Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium, and phosphate

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AGUS, ZALMAN S., LAURENCE B. GARDNER, LAURENCE H. BECK, AND MARTIN GOLDBERG. Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium, and phosphate. Am. J. Physiol. 224(5): 1143-1148. 1973. In order to study the relationships between sodium, calcium, and phosphate reabsorption in the proximal tubule and the effects of parathyroid hormone (PTH) on these relationships, recollection micropuncture and clearance studies were performed in dogs. Proximal tubular fluid/plasma ultrafiltrate ratios for calcium ([TF/UF]c) and phosphate ([TF/UF]p) were 1.1 and 0.7 during the non-diuretic state, and reabsorption of both ions was directly proportional to sodium and fluid reabsorption. PTH resulted in a parallel inhibition of proximal tubular sodium and calcium reabsorption but caused a disproportionate inhibition of phosphate reabsorption ([TF/UF]p rose to 0.9). The bulk of rejected phosphate was excreted in the urine. Sodium clearance rose slightly, whereas calcium clearance fell despite the proximal inhibition. Dibutyl cyclic adenosine monophosphate (cyclic AMP) produced identical effects in the proximal tubule and similar urinary changes. Thus, the reabsorption of calcium and phosphate is closely related to sodium transport in the proximal tubule. Parathyroid hormone, probably via cyclic AMP, inhibits the proximal reabsorption of all three ions, but in a nonparallel manner. Phosphaturia results because the proximally rejected phosphate is not reabsorbed distally to any great extent. On the other hand, PTH reduces calcium excretion by stimulating distal calcium reabsorption out of proportion to that of sodium.

cyclic AMP; proximal tubule; calcium transport; phosphate transport; sodium transport

THE REGULATION of the renal excretion of calcium is incompletely understood but is apparently influenced by several factors. In recent years, a large body of literature has accumulated suggesting a close relationship and possible interdependence between the renal tubular transport of sodium and calcium. Thus, as recently reviewed by Walser (18), changes in tubular sodium reabsorption induced by a variety of means such as diuretics, acute and chronic volume expansion, renal vasodilatation, and alterations in dietary sodium intake are often accompanied by parallel changes in the renal transport of calcium. Another factor thought to influence renal calcium excretion is parathyroid hormone (PTH). Infusions of parathyroid extract have usually been associated with a reduction in calcium excretion (7, 18), and acute parathyroidectomy has resulted in a calcinuria in some species despite a fall in serum calcium levels (2, 10, 16).

Previous studies in our laboratory (1) indicate that parathyroid hormone, probably via stimulation of renal cortical adenyl cyclase activity, is a potent inhibitor of proximal tubular sodium as well as phosphate reabsorption. The results of these latter studies were not able to demonstrate conclusively an inhibition of phosphate reabsorption by PTH out of proportion to the inhibition of sodium and fluid reabsorption in the proximal tubule. In the distal nephron, however, PTH has little, if any, inhibitory effect on sodium transport as evidenced by a minimal natriuresis following administration of the pure hormone. Thus, the previously described hypocalciuric effects of PTH in the presence of inhibition of proximal net sodium and phosphate reabsorption must be due to either a dissociation of sodium, calcium, and phosphate in the proximal tubule or to a stimulation of calcium transport in the distal nephron.

The present study was undertaken to study the interrelationships between proximal tubular sodium, calcium, and phosphate reabsorption and the effects of parathyroid hormone and dibutyryl cyclic AMP on these relationships, utilizing micropuncture techniques in the dog and a new and sensitive ultramicroanalytical technique, electron probe analysis.

METHODS

Mongrel dogs of either sex, weighing 15-20 kg and fasted for 12 hr, were anesthetized with intravenous sodium pentobarbital (20 mg/kg) and received supplemental doses during the experiment as required. The animals were intubated and ventilated with a Harvard respirator. Surgical preparation of the animals for clearance and recollection micropuncture studies was performed as previously described (1). 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 to maintain serum levels of inulin at approximately 100 mg/100 ml. Control samples were obtained by micropuncture techniques as previously described (1) from four to six surface proximal tubules per kidney, and urine was collected from both kidneys in 30- to 45-min periods. Following these initial control collections, five dogs received only the sustaining infusion, 11 received purified PTH at a rate of 50-60 U/hr (700-1,300 U/mg (Wilson Laboratories,
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Chicago, Ill.), and five received dibutylryl cyclic AMP (DB-cAMP) at 100 mg/hr. The FTH and DB-cAMP were added to the inulin-saline infusion, which was continued at a rate of 1.0 ml/min. Recollection micropuncture samples and two urine collections were obtained from both kidneys during the 2nd hr of infusion. Blood samples were drawn from the femoral artery at the beginning, midpoint, and end of the control and recollection periods.

Blood samples for calcium and phosphate, drawn anerobically, were ultrafiltered as previously described (1), and calcium in serum ultrafiltrate and urine was measured by atomic absorption spectrophotometry (Perkin-Elmer Co., Norwalk, Conn.). Sodium concentration in blood and urine samples was determined by flame photometry and insulin and phosphate was determined as previously described (1).

Tubular fluid inulin concentration was measured in duplicate in 6-nl aliquots using the fluorometric method of Vurek and Pegram (17). Tubular fluid calcium and phosphate concentration were determined by electron microprobe analysis using a modification of the method as described by Lechene (12). Six 0.3-nl aliquots of each tubular fluid sample were placed on a scored, polished, pure beryllium block under oil. The oil was removed with methylene and the samples were rapidly frozen in isopentane and precooled to -140°C with liquid nitrogen. The block was put into a freeze dryer (Virtis Co.), lyophilized under vacuum at -40°C for 18 hr, and then placed in an oven at 300°C for 5-10 min. The analysis was done in an Applied Research Laboratories microprobe using an accelerating potential of 11 kv, beam diameter of 75-150 μ, and a beam current of 0.3 μamp. Following the above procedure, each specimen consisting of fine crystals evenly distributed over a circular area of 70-100 μ in diameter, was counted for 100 sec, and the calcium and phosphate concentrations of each sample were determined from standard curves as the mean of six replicates. A set of 22 serum ultrafiltrates were analyzed by the macromethods described above for calcium and 36 ultrafiltrates for phosphate, and aliquots of each were analyzed as unknowns by the microprobe technique. The mean ± standard error of the quotients of results obtained by the microprobe divided by the results from the macromethods were 0.99 ± 0.02 for calcium and 1.02 ± 0.02 for phosphate.

Clearances of sodium (CNa), inulin (Cr), ultrafilterable calcium (Cc), and ultrafilterable phosphate (Cp) were calculated in the usual manner. Fractional sodium and water reabsorption in the proximal tubule was calculated as 1 - plasma inulin concentration/tubular fluid inulin concentration (1 - P/TF)in. Fractional calcium reabsorption was calculated as 1 - [(TF/UF)Ca/(TF/P)in] where (TF/UF)Ca is the ratio of calcium concentration in tubular fluid to that in serum ultrafiltrate and (TF/P)in is the tubular fluid/plasma ratio of inulin. Fractional phosphate reabsorption was calculated as 1 - [(TF/UF)p/(TF/P)in]. For statistical analysis, the mean control values for Cr, CNa, Cc, Cp, (TF/P)in, (TF/UF)Ca, (TF/UF)p, 1 - [(TF/UF)Ca/(TF/P)in], and 1 - [(TF/UF)p/(TF/P)in] for the experimental kidney in each dog were treated as single observations and compared with the mean value 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 (4).

RESULTS

Hydropenia. Data were obtained in 67 tubules from 16 dogs during the control period prior to administration of either parathyroid hormone or dibutylryl cyclic AMP. (TF/UF)Ca and (TF/UF)p tended to remain constant throughout the accessible length of the proximal tubule with means ± standard error of 1.14 ± 0.04 and 0.71 ± 0.04, respectively. In Fig. 1 (TF/UF)Ca and (TF/UF)p are plotted against (TF/P)in. The value for (TF/UF)p is not significantly different from the value of 0.72 ± 0.06 pre-

FIG. 1. Relationship between proximal tubular fluid/plasma ultrafiltrate calcium ((TF/UF)Ca) and (TF/UF)p and (TF/P)in during hydropenia in individual tubules from 16 dogs.

FIG. 2. Relationship between proximal tubular fractional reabsorption of water (1 - (P/TF)in) and fractional reabsorption of sodium, calcium and phosphate during hydropenia. Solid line represents fractional reabsorption of sodium, assuming isotonic reabsorption of sodium and water in proximal tubule. Symbols represent data from individual tubules from 16 dogs.
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or water and phosphate reabsorption was therefore altered was a proportionately greater fall in fractional phosphate reabsorption from 0.51 ± 0.02 to 0.31 ± 0.04 (P < 0.005) (Table 1), and the relationship between fractional sodium or water and phosphate reabsorption was therefore altered by PTH. The equation for the new relationship was y = 0.86x + 0.10 ± 0.04 with an r value of 0.70 (P < 0.01). By analysis of covariance, the slopes of the regression lines for the relationship between sodium and phosphate reabsorption before and after PTH are not significantly different, but the difference in the elevation (y intercept) of the two lines is significantly different at P < .005. In the final urine (Table 1), there was a small but significant rise in sodium clearance following PTH from 0.09 ± 0.03 to 0.16 ± 0.05 ml/min (P < 0.02) in contrast to calcium clearance, which fell from 0.13 ± 0.03 to 0.06 ± 0.006 ml/min (P < 0.05). Phosphate clearance rose markedly from 0.25 ± 0.03 to 0.15 ± 0.06 (P < .05) similar to our previous study (1), whereas inulin clearance was unchanged.

Dibutyryl cyclic AMP. DB-cAMP was infused systemically at a rate of 100 mg/hr in five dogs in which 22 collection-recollection pairs of proximal tubular fluid samples were obtained. Mean ± standard error (TF/UF)ca from 1.14 ± 0.03 to 1.14 ± 0.03, whereas (TF/UF)ca rose significantly from 0.75 ± 0.04 to 0.92 ± 0.04 (P < 0.005). The clearance and micropuncture observations are summarized in Table 1. There was a significant fall in proximal tubule fractional reabsorption of sodium from 0.34 ± 0.02 to 0.25 ± 0.03 (P < 0.02). Fractional reabsorption of calcium fell proportionately from 0.25 ± 0.04 to 0.15 ± 0.06 so that the relationship between sodium and calcium reabsorption in the proximal tubule was unchanged following PTH administration. The equation for this relationship following PTH was y = 1.28x - 0.18 ± 0.11 with an r value of 0.8 (P < 0.001); this is not significantly different from the relationship observed in the control tubules. In direct contrast, because of the rise in (TF/UF)ca, there was a proportionately greater fall in fractional phosphate reabsorption from 0.51 ± 0.02 to 0.31 ± 0.04 (P < 0.005) (Table 1), and the relationship between fractional sodium or water and phosphate reabsorption was therefore altered

Control dogs. We have previously shown that in continued hydropenia there is no significant change in (TF/UF)ca, (TF/UF)Ca, and 1 - [(TF/UF)Ca/(TF/P)In] between the control and recollection samples (1). In the present study, in 46 tubules obtained from nine dogs, the mean ± standard error (TF/P)In during the control period was 1.42 ± 0.03 and (TF/UF)Ca was 1.10 ± 0.02. After 1 hr of continued hydropenia, recollection values were 1.37 ± 0.03 for (TF/P)In and 1.10 ± 0.02 for (TF/UF)Ca. Neither recollection value was significantly different from the values in the initial collections.

Parathyroid hormone. The effects of a systemic infusion of purified PTH, 50-60 U/hr, were studied in 11 dogs in which 47 collection-recollection pairs of proximal tubular fluid samples were obtained. There was a significant fall in mean ± standard error (TF/P)In from 1.57 ± 0.07 to 1.38 ± 0.06 (P < 0.05) similar to the changes observed by us in a previous study (1). Figure 3 depicts the values for (TF/UF)Ca and (TF/UF)P for individual tubules and the mean for each animal during control and recollection periods. There was no significant change in (TF/UF)Ca from 1.14 ± 0.03 to 1.14 ± 0.03, whereas (TF/UF)P rose significantly from 0.75 ± 0.04 to 0.92 ± 0.04 (P < 0.005). The clearance and micropuncture observations are summarized in Table 1. There was a significant fall in proximal tubule fractional reabsorption of sodium from 0.34 ± 0.02 to 0.25 ± 0.03 (P < 0.02). Fractional reabsorption of calcium fell proportionately from 0.25 ± 0.04 to 0.15 ± 0.06 so that the relationship between sodium and calcium reabsorption in the proximal tubule was unchanged following PTH administration. The equation for this relationship following PTH was y = 1.28x - 0.18 ± 0.11 with an r value of 0.8 (P < 0.001); this is not significantly different from the relationship observed in the control tubules. In direct contrast, because of the rise in (TF/UF)ca, there was a proportionately greater fall in fractional phosphate reabsorption from 0.51 ± 0.02 to 0.31 ± 0.04 (P < 0.005) (Table 1), and the relationship between fractional sodium or water and phosphate reabsorption was therefore altered.
Discussion

A significant body of data exists that suggests a close relationship between the tubular handling of sodium and calcium (18). Previous micropuncture studies in the rat (11) and dog (5) have suggested that calcium concentration tends to remain constant along the length of the proximal tubule, implying paracellular reabsorption of sodium, calcium, and water. Simultaneous measurements of calcium and inulin concentrations were not performed, however. In the present study, the use of a sensitive technique, microprobe analysis, requiring only very small volumes of tubular fluid, allowed a more precise evaluation of the relationship of sodium and calcium reabsorption in the proximal tubule.

The data confirm that there is no change in TF/UF calcium along the accessible portion of the proximal tubule and indicate that there is a direct linear relationship between the fractional reabsorption of these two ions in the nondiuretic dog.

The mean value for (TF/UF)c,a in the proximal tubule was approximately 1.1, and this was significantly different from unity. This value is similar to published preliminary reports involving measurements of calcium concentration in proximal tubular fluid of the dog (5, 6). As (TF/UF)c,a showed no tendency to vary with (TF/P)r,a, it implies either a rate of calcium reabsorption slower than fluid reabsorption very early in the tubule (where (TF/P)r,a is less than 1.1) or transient net secretion of calcium very early in the tubule followed by net reabsorption. The number of very early proximal samples are too few, and the possible errors in this ratio at these levels of (TF/P)r,a are so great, however, that a definite conclusion concerning early proximal calcium secretion is not warranted. The value of 1.1 for the ratio of (TF/UF)c,a/(TF/P)r,a in the proximal tubule is compatible with passive distribution of calcium at electrochemical equilibrium assuming the existence of a small negative transtubular potential difference (−3 mV) in the dog proximal tubule as recently demonstrated by Boulpaep and Seely (3). It is also possible, however, that assessment of ultrafilterate calcium using artificial membranes may not be truly indicative of glomerular ultrafiltrability. To definitively settle this question, direct sampling and analysis of glomerular ultrafiltrate will ultimately be required, a procedure not yet feasible in the dog.

Previous micropuncture studies of calcium reabsorption have suggested the existence of active proximal calcium reabsorption based on a fall in tubular fluid calcium concentration during mannitol diuresis (5, 11). Mannitol
diuresis in the dog, however, is associated with a minimal fall in proximal tubular sodium reabsorption (14), and thus a linkage between sodium and calcium transport in the proximal tubule could adequately explain these data without invoking the existence of a separate calcium transport system. Further studies with simultaneous direct measurements of sodium and calcium reabsorption during mannitol diuresis are required to evaluate these possibilities.

We have previously shown that parathyroid hormone, probably via cAMP, inhibits the reabsorption of sodium, as well as phosphate, in the proximal tubule, resulting in a small natriuresis and a marked phosphaturia (1). Earlier clearance studies have suggested a stimulatory effect of PTH on tubular calcium reabsorption (2, 7, 10, 16, 19), implying a dissociation between sodium and calcium reabsorption somewhere in the nephron. In the present study, the data indicate that PTH inhibits the reabsorption of calcium and sodium in the proximal tubule in parallel so that the linear relationships between the reabsorption of these ions remain unchanged. In the final urine, however, there was a small natriuresis without an associated calciuria. Therefore, PTH exerts a dual action on the nephron resulting in inhibition of calcium and sodium reabsorption in the proximal tubule and a stimulation of calcium reabsorption in the distal nephron. It should be pointed out that in view of marked proximal inhibition of sodium reabsorption and increased delivery to the distal nephron, the small natriuresis observed implies an increase in sodium reabsorption in the distal nephron similar to that seen with albumin infusions (9). Thus, a large fraction of the calcium reabsorbed distally may be associated with parallel changes in sodium reabsorption. The small fraction reabsorbed in excess of sodium, producing a decrease in calcium excretion with a slight natriuresis, may reflect either the stimulation by PTH of a separate calcium transport system, or a PTH-induced change in membrane permeability in the distal nephron altering the sodium-calcium relationships in the reabsorbate.

Our previous micropuncture observations (1) plus the data in this study strongly suggest that the action of PTH to inhibit proximal calcium reabsorption along with phosphate and sodium is mediated by cyclic AMP. The data are also compatible with the conclusion that the hypocalciuric effect of PTH may be mediated via cyclic AMP in the distal nephron. In our studies calcium excretion, in contrast to sodium excretion, did not increase with DB-cAMP administration, despite an increase in filtered load and a marked inhibition of proximal tubular reabsorption of both of these ions, implying a stimulation of net calcium transport relative to sodium within the distal nephron.

The phosphate data reported in this study, similar to those previously reported by us (1), reveal inhibition of both phosphate and sodium reabsorption in the proximal tubule, but the use of a more sensitive technique in this study, electron probe analysis, has allowed a more precise evaluation of sodium-phosphate relationships than was previously possible. Thus, there is a direct linear relationship between the fractional reabsorption of sodium and phosphate in the dog proximal tubule similar to that of calcium. The mean (TF/UF)_P of 0.7, however, implies that very early in the tubule there is reabsorption of a large fraction of filtered phosphate in excess of sodium. Inspection of Fig. 2 reveals that during the first 10–20% of sodium and fluid reabsorption, 40% of filtered phosphate is reabsorbed, whereas an additional 40% of filtered phosphate is reabsorbed while fractional fluid reabsorption is increasing from 20–60%. As with calcium, this is compatible with passive distribution of phosphate at electrochemical equilibrium early in the tubule producing the (TF/UF)_P of 0.7, which is then maintained by a direct relationship between sodium and phosphate reabsorption throughout the remainder of the proximal tubule accessible to micropuncture.

The administration of PTH produced an increase in TF/UF phosphate, in contrast to TF/UF calcium, indicating that the hormone altered the relationship between proximal tubular sodium and phosphate reabsorption. The slopes of the regression lines relating the fractional phosphate and the fluid reabsorption before and after PTH are not different, but a statistically significant difference in the intercepts was demonstrable. Thus, a linear relationship between sodium and phosphate reabsorption persists but
with a higher (TF/UF)_P. This could be explained either by a change in the transtubular potential difference or by a change in membrane permeability to phosphate altering the equilibrium value for (TF/UF)_P. Our data cannot differentiate these possibilities, but, in view of the lack of change in TF/UF calcium, they suggest that a change in permeability to phosphate is the more likely explanation. An alternate possibility is a specific effect of PTH directly on a renal tubular phosphate transport system unrelated to its effect on sodium reabsorption. The specificity of this effect of PTH is yet to be determined, since studies in our laboratory have revealed a tendency toward a rise in proximal tubular (TF/UF)_P with saline infusion (13).

These data indicate the important role of the distal tubule in the regulation of differential excretion of sodium, calcium, and phosphate ions. Thus, following the establishment of equilibrium ratios very early in the proximal tubule, these three ions are reabsorbed in parallel. The nature of this parallelism is unclear but could be explained either by movement of calcium and phosphate according to electrochemical gradients or by reabsorptive bulk flow of tubular fluid from lumen to intercellular space in response to osmotic gradients, perhaps via intercellular shunt pathways (8). The separation of these ions occurs in the distal tubule. As distal phosphate reabsorption appears to be relatively small (5) in the presence of PTH, most of the phosphate delivered out of the proximal tubule is excreted in the final urine. Sodium and calcium are reabsorbed throughout the nephron but can be dissociated distally by PTH. The latter hormone, probably via the adenyl cyclase system, stimulates net reabsorption of calcium, despite the marked proximal inhibition of reabsorption. This results in a fall in calcium excretion and a slight natriuresis.

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REFERENCES


