mammalian kidney is the proximal tubule. Although net tubular secretion of phosphate has been demonstrated in the chicken (3) and aglomerular fish (4) its occurrence in mammals has not been documented (5, 6). Recent studies have demonstrated an inhibition of tubular phosphate reabsorption after extracellular fluid (ECF) expansion in the rat (7), thyroparathyroidectomized dog (8, 9), and normal man (10). In addition, certain diuretics such as acetazolamide and furosemide, whose renal sites of action include the proximal tubule (11, 12) have been shown to be consistently phosphaturic (13) while others, which appear to possess a natriuretic action more limited to the distal nephron (14–16) do not increase phosphate excretion (13, 16). The above observations suggest a relationship between sodium and phosphate reabsorption in the proximal tubule.

An abstract of this work was published previously (1970. *J. Clin. Invest.* **49**: 77a.).

During the tenure of this work, Dr. Puschett was a Research Associate of the Veterans Administration.

Received for publication 5 August 1970 and in revised form 27 October 1970.

Parathyroid hormone (PTH) has for many years been considered a major factor in the regulation of phosphate excretion. The mechanism of its phosphaturic effect is probably related to an inhibitory action on tubular phosphate reabsorption (17, 18). An effect of PTH also upon urinary excretion of sodium has been periodically observed since 1935 (19) but, as many preparations of PTH have had variable effects on renal blood flow and GFR (20), the physiological mechanism of the observed rise in sodium excretion and its relationship to the phosphaturia is unclear.

In recent years, evidence has accumulated to suggest that the phosphaturic effects of PTH may be mediated via stimulation of adenyl cyclase activity in tubular cells of the renal cortex causing an increase in intracellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (21–25). Thus while cyclic AMP may mimic the phosphaturic effect of PTH (26) its influence on the tubular handling of sodium and the relationship between tubular transport of sodium and phosphate have not been defined.

This study was designed to investigate via micropuncture techniques, the relationships between sodium and phosphate reabsorption in the proximal tubule of the dog nephron as affected by a purified preparation of PTH, dibutyryl cyclic AMP, and moderate expansion of the ECF space with saline. The data reveal that all three of these experimental maneuvers inhibit both sodium and phosphate reabsorption similarly in the proximal tubule. The bulk of the sodium rejected proximally did not appear in the final urine which contained a major portion of the unreabsorbed phosphate. These studies are consistent with the thesis that phosphate reabsorption is closely related to proximal tubular sodium transport. Therefore, the phosphaturic action of PTH, via cyclic AMP, might be mediated by a direct inhibition of proximal sodium reabsorption.

METHODS

Mongrel dogs of either sex, weighing 15–20 kg, which had been fasted for 12 hr were anesthetized with sodium pentobarbital (20 mg/kg.). The animals were intubated and ventilated with a Harvard respirator pump. Cannulas were inserted into the left femoral vein for sustaining infusions and into the left femoral artery for blood sampling and blood pressure recording. Polyethylene cannules were placed into both ureters via a mid-line suprapubic incision for timed collections of urine. The left kidney was exposed through a subcostal incision and a catheter was inserted via the right femoral artery into the abdominal aorta to the level of the left renal artery for subsequent infusion of lissamine green. A priming dose of 200 mg/kg of inulin was given followed by a sustaining infusion in 0.9% saline at a rate of 1.0 ml/min.

The left kidney was suspended on a Lucite holder and a 1 cm^2 area of capsule was removed. This area was bathed with mineral oil and illuminated via a fiber optic illuminator. Surface convolutions of proximal tubules were located by observing the passage of lissamine green dye which was injected rapidly (0.75 ml of a 10% solution) into the aorta. The selected tubules were marked for subsequent collections with nigrosine injected with a sharpened micropipette (tip diameter 6–7 μ). By use of sharpened micropipettes (tip diameter 9–11 μ) an oil block colored with Sudan black, with a length of 2–4 tubular diameters was placed into the tubule and allowed to flow past the micropipette tip, followed by an initial aspiration to begin fluid collection into the pipette. The rate of collection of fluid was adjusted to maintain the position of the oil block just distal to the point of puncture and timed collections were obtained for determinations of inulin and phosphate. Volume measurements for determination of single nephron glomerular filtration rate (SNGFR) were performed only on those tubules in which the spontaneous rate of collection was sufficient to maintain the position of the oil block so that no aspiration was required, and after completion of collection the oil drop spontaneously flowed distally. After collection of three to six tubular fluid samples, infusions were continued as per the protocols described below and recollections from the previously punctured tubules were obtained in a similar manner. The clearance of sodium, phosphate, and inulin were measured in two periods during the control and recollection periods. Blood samples were drawn from the femoral artery at the beginning, mid-point, and end of the control and recollection periods and blood pressure was recorded with an aneroid manometer from the left femoral artery.

Controls. 12 dogs received a continuous infusion of isotonic saline containing inulin for 3–4 hr at a rate of 1.0 ml/min. In order to provide a "time" control for the other experimental groups. recollection punctures were performed 1–2 hr after completion of initial collections.

Parathyroid hormone. In eight dogs, following initial collections, a sufficient amount of a highly purified preparation of PTH, 740–1300 U/mg, (Wilson Laboratories, Chicago, Ill.) was added to the inulin-saline solution so as to deliver 50–60 U/hr. Recollection micropuncture samples were obtained 60–90 min after the initiation of the infusion, and two urine collections were obtained during this period.

Dibutyryl cyclic AMP. In six dogs dibutyryl cyclic AMP was added to the intravenous infusate to deliver 100 mg/hr at 1 ml/min. After obtaining the control micropuncture samples, recollections were obtained as described above. In eight dogs, a 23 gauge needle was inserted into the left renal artery, directed toward the aorta, and 0.9% normal saline at a rate of 0.1 ml/min was infused during the control periods. After completion of the control collections, sufficient dibutyryl cyclic AMP was added to this solution to allow infusion of either 0.014 mg/kg per hr (four dogs) or 0.75 mg/kg per hr (four dogs). Urine collections were obtained from both kidneys and recollection samples were obtained during the second hour of infusion.

Adenosine 5′-monophosphate (5′-AMP). In four dogs, after obtaining initial control samples, 5′-AMP was added to the inulin-saline infusion solution in amounts sufficient to deliver 100 mg/hr at a rate of 1 ml/min. Recollection micropuncture samples and two urine collection periods were obtained during the second hour of infusion.

Saline. After the control period, six dogs were expanded with 25 ml/kg of a solution containing 125 mEq/liter sodium, 20 mEq/liter bicarbonate, 109 mEq/liter chloride, 3.6 mEq/liter calcium, 4 mEq/liter potassium, infused at a rate of 20 ml/min. Following the initial expansion, urine losses of water and electrolytes were continuously replaced and recollection micropuncture samples and urine collections obtained in the second hour after completion of expansion.

After collection, tubular fluid samples were transferred under oil to quartz tubing and aliquots were removed for determination of inulin and phosphate. The volume of tubular fluid collected was then measured using constant bore quartz tubing of known inner diameter and a cathetometer. Inulin concentration was measured in duplicate in 6-nl aliquots using the fluorometric method of Vurek and Pegram (27).

Tubular fluid phosphate concentrations were measured in duplicate using a modification of the ultra-micro method of Howell, Pita, Marquez, and Madruga (28). 2 μl of distilled water were placed in the middle of an oil column in a piece of Pyrex glass tubing approximately 1.0 mm I.D. 20–30 nl of sample, 9.5 μl of molybdate reagent, and 0.5 μl of stannous chloride solution were added and mixing was accomplished with a small capillary pipette. The resultant color was read in an ultramicrocuvette at 660 mμ as described by Howell, Pita, and Marquez (29) using a Zeiss PMQ II Spectrophotometer. The recovery in urine over a concentration range of 3–11 mg/100 ml was 100.9 ±2.3% using 25-nl samples.

Blood samples for calcium and phosphate, drawn anaerobically, were immediately placed under oil, allowed to clot, and centrifuged. 5 ml of serum was then transferred under oil to Centriflo membrane cones (Amicon Corp., Lexington, Mass.) and centrifuged at 2000 rpm for 30 min. Calcium in serum and ultrafiltrates was measured with a Perkin-Elmer Atomic Absorption Spectrophotometer and phosphate in serum and ultrafiltrates and urine were measured as previously described (13). Sodium concentration in blood and urine samples was determined by flame photometry and inulin was determined with an AutoAnalyzer using a modification of previously described anthrone methods (30, 31).

Calculations. Clearances of sodium (C_{Na}), inulin (C_{In}), and phosphate (C_P) were calculated in the usual manner. Fractional sodium and water reabsorption in the proximal tubule to the point of puncture was calculated as 1 − (plasma/tubular fluid) inulin and fractional phosphate reabsorption as $1 - \left[\left(\frac{TF}{UF}\right)_P \Big/ (TF/P)_{In}\right]$ where $(TF/UF)_P$ is the ratio of phosphate concentration in tubular fluid to that in plasma ultrafiltrate and $(TF/P)_{In}$ is the tubular fluid/plasma ratio of inulin. Single nephron GFR was calculated from the following, SNGFR = volume of tubular fluid collected per minute $\times$ $(TF/P)_{In}$. For statistical analysis, the mean control values for C_{In}, C_{Na}, C_P, $(TF/P)_{In}$, $(TF/UF)_P$,

$$\left[\left(\frac{TF}{UF}\right)_P \Big/ (TF/P)_{In}\right],$$

and SNGFR for the experimental kidney in each dog were treated as single observations and compared with the mean values during the recollection period. The significance of the mean difference between control and experimental observations was determined by the *t* test for paired or nonindependent variables (32).

RESULTS

Table I is a summary of the micropuncture and clearance data from all experiments in which tubular fluid samples were obtained. The clearance data are those of the micropunctured kidneys.

Controls. Data were obtained in 12 dogs that received only the inulin-saline sustaining infusion for 1–2 hr after initial control collections. Recollections of tubule fluid were obtained in 54 tubules. Fig. 1 depicts the $(TF/P)_{In}$ and $(TF/UF)_P$ values for individual tubules during control and recollection periods. Mean values (as represented in the figures by triangles) ±SEM for $(TF/P)_{In}$ were 1.40 ±0.04 and 1.38 ±0.03 and for $(TF/UF)_P$ 0.72 ±0.06 and 0.68 ±0.04 during control and recollection respectively. Neither of these changes were statistically significant. Table I summarizes the micropuncture values for fractional sodium and phosphate reabsorption in the proximal tubule and GFR, fractional sodium, and phosphate excretion in the experimental kidney as a whole. There were no significant changes in any of these parameters in these control animals.

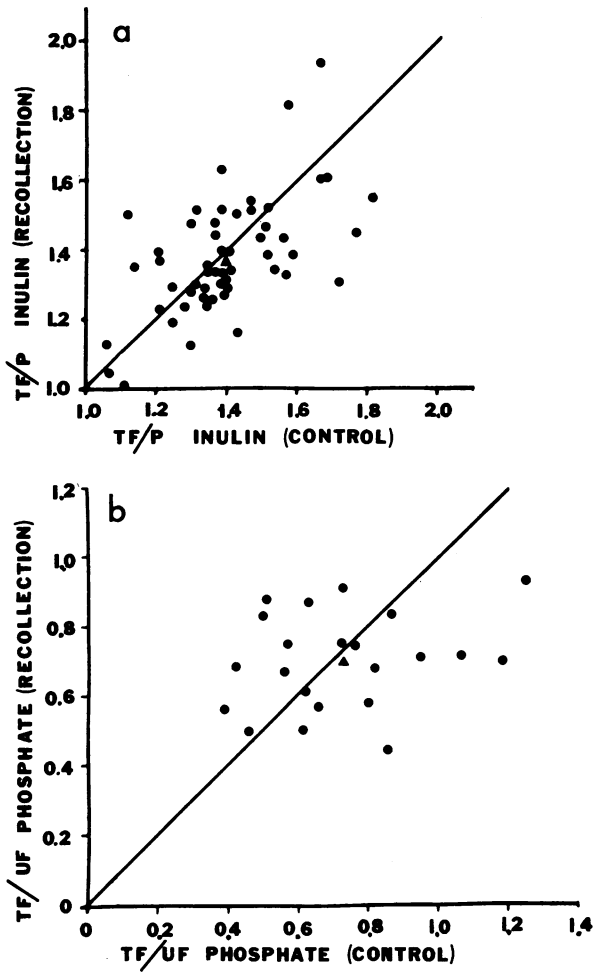

FIGURE 1 Micropuncture results in 12 control dogs receiving only 1 ml/min inulin-saline sustaining infusion. (*a*) Collection-recollection proximal (TF/P) inulin. (*b*) Collection-recollection proximal (TF/UF) phosphate. The solid triangle represents mean value for control and recollection periods.

Parathyroid hormone. The effects of a systemic infusion of purified PTH, 50–60 U/hr, were studied in

TABLE I

*Summary of Clearance and Micropuncture Data**

	Micropuncture data						Clearance data					
	Fractional sodium reabsorption‡		Fractional phosphate reabsorption		SNGFR		C_{In}		C_{Na}/C_{In}		C_P/C_{In}	
	Control	Recollection	Control	Recollection	Control	Recollection	Control	Recollection	Control	Recollection	Control	Recollection
					nl/min		*ml/min*					
A. Control experiments												
No. dogs	12		8				12		12		8§	
No. tubules	54		22									
Mean	0.29	0.28	0.40	0.41			20.7	20.1	0.008	0.006	0.04	0.085
SEM	0.02	0.02	0.04	0.03			2.3	2.7	0.001	0.001	0.01	0.02
SEMD	0.02		0.03				2.2		0.002		0.023	
P	NS		NS				NS		NS		NS	
B. Parathyroid hormone												
No. dogs	8		6		6		8		8		6	
No. tubules	49		23		23							
Mean	0.29	0.19	0.58	0.46	55	44	43.1	39.4	0.003	0.005	0.039	0.24
SEM	0.03	0.03	0.05	0.04	7	9	13.2	13.2	0.001	0.001	0.018	0.039
SEMD	0.02		0.02		6.7		1.4		0.0004		0.037	
P	<0.001		<0.001		NS		<0.05		<0.01		<0.001	
C. Dibutyryl 3′5′-cyclic AMP (systemic infusion)												
No. dogs	6		5		3		6		6		5	
No. tubules	28		16		9							
Mean	0.28	0.15	0.42	0.26	43	41	25.9	25.8	0.004	0.008	0.07	0.34
SEM	0.04	0.03	0.07	0.04	2.7	2.1	4.6	4.2	0.001	0.001	0.04	0.07
SEMD	0.04		0.05		2.9		1.3		0.001		0.05	
P	<0.025		<0.05		NS		NS		<0.02		<0.01	
D. Dibutyryl 3′5′-cyclic AMP (renal arterial infusion)												
No. dogs	6		3		3		6		6		3	
No. tubules	27		9		14							
Mean	0.27	0.19	0.69	0.41	65	66	42.0	43.8	0.007	0.010	0.05	0.24
SEM	0.03	0.04	0.05	0.07	15	11	4.5	5.7	0.002	0.002	0.03	0.05
SEMD	0.01		0.04		4.3		2.5		0.002		0.04	
P	<0.005		<0.02		NS		NS		>0.1		<0.05	
E. 5′-AMP												
No. dogs	3						3		3		3	
No. tubules	14											
Mean	0.35	0.35					63.6	53.5	0.004	0.003	0.04	0.05
SEM	0.04	0.05					1.1	4.1	0.002	0.001	0.02	0.03
SEMD	0.01						5.1		0.001		0.01	
P	NS						NS		NS		NS	
F. Saline												
No. dogs	6		6				6		6		6	
No. tubules	25		19									
Mean	0.31	0.17	0.47	0.32			30.1	33.1	0.008	0.017	0.082	0.17
SEM	0.02	0.04	0.04	0.04			3.7	4.7	0.002	0.004	0.02	0.05
SEMD	0.03		0.06				1.6		0.002		0.027	
P	<0.01		<0.05				NS		<0.02		<0.02	

* Abbreviations: SNGFR = single nephron glomerular filtration rate, C_{In}, C_{Na}, C_P = clearance of inulin, sodium and phosphate respectively; C_{Na}/C_{In} and C_P/C_{In} = fractional excretion of sodium and phosphate; control = mean of micropuncture and clearance data simultaneously obtained during two consecutive control periods; recollection = mean of recollection micropuncture and clearance data obtained during two consecutive periods 60–90 min after initiation of experimental infusion. SEMD = standard error of the mean difference.

‡ Fractional sodium reabsorption to the point of puncture in the proximal tubule is calculated as $1 - \left(\frac{\text{Plasma}}{\text{Tubular Fluid}}\right)$ Inulin and fractional phosphate reabsorption as $1 - \left[\frac{\left(\frac{\text{tubular fluid}}{\text{ultrafiltrate}}\right)\text{phosphate}}{\left(\frac{\text{tubular fluid}}{\text{plasma}}\right)\text{inulin}}\right]$.

§ Represents the C_P/C_{In} results only in those dogs in which tubular fluid samples were analyzed for phosphate.

eight dogs in which 49 collection-recollection pairs of proximal tubule fluid samples were obtained. As shown in Fig. 2, there was a significant fall in $(TF/P)_{In}$ from 1.40 ±0.05 to 1.23 ±0.05 ($P < 0.001$) and a tendency toward a rise in $(TF/UF)_P$ from 0.59 ±0.05 to 0.66 ±0.04 ($P < 0.1$). As summarized in Table I, there was a significant fall in proximal tubule fractional reabsorption of sodium from 0.29 ±0.03 to 0.19 ±0.03 ($P < 0.001$) and fractional reabsorption of phosphate from 0.58 ±0.05 to 0.46 ±0.04 ($P < 0.001$). Fractional phosphate excretion in the final urine rose markedly from 0.039 ±0.018 to 0.24 ±0.039 ($P < 0.001$) while fractional sodium excretion rose minimally from 0.003 ±0.001 to 0.005 ±0.001 ($P < 0.01$).

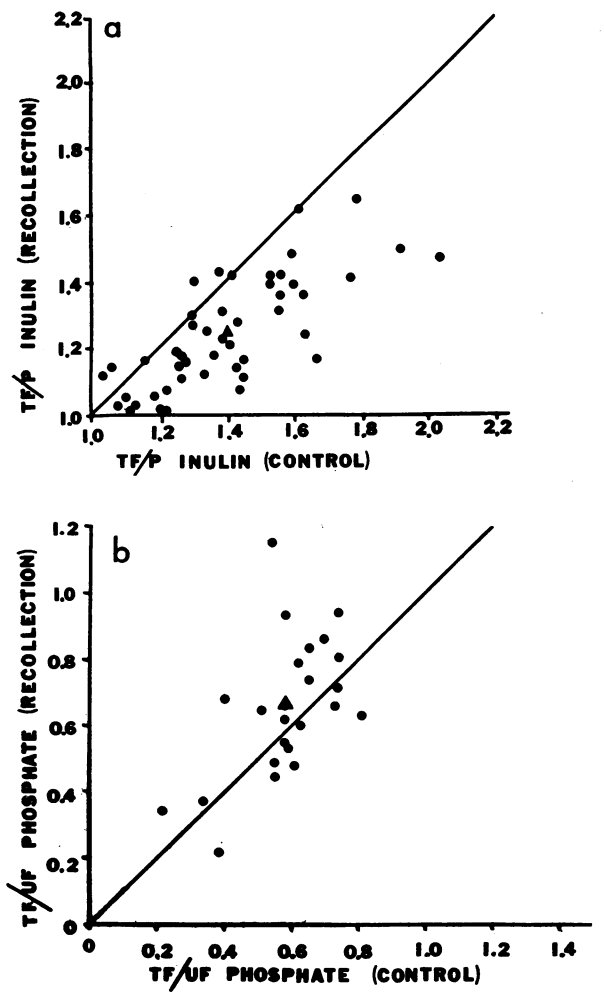

FIGURE 2 Effects of infusion of parathyroid hormone, 50–60 U/hr. (*a*) Collection-recollection proximal (TF/P) inulin in eight dogs. (*b*) Collection-recollection proximal (TF/UF) phosphate in six dogs. The solid triangle represents mean value for control and recollection periods.

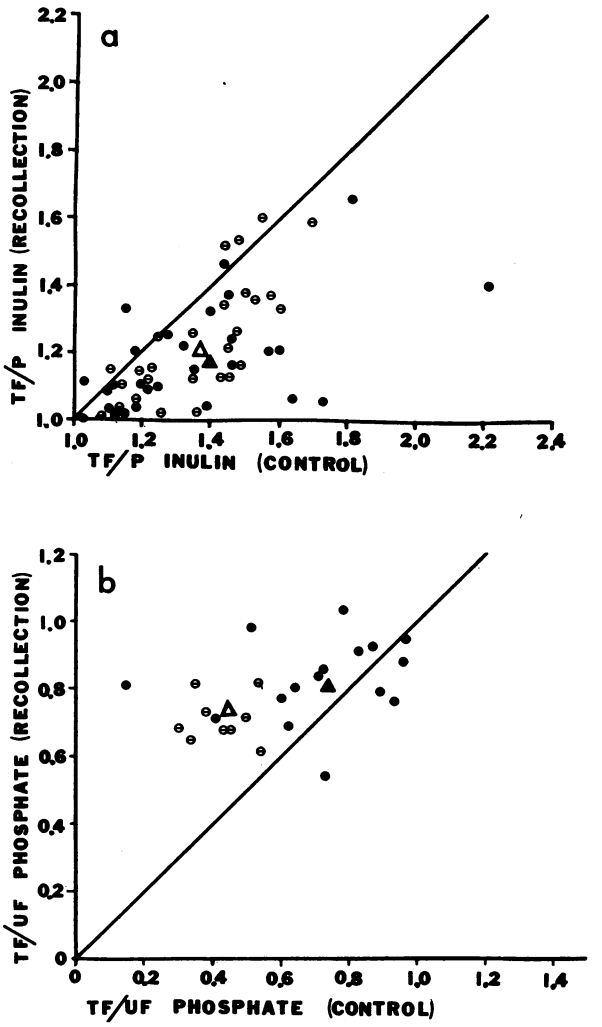

FIGURE 3 Effects of infusion of dibutyryl cyclic AMP. (*a*) Collection-recollection proximal (TF/P) inulin in 12 dogs. (*b*) Collection-recollection proximal (TF/UF) phosphate in eight dogs. The solid circles represent those values obtained in the dogs receiving intravenous infusion, and open circles those receiving intra-arterial administration. The triangles represent mean value for control and recollection periods.

Single nephron GFR was measured in 23 pairs of tubule fluid collections in six of the eight dogs receiving PTH. As summarized in Table I, there was no significant change in SNFGR, whereas total kidney GFR fell slightly from 43 ±13.2 to 39 ±13.2 ml/min ($P < 0.05$). C_{PAH} measured in five dogs was 104 ±21 ml/min during control and 104 ±27 ml/min during the experimental periods.

Dibutyryl cyclic adenosine 3′,5′-monophosphate. Dibutyryl cyclic AMP was infused systemically, 100 mg/hr, into six dogs and into the left renal artery in eight

TABLE II

*Experiment Illustrating Effects of Unilateral Arterial Infusion of Dibutyryl Cyclic AMP**

Time	$(TF/P)_{In}$	C_{In}		C_{PAH}		C_{Na}		C_P		C_{Na}/C_{In}		C_P/C_{In}	
		Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
min		*ml/min*		*ml/min*		*ml/min*		*ml/min*					
−120		Surgery and preparation of left kidney for micropuncture											
−60		Begin infusion of isotonic saline at 0.1 ml/min into left renal artery											
		Priming doses of inulin and PAH, begin sustaining infusion at 1 ml/min											
0–46		44	45	92	96	0.4	0.4	1.3	1.2	0.008	0.009	0.027	0.027
47–71	1.49 ±0.06‡	62	59	105	98	0.3	0.5	2.6	3.2	0.005	0.008	0.049	0.047
		Begin infusion of 0.014 mg/kg per hr dibutyryl cyclic AMP into left renal artery at 0.1 ml/min											
72–91		55	58	99	101	0.1	0.3	2.4	4.2	0.002	0.005	0.043	0.072
92–111		65	58	119	106	0.1	0.3	3.2	5.7	0.002	0.004	0.049	0.099
112–131	1.37 ±0.01‡	72	55	139	108	0.1	0.2	4.0	8.8	0.001	0.003	0.056	0.160
132–160		67	66	133	126	0.1	0.2	4.6	14.1	0.001	0.004	0.068	0.210

* Abbreviations as in Table I: $(TF/P)_{In}$ = tubular fluid/plasma inulin; C_{PAH} = clearance of paraaminohippurate. Right and left refer to right and left (experimental) kidneys respectively.
‡ Values given are for mean ±SEM for six tubules.

dogs, four of which received a dose of 0.014 mg/kg per hr and four received 0.75 mg/kg per hr. Unilateral effects were obtained in all four dogs receiving the lower dose and bilateral responses were observed in all animals receiving the higher dose. Recollection micropuncture studies were performed in all dogs receiving the systemic infusion and six of the eight dogs receiving intra-arterial dibutyryl cyclic AMP. The results obtained in the experimental kidney from the 12 micropunctured dogs receiving cyclic AMP are depicted in Fig. 3 and Table I. There was a statistically significant fall in $(TF/P)_{In}$ from 1.39 ±0.09 to 1.17 ±0.04 ($P < 0.02$) in those animals receiving the systemic infusion and from 1.37 ±0.05 to 1.23 ±0.06 ($P < 0.025$) in the animals receiving intra-arterial cyclic AMP. $(TF/UF)_P$ rose significantly in the group receiving intraarterial cyclic AMP ($P < 0.01$), whereas in the group receiving the systemic infusion the rise in $(TF/UF)_P$ did not

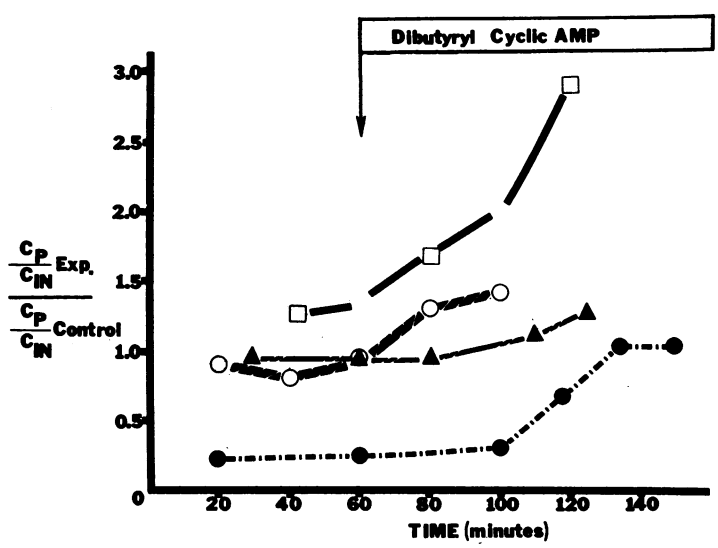

FIGURE 4 Effects of infusion of dibutyryl cyclic AMP 0.014 mg/kg per hr into the left renal artery of four dogs upon fractional phosphate excretion. Exp. refers to the experimental kidney (left), and control refers to the control kidney (right).

achieve statistical significance. As seen in Table I, the significant decreases in proximal tubular fractional reabsorption of sodium and phosphate in both groups were of the same order of magnitude as those occurring following PTH. Also, similar to PTH, the urinary fractional excretion of phosphate rose markedly while urinary fractional sodium excretion rose only slightly in both groups in contrast to the marked changes in proximal tubule fractional sodium reabsorption. Total kidney GFR did not change significantly in either group and single nephron GFR was also unaffected by either the systemic or intra-arterial administration of dibutyryl cyclic AMP. C_{PAH}, measured in six dogs receiving systemic dibutyryl cyclic AMP, was 96 ±11 ml/min during control and 92 ±7 ml/min after infusion.

A protocol of an experiment with the lower dose of intra-arterial dibutyryl cyclic AMP (0.014 mg/kg per hr) which demonstrates a unilateral response is shown in Table II and data illustrating a unilateral phosphaturia in all four dogs receiving this dose are depicted in Fig. 4. Fractional phosphate excretion in the experimental kidney rose markedly after the initiation of the infusion. There was no change in GFR in either control or experimental kidneys and at a time when the phosphaturia was predominantly unilateral, $(TF/P)_{In}$ measured in 12 control recollection pairs of tubules in two dogs (one of which is represented in Table II) fell from 1.45 to 1.33 reflecting a fall in fractional sodium reabsorption from 0.31 to 0.23. These results agree closely with the micropuncture data for the entire group receiving the intra-arterial infusion as well as the group receiving intravenous dibutyryl cyclic AMP (Table I).

Adenosine 5'-monophosphate. In order to study the specificity of the results obtained with dibutyryl cyclic AMP, another adenine nucleotide, 5'AMP, was infused systemically, 100 mg/hr, into four dogs (Fig. 5). In one, separately identified in Fig. 5, GFR fell by 60% associated with a marked rise in $(TF/P)_{In}$ in the three tubules studied. In the other three dogs, in which the changes in GFR were less than 15%, the mean $(TF/P)_{In}$ in 14 control recollection pairs of tubules of 1.55 ±0.05 during control and 1.55 ±0.07 during recollection as shown in Fig. 5 were not significantly different. The results in Table I represent those obtained in the three dogs which did not experience a marked fall in GFR. It is apparent that there was no depression of proximal tubular fractional sodium reabsorption while fractional sodium and phosphate excretion were essentially unchanged.

Saline. For purposes of comparison, the results of the administration of 25 ml/kg of a modified saline solution (a moderately small saline load) to six dogs,

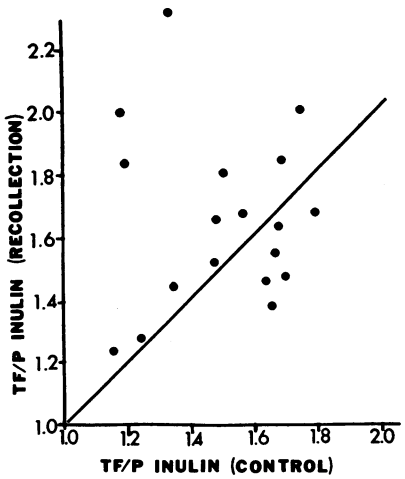

FIGURE 5 Effects of infusion of 5'-AMP, 100 mg/hr on proximal (TF/P) inulin in four dogs. Open circles represent data obtained from single dog with marked fall in GFR.

are presented in Table I. The data reveal a fall in proximal tubular fractional reabsorption of sodium from 0.31 ±0.02 to 0.17 ±0.04 ($P < 0.01$) and phosphate from 0.47 ±0.04 to 0.32 ±0.04 ($P < 0.05$). Fractional phosphate excretion rose from 0.082 ±0.02 to 0.17 ±0.05 ($P < 0.02$) whereas fractional sodium excretion rose only from 0.008 ±0.002 to 0.017 ±0.004 ($P < 0.02$). These results will be presented in greater detail in another publication.[1]

Ultrafilterable calcium and phosphate. Per cent ultrafilterable calcium ranged from 48.1 to 79.5% with a mean ±SE of 68.7 ±1.44% when corrected for plasma water. The per cent ultrafilterable phosphate in plasma water ranged from 95.9 to 100% with a mean value of 98.9 ±2.0%. The variability of serial specimens from a given animal was ±5% and there was no significant alteration in either total or ultrafilterable calcium and phosphate associated with the administration of PTH, saline, dibutyryl cyclic AMP, or 5'-AMP.

DISCUSSION

The data demonstrate that PTH and cyclic AMP inhibit both the absolute and fractional reabsorption of phosphate and sodium in the proximal tubule. In none of the experiments did the data suggest net tubular secretion of phosphate. In the final urine, these agents are associated with a marked increase in the excretion of phosphate and only a small increase in sodium excretion despite the marked proximal inhibition. Previous micro-

[1] Puschett, J. B., Z. S. Agus, D. J. Senesky, and M. Goldberg. 1970. Study of the relationship between the tubular reabsorption of phosphate and sodium in the proximal tubule. Submitted for publication.

puncture studies in the rat (2) and stop-flow studies in the dog (1) have indicated that a relatively small fraction of filtered phosphate is reabsorbed in the distal tubule in mammalian kidneys. Sodium reabsorption, of course, occurs in both the proximal and distal segments of the nephron. Thus, the apparent selective phosphaturia produced by PTH, cyclic AMP, and moderate saline loads, seen in our studies, are actually the result of the simultaneous proximal tubular inhibition of both sodium and phosphate reabsorption in parallel. The phosphate delivered out of the proximal tubule, however is excreted into the final urine, while the bulk of the sodium rejected proximally is reabsorbed in the distal nephron.

Many of the earlier studies on the renal tubular action of PTH have been complicated by simultaneously occurring changes in glomerular filtration rate and renal blood flow (20). The use of a purified preparation of PTH in our experiments was not associated with increases in either total kidney or single nephron glomerular filtration rate or C_{PAH}, demonstrating conclusively a direct tubular action of PTH on sodium and phosphate reabsorption. Thus, the previously described effects of PTH on renal hemodynamics were probably related to the presence of foreign substances in impure extracts.

Recent evidence has suggested a relationship between the phosphaturic action of PTH and the renal cortical adenyl cyclase system. Infusion of PTH has resulted in a rise in the urinary excretion of cyclic AMP in the rat (21) and man (23) and a selective stimulation of renal cortical adenyl cyclase in the rat (24) and rabbit (22). The site of stimulation of cortical adenyl cyclase has recently been more precisely localized to the cortical tubule (25). In addition, systemic infusion of dibutyryl cyclic AMP has resulted in increased phosphate excretion in both the rat (26) and dog (33). In our studies, the physiological effects of PTH and dibutyryl cyclic AMP both in the proximal tubule and in the final urine were indistinguishable, while 5′-AMP had no inhibitory effect in the proximal tubule. The data, therefore, support the hypothesis that the renal effects of PTH are mediated via stimulation of renal cortical adenyl cyclase.

The mechanism of renal tubular phosphate reabsorption is still unclear. There are, however, a number of observations in which changes in the renal handling of phosphate are associated with parallel changes in sodium excretion. Thus, phosphaturia occurs after the administration of furosemide (13) and acetazolamide (13), and after extracellular fluid volume expansion (7–10). Another feature common to these agents is depression of proximal tubular fractional sodium reabsorption (11, 12, 34). In contrast, other diuretics such as the thiazides, ethacrynic acid, triflocin, and mannitol with natriuretic sites of action more limited to the distal nephron (14–16, 35) do not consistently increase phosphate excretion (13, 16). In our studies, PTH, an agent previously thought to be specifically phosphaturic, effected an inhibition of proximal tubular sodium transport of 32% equivalent to that produced by the saline loading experiments. These data suggest that sodium and phosphate transport in the proximal tubule might be intimately related.

The mean $(TF/UF)_P$ in the proximal tubule in the control animals was approximately 0.7, similar to that observed by others in the rat (2), and rose after the administration of PTH, dibutyryl cyclic AMP, and saline. As sodium reabsorption in the proximal tubule is isosmotic, the sodium/phosphate ratio was lower in the reabsorbate than in the filtrate where the molar ratio is 140/1. In our studies, at a point in the proximal tubule where approximately 30% of filtered sodium had been reabsorbed, 42% of filtered phosphate was reabsorbed. Thus, each mmole of phosphate in the reabsorbate was associated with approximately 100 mmoles of sodium. If the inhibition of proximal sodium reabsorption due to PTH and cyclic AMP were merely a consequence of a primary effect of these agents on phosphate, and secondarily, a passive ionic association of sodium with phosphate as Na_2HPO_4 or NaH_2PO_4, then one would expect a sodium/phosphate molar ratio in the rejectate no greater than 2/1. A rough calculation of our data based on SNGFR, $(TF/P)_{In}$, and $(TF/UF)_P$ indicates a sodium/phosphate molar ratio in proximal tubular rejectate following these agents of approximately 75/1. Based upon these data and studies by others discussed above, we propose that proximal tubular reabsorption of phosphate might be dependent upon the tubular reabsorption of sodium. Accordingly, factors which enhance phosphate excretion would do so by primarily inhibiting proximal tubular sodium transport. Unless there is a simultaneous distal inhibitory effect on sodium this proximal action would result in a phosphaturia with little or no natriuresis. The data as reported here, however, do not conclusively prove the existence of such a relationship and further studies under a variety of experimental conditions are required.

The precise nature of the proposed linkage between sodium and phosphate reabsorption is not clear from our data. A simple solvent drag phenomenon cannot be invoked since this mechanism cannot account for a phosphate concentration in the reabsorbate higher than in tubular fluid. The observed $(TF/UF)_P$ of 0.7 is compatible with passive distribution of phosphate at electrochemical equilibrium according to the Nernst equation, assuming the existence of a small transtubular potential difference of -4 mv in the dog proximal tubule (36).

Such calculations are, of course, subject to multiple criticisms and do not prove the passive nature of phosphate reabsorption. Carrier-mediated phosphate transport could be linked to sodium by a double-ion carrier, perhaps similar to that proposed by Diamond in the gall bladder (37). Further more direct studies are required to evaluate these possibilities.

ACKNOWLEDGMENTS

We thank Marta McCusker for her invaluable technical assistance and support. We are also grateful to Jeanne Tschorn Baldino for technical aid and to Lydia Kosolapovs, Leonids Kosolapovs, Carmen D'Angelo, Elizabeth Collona, Katherine Wishnevski, Frances McKee, and Diane Sylk for the laboratory analyses. We are indebted to Doctors John H. Dirks and James R. Clapp for their advice in helping us initiate our micropuncture laboratory, and to Dr. David S. Howell for his advice and assistance in developing the ultra-micro phosphate technique.

This work was supported by U. S. Public Health Service Grants HE 00340 and HE 07284 from the National Institutes of Health, by training Grant 1 TO1 AM05634-01, and also by a grant from the Hoechst Pharmaceuticals, Inc., Cincinnati, Ohio. Part of Dr. Agus' support was through a special fellowship (1-F03-AM-38,709-01) from the National Institute of Arthritis and Metabolic Diseases.

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