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PARATHYROID HORMONE INHIBITION OF PHOSPHATE TRANSPORT IN RENAL BRUSH BORDER VESICLES FROM PHOSPHATE-DEPLETED DOGS

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Dietary phosphate (P_i) restriction increases renal P_i reabsorption and induces resistance to the phosphaturic action of parathyroid hormone. Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from the renal brush border of experimental animals has been shown to parallel changes in renal P_i reabsorption induced by dietary P_i restriction and in vivo administration of parathyroid hormone. Dietary P_i restriction has been shown to markedly inhibit the phosphaturic response to parathyroid hormone in rats and dogs. Parathyroid hormone has been reported not to decrease the Na^+-gradient-stimulated transport of P_i in brush border membrane vesicles isolated from dietary P_i restricted rats unless the rats were administered an acute P_i load prior to killing, however, thyroparathyroidectomy of rats fed a low P_i diet has been reported to increase Na^+-gradient-stimulated P_i transport. Using the dietary P_i restricted dog, we demonstrated no significant decrease in renal reabsorption of P_i in response to parathyroid hormone administration. However, significant decreases in P_i transport in brush border membrane vesicles isolated from the kidneys of dietary P_i restricted dogs were observed in response to in vivo parathyroid hormone administration. These data demonstrate that the resistance to the phosphaturic action of parathyroid hormone observed in vivo does not include resistance to the inhibitory effect of parathyroid hormone on P_i transport in brush border membrane vesicles. Thus, the data suggest that parathyroid hormone continues to alter P_i transport characteristics of the brush border membrane in states of P_i depletion despite the resistance to parathyroid hormone seen in vivo.

Introduction

Renal phosphate (P_i) reabsorption is increased by dietary P_i restriction [1,2] and decreased by parathyroid hormone [3]. Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from the renal brush border is increased when the vesicles are isolated from kidneys from animals fed a P_i restricted diet [4–7] and decreased when the vesicles are isolated from kidneys of animals given parathyroid hormone in vivo [8]. Studies in intact rats [9–11] and dogs [12] and micropuncture studies in dogs [13] have indicated that dietary P_i restriction renders the kidney and renal proximal tubule refractory to the phosphaturic effect of parathyroid hormone.

We have previously demonstrated that decreases in renal P_i reabsorption measured in vivo, which are induced by administration of parathyroid hormone to intact dogs are reflected in decreased rates of Na^+-gradient-stimulated P_i transport in membrane vesicles from the renal brush border isolated from the kidneys of those dogs [14]. The use of dogs allowed us to isolate a sufficient quantity of membrane vesicles so

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as to utilize a control kidney (prior to parathyroid hormone administration) and an experimental kidney (after parathyroid hormone administration) from the same animal for preparation of membrane vesicles.

Previous studies which examined the interaction of dietary P_i restriction and in vivo parathyroid hormone administration on P_i transport in isolated rat brush border membrane vesicles have yielded somewhat conflicting results. One study demonstrated an inhibitory effect of parathyroid hormone on Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from rats fed a low P_i diet only if rats were administered an acute P_i load 1 h prior to killing [7]. In this study parathyroidectomy of rats fed a low P_i diet did not increase Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from the kidneys of those rats. Measurements of in vivo renal P_i reabsorption were not presented in this study. However, thyroparathyroidectomy of rats fed a low P_i diet was reported to increase Na^+-gradient-stimulated P_i transport in a second study [6]. This increase in Na^+-gradient-stimulated P_i transport was paralleled by an increase in renal P_i reabsorption measured in vivo. These latter findings lead the authors to suggest that alterations in P_i transport at the level of the brush border membrane could represent a common final mechanism to account for alterations in renal P_i reabsorption induced by dietary P_i restriction and parathyroid hormone. Because of the importance of this hypothesis to our understanding of phosphate transport, we have re-examined the effects of P_i depletion in the dog. We have analyzed the resistance to the action of parathyroid hormone both in vivo, and in brush border membrane vesicles isolated from P_i-depleted dogs.

Materials and Methods

Mongrel dogs, weighting 14–25 kg and fed a high protein Purina Dog Chow (Ralson Purina, St. Louis, MO) or fed a low P_i diet (Casein base, phosphate content 0.0002%, ICN Pharmaceuticals Inc., Cleveland, OH) for six weeks prior to killing so as to effect P_i depletion, were anesthesized with pentobarbitol (0.12 g/kg given slowly intravenously) and ventilated mechanically through an endotrachael tube. A jugular vein catheter was placed for obtaining blood samples and for infusion of solutions. A urinary bladder catheter was placed for collection of urine. Baseline collections for determinations of endogenous creatinine and P_i clearances were obtained after which the left kidney (control) was removed. After the unilateral nephrectomy, a second baseline clearance period was obtained from the now single experimental kidney. Then, bovine parathyroid hormone (b-PTH 1-84) (Inolex, Park Forest, IL, 1 500 U/mg in a rat hypercalcemic assay) was administered intravenously at a dose of 2 μg/kg of body weight. 30 min after administration of parathyroid hormone, a 15-min clearance period was performed followed immediately by right nephrectomy.

After each nephrectomy the renal artery was immediately catheterized and 75–100 ml of ice-cold saline was infused through the aterial catheter, and the kidneys were placed in ice.

Vesicles of membranes from the brush border of the kidney were isolated by a $CaCl_2$ precipitation technique described previously in detail [15,16]. The vesicles were preloaded by suspending them in 300 mM mannitol containing 5 mM Hepes/Tris, pH 7.5 (5 mM Hepes adjusted with Tris hydroxide). The enrichment of the membrane preparation was evaluated randomly by specific enzyme markers [16,17] and by electron microscopy. Protein concentrations were determined by a standard procedure [18] using bovine serum albumin as the reference protein.

Uptake of [^{32}P]orthophosphoric acid ($^{32}P_i$), D-[2-^{3}H]glucose and ^{22}NaCl by the vesicles was measured by a Millipore filtration technique [14,16,17] using 0.65 μm Millipore filters. All incubations were carried out in triplicate with freshly prepared membrane vesicles. The results are expressed as the mean ± S.E. All experiments were performed on at least three separate occasions using at least nine separate incubation mixtures unless otherwise noted.

Apparent K_m and V values for Na^+-dependent P_i transport in isolated membrane vesicles were calculated as previously described [14].

$^{32}P_i$, D-[2-^{3}H]glucose (18.1 Ci/mmol), and ^{22}NaCl were obtained from New England Nuclear, Boston, MA. Other chemicals were of the highest purity available from commercial sources. All solutions were filtered through 0.45 μm millipore filters prior to use [19].

Plasma and urine levels of creatinine and phosphorus were determined by methods previously

described [14]. Ultrafiltrable phosphorus was determined by centrifugation of anaerobically obtained plasma samples through Amicon XM50 cones and measurement of P_i in the filtrate. Creatinine clearance tubular reabsorption of phosphate were determined by standard calculations. The absolute P_i reabsorption (^TP_i) was determined by differences between the filtered load of phosphorus and the absolute urinary excretion of P_i.

Analysis of variance was utilized to analyze differences between 'overshoots' in Fig. 1. A Student's *t*-test was employed to analyze statistical differences in Table I and the multiple comparison procedure of Dunnett [20] was utilized in Tables II and III.

Results

Characterization of renal brush border membrane vesicles

Enrichment for specific marker enzymes [14] in isolated dog membrane vesicle preparations compared to crude renal cortical homogenates was determined using membrane vesicles and cortical homogenates from normal and dietary P_i restricted dogs. Enzyme content of membrane vesicles from the brush borders as compared to crude homogenates was not enriched for a mitochondrial enzyme marker (glutamate dehydrogenase was 0.007 to 0.009 of crude homogenate specific activity), lysozomal enzyme marker (acid phosphatase was 0.01 to 0.07 of crude homogenate specific activity), endoplasmic reticulum (glucose-6-phosphatase was 0.002 to 0.004 of crude homogenate specific activity), or basal lateral membrane enzyme marker (ouabain-sensative(Na^+ K^+)ATPase was 0.4 to 0.65 of crude homogenate specific activity). Enrichment for alkaline phosphatase, a marker enzyme for brush border membrane was 10 to 12-fold. No significant differences were observed between membrane vesicle preparations originating from kidneys from normal or P_i restricted dogs.

Plasma calcium, creatinine clearance and tubular reabsorption of P_i in normal and dietary P_i restricted dogs

Plasma calcium (Ca) and P_i, clearance of creatinine and tubular reabsorption of P_i were measured prior to the first nephrectomy, after the first nephrectomy prior to parathyroid hormone administration, and after parathyroid hormone adminstration in normal dogs and dogs fed P_i restricted diets (Table I). The low P_i diet induced severe hypophosphatemia. Previous studies have demonstrated that imposition of a low P_i diet lowers creatinine clearance in the dog [21]. Every normal dog responded to parathyroid hormone with a decrease in tubular reabsorption of P_i. Parathyroid hormone administration to dietary P_i restricted dogs did not significantly decrease tubular reabsorption of P_i. The absolute P_i reabsorption was 0.58 ± 0.02 mg P_i/min in dietary P_i restricted dogs prior to parathyroid hormone administration and 0.52 ± 0.02 mg P_i/min after parathyroid hormone administration. This did not represent a significant decrease (Student's *t*-test).

TABLE I

PLASMA CALCIUM AND P_i, CREATININE CLEARANCE AND TUBULAR REABSORPTION OF P_i IN NORMAL AND DIETARY P_i RESTRICTED DOGS

Plasma calcium (Ca) and P_i, creatinine clearance (Ccr) and tubular reabsorption of P_i (TRP) were measured in normal dogs and dietary P_i restricted dogs: (a) after anesthesia prior to the first nephrectomy; (b) following the first nephrectomy prior to the administration of parathyroid hormone (PTH); (c) 30 min following administration of PTH. Data are reported as mean ± S.E. for three animals in each group.

Animals	Ca^{2+} (mg/100 ml)	P_i (mg/100 ml)	Ccr (ml/min)	TRP (%)
Normals				
a	9.7 ± 0.8	4.2 ± 0.2	74 ± 5	92 ± 4
b	9.4 ± 0.2	3.8 ± 0.4	38 ± 4	88 ± 2
c	9.4 ± 0.1	3.9 ± 0.1	37 ± 3	64 ± 1 *
Dietary restricted				
a	9.6 ± 0.5	1.4 ± 0.4	65 ± 7	100 ± 0
b	9.5 ± 0.3	1.4 ± 0.4	37 ± 6	100 ± 0.3
c	9.4 ± 0.2	1.4 ± 0.5	35 ± 7	98 ± 0.3 **

* Normal + PTH < normal; $P < 0.05$.

** Dietary P_i restricted + PTH < dietary P_i restricted; not significant.

Na^+-gradient-stimulated P_i transport in isolated vesicles from kidneys of normal and dietary P_i restricted dogs

Na^+-gradient-stimulated P_i transport [14] was measured in membrane vesicles isolated from kidneys of normal dogs, normal dogs given parathyroid hor-

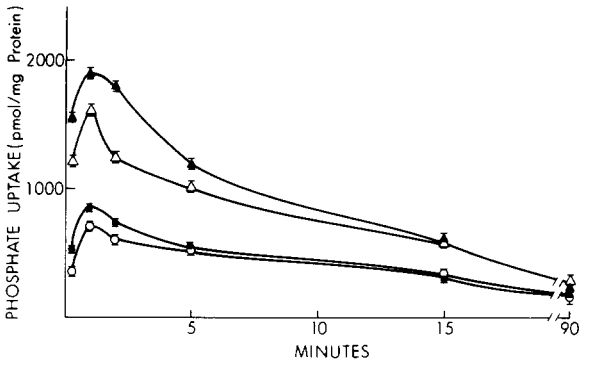

Fig. 1. The time course of uptake of 25 μM P_i in renal brush border membrane vesicle uptake was measured in the presence of an extravesicular to intravesicular gradient of NaCl (100 mM). Brush border vesicles were isolated form normal dogs (●——●), normal dogs given parathyroid hormone (○——○), dietary P_i restricted dogs (▲——▲) and dietary P_i restricted dogs given parathyroid hormone (△——△). Data are reported as mean ± S.E.

mone in vivo, dietary P_i restricted dogs and dietary P_i restricted dogs given parathyroid hormone in vivo. Fig. 1 demonstrates a significantly increased 'overshoot' of Na^+-stimulated P_i transport in membrane vesicles originating from kidneys from dietary P_i restricted dogs compared to vesicles isolated from kidneys of normal dogs. Parathyroid hormone administration to normal or dietary P_i restricted dogs resulted in significantly decreased 'overshoots' in membrane vesicles isolated from kidneys originating from both normal dogs and dietary P_i restricted dogs.

Initial rates [14,17] of Na^+-gradient-stimulated P_i transport, Na^+-gradient-stimulated D-glucose transport and $^{22}Na^+$ uptake expressed in terms of percent of steady state (90 min) P_i, D-glucose or $^{22}Na^+$ uptake in membrane vesicles [6,7,14,17] are compared in Table II. A significant increase in the initial rate of P_i, but not D-glucose transport was demonstrated in vesicles from kidneys of dietary P_i restricted dogs compared to vesicles isolated from kidneys of normal dogs. Significant decreases in initial rates of P_i, but not D-glucose transport in isolated vesicles were effected by administration of parathyroid hormone to normal or dietary P_i restricted dogs. Initial rates of 25 μM $^{22}NaCl$ uptake were not changed by parathyroid hormone administration to normal or dietary P_i restricted dogs. Initial rates of P_i and D-glucose transport measured in the presence of an initial KCl gradient were small [14] and did not vary significantly among groups of dogs studied (data not shown).

The effect of parathyroid hormone administration to dietary P_i restricted dogs on apparent K_m and V

TABLE II

INITIAL RATE OF TRANSPORT AND STEADY STATE P_i, D-GLUCOSE AND $^{22}Na^+$ UPTAKES IN BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM NORMAL AND DIETARY P_i RESTRICTED DOGS

Initial rates of 25 μM P_i and 25 μM D-glucose transport and $^{22}Na^+$ uptake in membrane vesicles (uptake per 20 s) were determined as previously described [14] as were steady-state levels of 25 μM P_i, 25 μM D-glucose and 25 μM $^{22}Na^+$ uptake (90 min). The uptake at 20 s was divided by the uptake at 90 min and multiplied by 100 to obtain the initial rate of transport expressed as a percentage of the steady-state uptake [6,7]. Data are expressed as mean ± S.E. PTH, parathyroid hormone.

Animals	Initial rate of transport (% uptake at steady state per 20 s)			Uptake at steady state (pmol/mg protein)		
	P_i	D-Glucose [d]	$^{22}Na^+$ [e]	P_i	D-Glucose	$^{22}Na^+$
Normal	337 ± 4.0 [c]	627 ± 27	16.1 ± 2.0	173 ± 2.1	29.2 ± 1.0	87.0 ± 2.0
Normal + PTH	233 ± 7.7 [a]	630 ± 19	16.2 ± 3.0	169 ± 3.0	27.0 ± 0.5	88.5 ± 2.5
Dietary P_i restricted	566 ± 17	686 ± 28	20.6 ± 4.5	209 ± 24	24.6 ± 1.0	77.6 ± 5.9
Dietary P_i restricted E PTH	485 ± 15 [b]	680 ± 10	18.2 ± 4.1	218 ± 13	25.1 ± 6.2	86.7 ± 3.8

[a] Normal + PTH < normal; $P < 0.05$.
[b] Dietary P_i restricted + PTH < dietary P_i restricted; $P < 0.05$.
[c] Normal < dietary P_i restricted; $P < 0.01$.
[d] No significant difference among groups.
[e] No significant difference among groups.

TABLE III

EFFECT OF EXPERIMENTAL MANIPULATIONS ON K_m AND V FOR P_i TRANSPORT IN BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM NORMAL AND DIETARY P_i RESTRICTED DOGS

Double reciprocal plots of the Na^+-gradient-dependent P_i transport system were obtained by subtracting the P_i uptake observed in the presence of an initial 100 mM NaCl gradient from that observed in the presence of an initial 100 mM KCl gradient [14]. The P_i concentration of the incubation media was varied from 0.01 to 0.5 mM. Apparent K_m and V values were calculated from regression lines obtained from least squares analysis in Lineweaver-Burk plots (regression coefficients = 0.96–0.99). Six different concentrations of P_i were used and the transport experiments were performed in triplicate on at least three different occasions using kidneys from different animals. Data are reported as mean ± S.E. PTH, parathyroid hormone.

Animals	K_m [c] (μM)	V (pmol/20 s per mg protein)
Normal	51 ± 2	1585 ± 20
P_i restricted	39 ± 6	3364 ± 75 [b]
P_i restricted ± PTH	36 ± 3	2680 ± 69 [a]

[a] P_i restricted > P_i restricted + PTH; $P < 0.01$.
[b] P_i restricted > normal; $P < 0.01$.
[c] No significant difference among groups.

[8,14] for P_i transport in membrane vesicles isolated from kidneys of those dogs is shown in Table III. parathyroid hormone administered in vivo significantly decreased the apparent V for P_i transport in vesicles isolated from P_i restricted dogs. The V in vesicles isolated from P_i restricted dogs was significantly greater than the V in vesicles isolated from normal dogs. It has been previously demonstrated [14] that parathyroid hormone administered in vivo decreases the apparent V for P_i transport in vesicles isolated from normal dogs.

Discussion

An absolute or relative unresponsiveness to the phosphaturic effects of parathyroid hormone has been demonstrated in rats and dogs under the conditions of dietary P_i restriction [9–13]. It is known that changes in renal P_i reabsorption induced by P_i restriction or by parathyroid hormone administration are reflected by changes in Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from the renal brush border of dietary P_i restricted or parathyroid hormone-treated animals [4–8,14]. Stoll et al. [7] using rats which had undergone dietary P_i restriction was unable to demonstrated an effect of in vivo administered parathyroid hormone upon Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from the renal brush border of the dietary P_i restricted rats. Subsequent studies by Stoll et al. [6] demonstrated that thyroparathyroidectomy of rats fed a low P_i diet increased whole kidney P_i reabsorption and Na^+-gradient-stimulated P_i transport in brush border membrane vesicles isolated from those rats and lead the authors to suggest that changes in P_i reabsorption induced by dietary P_i and parathyroid hormone might be accounted for by alterations in P_i transport at the level of the brush border membrane induced by common final alterations in brush border membrane structure.

The studies reported here demonstrate increased rates of Na^+-gradient-dependent P_i transport in membrane vesicles isolated from P_i-depleted dogs as compared to rates of Na^+-gradient-dependent P_i transport in brush border membrane vesicles isolated from normal dogs. Parathyroid hormone administration in vivo decreased the renal reabsorption of P_i in normal dogs but not in P_i-depleted dogs. The resistance to the phosphaturic action of parathyroid hormone which was demonstrated in the P_i-depleted state invivo was not reflected in the brush border membrane vesicles. Treatment of P_i-depleted dogs with parathyroid hormone prior to nephrectomy and preparation of the brush border membrane vesicles, resulted in significant reductions of the inital rates and heights of the overshoots of P_i transport and in the V for P_i transport. This, parathyroid hormone was active in decreasing P_i transport in brush border membrane vesicles from P_i-depleted dogs. This decrease in Na^+-gradient-stimulated P_i transport in membrane vesicles was observed in the face of no significant decrease in tubular reabsorption of P_i or ^TP_i.

Our findings are consistent with the hypothesis that dietary P_i restriction and parathyroid hormone both alter renal reabsorption of P_i by altering Na^+-dependent P_i transport at the level of the renal brush border membrane [6]. They demonstrate in addition, however, that parathyroid hormone-induced altera-

tions in Na^+-dependent P_i transport across the renal brush border membrane, as reflected by alterations in Na^+-gradient-dependent P_i transport in isolated brush border membrane vesicles, are masked in vivo by alterations in Na^+-dependent P_i transport induced by dietary P_i restriction.

It is possible that the effects of dietary P_i restriction and parathyroid hormone upon P_i reabsorption are mediated dissimilarly such as through initiation of protein synthesis in the case of dietary P_i restriction [22] and through protein phospharylation in the case of parathyroid hormone [6,23]. This being the case it would be reasonable to suggest that differences exist in the manner by which and degree to which changes in P_i reabsorption induced by dietary P_i restriction and by parathyroid hormone administration persist in the form of alterations in Na^+-gradient-stimulated P_i transport in isolated brush border membrane vesicles. We interpret our data as demonstrating that the in vivo phosphaturic effects of parathyroid hormone are not expressed in the dietary P_i restricted dog because they are masked by the antiphosphaturic effects of dietary P_i restriction on renal tubular cells. Parathyroid hormone, nonetheless, alters the renal brush border membrane in vivo in the dietary P_i restricted dog. The alteration is reflected in decreased Na^+-gradient-stimulated P_i transport in membrane vesicles isolated from the parathyroid hormone-treated dietary P_i restricted dog. The effect of parathyroid hormone is masked in vivo, but not vitro, indicating that the whole cellular effect of dietary P_i depletion results in inhibition of the phosphaturic action of parathyroid hormone, but that the biochemical alterations of the brish border membrane induced by parathyroid hormone, reflective of its phosphaturic action, are still produced.

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