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Effect of phosphonoformic acid, dietary phosphate and the *Hyp* mutation on kinetically distinct phosphate transport processes in mouse kidney *

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We examined the kinetics of phosphate transport in mouse renal brush-border membrane vesicles under initial rate (6 s), trans zero, voltage clamp conditions. Two kinetically distinct Na^+-dependent phosphate transport processes were identified: a high-affinity, low-capacity system (K_m, 0.09 ± 0.02 mM; V_{max}, 539 ± 50 pmol/mg protein per 6 s) and a low-affinity, high-capacity system (K_m, 1.28 ± 0.35 mM; V_{max}, 1677 ± 198 pmol/mg protein per 6 s). The high-affinity system was inhibited competitively by 1 mM phosphonoformic acid (PFA) (apparent K_i, 0.31 ± 0.03 mM) and completely abolished by 20 mM PFA; the low-affinity system was insensitive to 1 mM PFA and was inhibited competitively by 20 mM PFA (apparent K_i, 9.03 ± 1.21 mM). Dietary phosphate deprivation elicited a significant increase in V_{max} of both high- and low-affinity phosphate transport systems whereas the X-linked *Hyp* mutation caused a 50% decrease in V_{max} of the high-affinity system with no change in the low-affinity system. Phosphate deprivation of *Hyp* mice elicited a 3.5-fold increase in V_{max} of the high-affinity system. Neither diet nor mutation significantly altered the apparent K_m values of either phosphate transport process. We conclude that (1) mouse kidney brush-border membranes have two distinct Na^+-dependent phosphate transport systems which differ in affinity and capacity; (2) both processes participate in the adaptive response to dietary phosphate restriction; (3) only the high-affinity system is impaired by the X-linked *Hyp* mutation.

Introduction

The reabsorption of filtered phosphate by the kidney is a major determinant of phosphate homeostasis and, therefore, crucial to normal growth and bone development [1]. Evidence from micropuncture and microperfusion studies indicates that the bulk of filtered phosphate is reabsorbed in the proximal tubule, with the S_1 segment, or proximal convoluted tubule, exhibiting a higher capacity for phosphate reabsorption than the more distal S_2 and S_3 segments, comprising the proximal straight tubule or pars recta [2]. Studies in renal cortical brush-border membrane vesicles isolated from a number of mammalian species have established that the initial step in phosphate reabsorption across the luminal surface is concentrative, Na^+-dependent, and mediated by a high-affinity, phosphate transport system [3,4]. *In vitro* measurement of phosphate transport into brush-border membrane vesicles has been shown to accurately reflect the phosphate reabsorptive capacity of the whole kidney as assessed by *in vivo* clearance studies [5]. Accordingly, brush-border membrane phosphate transport has been widely used to characterize renal phosphate transport and its regulation by hormonal [6], environmental [7], and genetic factors [8].

Recent studies have provided evidence for more than one phosphate transport system in brush-border membranes of rat [9,10] and pig kidney [11]. In addition to the previously described high-affinity system [3,4], a low-affinity, high-capacity system was also identified [9–11]. The demonstration that both systems reside in the outer cortex of pig kidney while only the high-affinity system is found in the outer medulla suggested that the low-affinity, high-capacity system is located in the

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early proximal tubule (S_1), where the bulk of filtered phosphate is reabsorbed whereas the high-affinity, low-capacity system is situated throughout the proximal tubule, where it reclaims residual tubular phosphate [11].

The present study was undertaken to characterize further the two phosphate transport processes in mouse renal brush-border membrane vesicles. The interaction of each phosphate transport system with phosphonoformic acid (PFA), a specific inhibitor of brush-border membrane phosphate transport [12], was examined. In addition, we studied the effects of environmental and genetic determinants, namely dietary phosphate restriction and the X-linked *Hyp* mutation, on the expression of the kinetically distinct brush-border membrane phosphate transport processes. The phosphate-deprived mouse exhibits an adaptive renal response which is characterized by a striking and specific increase in renal brush-border membrane phosphate transport activity [13]. The X-linked *Hyp* mouse is a murine homologue of X-linked hypophosphatemic rickets in man and exhibits a specific reduction in renal brush-border membrane phosphate transport function [8,14].

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Materials and Methods

Mice. C57BL/6 male mice (2–4 months old), maintained on Wayne Lab Blox containing 1% phosphate and 1.2% calcium, were used in all experiments except where otherwise indicated. To test the effect of dietary phosphate restriction on renal brush-border membrane phosphate transport, C57BL/6 mice were fed a low phosphate diet containing 0.03% phosphate and 1% calcium for 4 days. Control mice were fed the identical diet supplemented with 1% phosphate for 4 days. Mice were killed by decapitation and trunk blood was collected. Plasma phosphate, determined as described previously [14], fell from 2.68 ± 0.11 mM on the control diet to 1.71 ± 0.08 mM on the low phosphate diet (mean ± S.E., $n = 10$, $P < 0.001$).

Mutant *Hyp* mice (male, *Hyp*/Y; female, *Hyp*/+) and normal littermates (male, +/Y; female, +/+) were obtained by crossing C57BL/6J males with *Hyp*/+ females [14]. Breeders were maintained on Wayne Mouse Breeder Blox containing 0.98% phosphate and 1.2% calcium. Pups were weaned at 25 days of age and switched to Wayne Lab Blox. Mice were used for experiments at 3–4 months of age. *Hyp* mice were significantly hypophosphatemic when compared to normal littermates (plasma phosphate of 1.40 ± 0.05 mM vs. 2.70 ± 0.07 mM, mean ± S.E., $n = 10$, $P < 0.001$). *Hyp* mice fed the control and low-phosphate diets for 4 days had a plasma phosphate of 1.70 ± 0.08 mM and 0.98 ± 0.05 mM, respectively (mean ± S.E., $n = 10$, $P < 0.001$).

Brush-border membrane isolation and transport studies. Brush-border membrane vesicles were prepared from fresh kidney cortex, or from kidney cortices which had been quick-frozen in liquid nitrogen and stored at −85°C, by the Mg^{2+} precipitation procedure as described previously [8]. No differences in yield, enrichment or transport activity could be found between brush-border membrane vesicles prepared from frozen and fresh kidneys. Membrane vesicles were suspended in 20 mM KCl, 260 mM mannitol, 20 mM Hepes-Tris (pH 7.4) to a final protein concentration of 4–6 mg/ml. Valinomycin, dissolved in 100% dimethylsulphoxide (DMSO), was added to the vesicles to yield a final valinomycin concentration of 62.5 μg/ml. The concentration of DMSO did not exceed 0.25%. Alkaline phosphatase activity, assayed at pH 10.0 using 5 mM *p*-nitrophenyl phosphate in 1.0 M diethanolamine, was used to estimate brush-border membrane enrichment [15]. Enrichments of 8–10-fold were routinely obtained and were similar for brush-border membrane vesicles prepared from normal, phosphate-deprived, and mutant *Hyp* mice. Protein concentrations of homogenates and brush border membrane vesicles were measured by the procedure of Lowry et al. [16] with bovine serum albumin as the standard.

The uptake of ^{32}P inorganic phosphate was measured at 6 s at 22°C under voltage-clamp, zero-trans sodium and phosphate conditions, by the rapid filtration technique using 0.45 μm Millipore filters [8]. Transport studies were performed in quadruplicate, in both 100 mM KCl, 100 mM mannitol, 20 mM Hepes-Tris, (pH 7.4) and 100 mM NaCl, 20 mM KCl, 60 mM mannitol, 20 mM Hepes-Tris (pH 7.4) over a phosphate concentration range of 0.01 mM to 10.0 mM. The reaction was stopped by the addition of ice-cold stop solution, containing 100 mM NaCl, 100 mM mannitol, 1 mM NaN_3, 10 mM Na_2HAsO_4 and 20 mM Hepes-Tris (pH 7.4). In experiments with PFA, the inhibitor was added to the ^{32}P-containing uptake medium.

To determine the Na^+-dependent component of transport, diffusional uptake, derived from pooled experiments ($n = 14$) conducted in the presence of the KCl medium, was subtracted from total uptake, derived from individual experiments measured in the NaCl medium. The combined results of all diffusion measurements clearly belonged to a single population in which uptake was a linear function of phosphate concentration, with a slope equal to 130 · (phosphate concentration, mM) · $(\text{mg protein})^{-1} \cdot (6\ \text{s})^{-1}$.

Eadie-Hofstee transformation of the data was used to calculate kinetic parameters. The plots were not linear but could be resolved into two straight lines: one, at low concentrations of phosphate, corresponding to

the high-affinity system only and one, at high concentrations of phosphate, corresponding to the sum of the high-affinity and low-affinity processes (see Fig. 2). This was accomplished with an iterative process which minimized the sum of the squares of the standard deviations of the experimental points from the lines. In all cases, the best fit was obtained when the junctions of the two lines occurred at a phosphate concentration between 0.5 mM and 1.0 mM, indicating that the contribution of the low-affinity system to the combined systems line becomes negligible below this range. The apparent K_m and V_{max} of the two processes were obtained directly from the slopes and intercepts of the two linear portions, i.e. K_m (high affinity) = slope of the right limb; V_{max} (high affinity) = extrapolated intercept of the right limb, $V/S = 0$; K_m (low affinity) = slope of the left limb − slope of right limb; V_{max} (low affinity) = intercept of left limb − V_{max} of high-affinity process (see Fig. 2 for corrected left limb). The standard errors of the slopes and intercepts were calculated with the help of published equations [17]. The slopes (apparent K_m) and intercepts (V_{max}) of regression lines were compared independently by Student's *t*-test.

Materials. PFA, trisodium salt, was purchased from Sigma. We determined that contamination of PFA by inorganic phosphate was less than 0.05%. Therefore, inhibition of transport by PFA could not be attributed to dilution of ^{32}P specific activity. Our initial breeding pairs (C57BL/6J males and *Hyp*/+ females) were obtained from the Jackson Laboratory, Bar Harbor, ME. C57BL/6 mice were purchased from Charles River Canada, Inc., St. Constant, Quebec, Wayne diets from Allied Mills, Inc., Chicago, IL, low phosphate (No. 86128) and control (No. 86129) diets from Teklad, Madison, WI, carrier-free ^{32}P from Dupont, Canada, and valinomycin from Boehringer Mannheim, Mannheim, F.R.G.

Results

Kinetic studies

A time course for phosphate uptake in mouse renal brush-border membrane vesicles is shown in Fig. 1. The results indicate that, under the present assay conditions (22°C, pH = 7.4), phosphate uptake is linear as a function of time for at least 6 s in both NaCl and KCl incubation media. The Na^+-dependent component of phosphate transport, used to derive kinetic parameters, is also linear for 6 s.

Our earlier studies of Na^+-dependent phosphate transport in mouse renal brush-border membrane vesicles, performed over a concentration range of 0.015mM to 0.8 mM phosphate, revealed a single transport system with an apparent K_m and V_{max} of 0.05 mM and 722 pmol/mg protein per 15 s, respectively [13]. In the present study, initial rate phosphate fluxes were

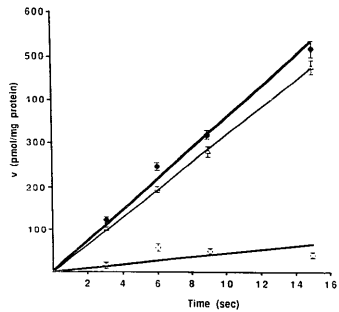

Fig. 1. Time course of ^{32}P-inorganic phosphate uptake into brush-border membrane vesicles prepared from normal mouse kidney cortex. Uptake was measured at 22° C as described in Materials and Methods. Uptake media contained 100 mM KCl, 100 mM mannitol, 20 mM Hepes-Tris, pH 7.4 (○) and 100 mM NaCl, 20 mM KCl, 60 mM mannitol, 20 mM Hepes-Tris, pH 7.4 (●). The sodium-dependent component of phosphate transport, obtained by subtracting uptake in KCl medium from uptake in NaCl medium, is also shown (△).

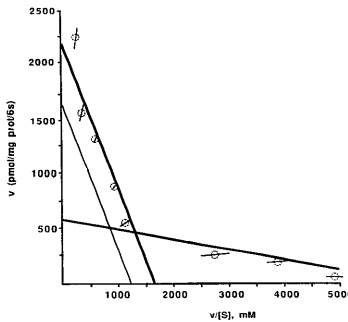

Fig. 2. Eadie-Hofstee plot of the Na^+-dependent component of phosphate transport. Renal brush-border membrane phosphate transport was measured at 6 s at phosphate concentrations between 0.01 mM and 10 mM. Uptake measured in the presence of KCl was subtracted from that in the presence of NaCl to obtain the Na^+-dependent component of phosphate flux. Computer transformation of the data was used to determine the regression lines and calculate the kinetic parameters as described in Materials and Methods. Data shown were derived from 10 different brush-border membrane preparations from normal mouse kidney cortex, with uptake measurements performed in quadruplicate. The corrected line depicting the low-affinity, high-capacity process is shown.

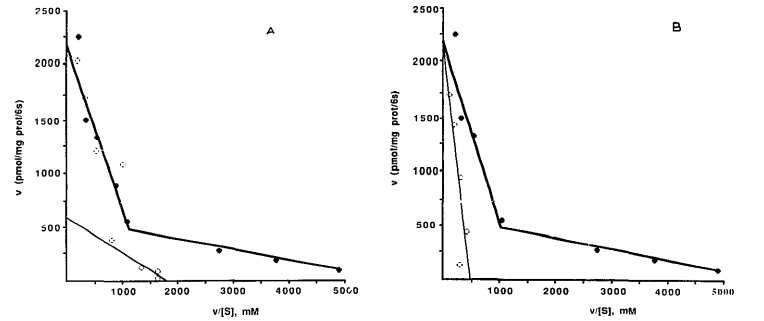

Fig. 3. Effect of phosphonoformic acid on the Na^+-dependent component of phosphate transport. Normal mouse renal brush-border membrane vesicles were prepared and kinetics of phosphate transport was examined as described in the legend to Fig. 2. (A) The Na^+-dependent component of phosphate transport was determined in the presence (○) and absence (●) of 1 mM PFA. (B) The Na^+-dependent component of phosphate transport was determined in the presence (○) and absence (●) of 20 mM PFA. Results are means of two representative brush-border membrane preparations, with uptake measurements performed in quadruplicate.

measured over a wider range of phosphate concentrations (0.01 mM to 10 mM). Fig. 2 depicts an Eadie-Hofstee plot of the data derived from ten separate brush-border membrane preparations. The plot is clearly non-linear and can be resolved into two linear segments. The best fit was obtained when the junction of the two segments occurred at a phosphate concentration between 0.5 mM and 1.0 mM. The kinetic constants of the two saturable components were determined as described in Materials and Methods. One system was of high capacity (V_{max}, 1677 ± 198 pmol/mg protein per 6 s) and relatively low affinity (apparent K_m, 1.28 ± 0.35 mM) and the other of lower capacity (V_{max}, 539 ± 50 pmol/mg protein per 6 s) and higher affinity (apparent K_m, 0.09 ± 0.02 mM).

TABLE I

Effect of phosphonoformic acid on kinetically distinct phosphate transport systems in mouse renal brush-border membrane vesicles

Uptake studies were performed in quadruplicate in mouse renal brush-border membrane vesicles as described in Materials and Methods over a phosphate concentration range of 0.01 mM to 10.0 mM. In experiments with PFA, values are means ± S.E. of three separate brush-border membrane vesicle preparations. Kinetic parameters for experiments in the absence of PFA are means ± S.E. of ten membrane preparations. Control vs. PFA: [1] $P < 0.001$; [2] $P < 0.05$.

High-affinity system		
	Control	1 mM PFA
V_{max} (pmol/mg protein per 6 s)	539 ± 50	629 ± 109
K_m (mM)	0.09 ± 0.02	0.37 ± 0.03 [1]
K_i (mM)	–	0.31 ± 0.03
Low-affinity system		
	Control	20 mM PFA
V_{max} (pmol/mg protein per 6 s)	1677 ± 198	2244 ± 609
K_m (mM)	1.28 ± 0.35	3.13 ± 0.73 [2]
K_i (mM)	–	9.03 ± 1.21

TABLE II

Effect of low-phosphate diet on kineticaily distinct phosphate transport systems in mouse renal brush-border membrane vesicles

Brush-border membrane vesicles were prepared from mice fed either a control or low-phosphate diet for 4 days. Uptake studies were performed in quadruplicate as described in Materials and Methods over a phosphate concentration range of 0.01 mM to 10 mM. [1] In another series of experiments, V_{max} of the low-affinity system was examined in the presence of 10 mM or 20 mM PFA, in order to inhibit the contribution of the high-affinity system. Values are means ± S.E. of at least four brush-border membrane vesicle preparations. Control vs. low-P_i diet: [2] $P < 0.001$, [3] $P < 0.03$.

High-affinity system		
	Control diet	Low-P_i diet
V_{max} (pmol/mg protein per 6 s)	518 ± 54	1288 ± 126 [2]
K_m (mM)	0.17 ± 0.03	0.11 ± 0.02
Low-affinity system		
	Control diet	Low-P_i diet
V_{max} (pmol/mg protein per 6 s)	1239 ± 183	1751 ± 401
K_m (mM)	1.25 ± 0.43	1.09 ± 0.50
V_{max}, with PFA [1] (pmol/mg protein per 6 s)	1897 ± 367	3105 ± 325 [3]
K_m, with PFA [1] (mM)	3.15 ± 0.80	3.76 ± 0.90

Effect of PFA

We examined the effect of PFA on these kinetically distinct phosphate transport processes in mouse renal brush-border membrane vesicles. 1 mM PFA inhibited the high-affinity system competitively, affecting K_m but not V_{max} (apparent K_i, 0.31 ± 0.03 mM, Table I). 1 mM PFA had no effect on the low-affinity component (Fig. 3A). 20 mM PFA completely eliminated the high-affinity system and inhibited the low-affinity process competitively (apparent K_i, 9.03 ± 1.21 mM, Table I, Fig. 3B).

Effect of phosphate deprivation

Table II compares kinetic parameters of renal brush-border membrane vesicles prepared from phosphate-deprived and control mice. Restriction of dietary phosphate elicited a 2.5-fold increase in V_{max} of the high-affinity system. Phosphate deprivation also increased V_{max} of the low-affinity process, although the diet-induced increase did not reach statistical significance. The apparent K_m values of both systems were not changed by dietary phosphate restriction (Table II).

To further examine the effect of phosphate restriction on V_{max} of the low-affinity transport process, additional kinetic studies were performed in the presence of 10 mM or 20 mM PFA. Under these conditions, the competitive inhibitor completely suppressed the contribution of the high-affinity system (data not shown and Fig. 3B). (In the absence of PFA, subtraction of the high-affinity system from the low-affinity system is achieved mathematically, as described in Methods and Materials). In the presence of high concentrations of PFA, low-phosphate diet elicited a significant increase in V_{max} and no change in apparent K_m of the low-affinity system, confirming the results obtained in the absence of inhibitor (Table II).

Effect of the Hyp mutation

Both phosphate transport systems were expressed in renal brush-border membrane vesicles prepared from *Hyp* mice (Table III). Kinetic parameters characterizing these processes are compared with those derived from brush-border membrane vesicles of normal littermates (Table III). The *Hyp* mutation significantly decreases the V_{max} of the high-affinity system but does not appear to affect the V_{max} of the low-affinity system. Table III also shows that the *Hyp* mutation does not significantly alter the apparent K_m of either transport process.

The effect of phosphate deprivation on phosphate transport kinetics in brush-border membrane vesicles from *Hyp* mice was also examined. Phosphate restriction increased the V_{max} of the high-affinity system from 267 ± 34 to 962 ± 62 pmol/mg protein per 6 s ($n = 4$, $P < 0.001$) but did not significantly alter the apparent K_m (0.06 ± 0.02 vs. 0.07 ± 0.01 mM for the control and low-phosphate diets, respectively). It is not yet clear whether phosphate deprivation influences the V_{max} of the low-affinity phosphate transport process in brush-border membranes of *Hyp* mice since kinetic analyses in the presence of high concentrations of PFA were not performed in the mutant strain. These conditions were necessary to demonstrate a significant diet-induced increase in V_{max} of the low-affinity system in brush-border membrane vesicles of normal mice (Table II).

TABLE III

Effect of Hyp mutation on kinetically distinct phosphate transport systems in mouse renal brush-border membrane vesicles

Brush-border membrane vesicles were prepared from *Hyp* mice and normal littermates. Uptake studies were performed in quadruplicate as described in Materials and Methods over a phosphate concentration range of 0.01 mM to 10 mM. Values are means ± S.E. of four separate brush-border membrane vesicle preparations derived from *Hyp* mouse kidney and eight membrane preparations from kidneys of normal littermates. Normal vs. *Hyp*: [1] $P < 0.001$.

High-affinity system		
	Normal	*Hyp*
V_{max} (pmol/mg protein per 6 s)	539 ± 50	253 ± 30 [1]
K_m (mM)	0.09 ± 0.02	0.08 ± 0.02
Low-affinity system		
	Normal	*Hyp*
V_{max} (pmol/mg protein per 6 s)	1677 ± 193	1868 ± 352
K_m (mM)	1.28 ± 0.35	3.45 ± 1.15

Discussion

Two kinetically distinct phosphate transport processes have been identified in brush-border membranes of rat [9,10] and pig kidney [11]: a low-affinity, high-capacity system, present only in the early proximal tubule in a position to reabsorb the bulk of filtered phosphate, and a high-affinity, low-capacity system located throughout the proximal tubule to reclaim residual filtered phosphate [11]. In the present study, we provide evidence for both phosphate transport processes in brush-border membranes of mouse kidney. We demonstrate that both systems are competitively inhibited by PFA. In addition, we demonstrate that the adaptive response to phosphate restriction involves both the high-affinity and low-affinity systems whereas the X-linked *Hyp* mutation impairs only the high-affinity process.

Several reports indicate that the detection of multiple phosphate transport processes in renal brush-border membranes is dependent on the ambient temperature at which uptake studies are performed [9,10]. In rat renal brush-border membranes, the low-affinity system was less evident at 25 °C than at 35 °C [19], and in another study, only a slight deviation from the linear Michaelis-

Menten kinetics was observed at 25°C [20]. In contrast, two phosphate transport systems were demonstrated in brush-border membranes of pig outer renal cortex at 21°C [11]. In the present study, both the high- and low-affinity phosphate transport processes were also clearly identified in mouse renal brush border membranes at 22°C. The reasons for these discrepancies are not understood but may be related to the relative proportion of S_1, S_2 and S_3 segments in the tissue used to isolate brush-border membrane vesicles, to species variations, or to pH and counter anions present in the uptake medium.

Multiple transport systems for solutes other than phosphate have also been demonstrated in renal proximal tubule brush-border membranes. A topological separation of Na^+-dependent D-glucose transport systems was obtained in rabbit kidney: a low-affinity, high-capacity system in the outer cortex (early proximal tubule) and a high-affinity, low-capacity system unique to the outer medulla (late proximal tubule [21]). In a more recent study, however, the high-affinity glucose transporter was also identified in the early proximal tubule, suggesting that this transporter, like the high-affinity phosphate transporter, reclaims filtered solute throughout the entire proximal tubule [11].

Our results with PFA confirm those of a previous report which demonstrated that it acts as a competitive inhibitor of the high-affinity phosphate transport system in renal brush-border membranes [12]. In addition, we show that at higher concentrations, PFA can also competitively inhibit the low-affinity, high-capacity phosphate transport system. At these concentrations, we demonstrate that PFA also completely inhibits the high-affinity phosphate transport process. Accordingly, under these conditions, direct comparisons of V_{max} for the low-affinity process can be made. This approach was useful to establish whether or not the low phosphate diet directly affected the low-affinity process.

Dietary phosphate is an important regulator of renal phosphate handling [1]. Phosphate restriction elicits a significant rise in proximal tubule brush-border membrane phosphate transport [7,13]. Kinetic studies over a narrow phosphate concentration range demonstrated a diet-induced increase in V_{max} and no change in apparent K_m [13,22] of what is now recognized as the high-affinity phosphate transport process, which extends throughout the proximal tubule. In the present study, we examined the effect of low phosphate diet on both phosphate transport processes, with particular emphasis on the low-affinity, high-capacity system in the early proximal segment. Our data on the effect of phosphate restriction on the high-affinity system are consistent with previous findings [13,22]. In addition, we demonstrate that the low-affinity system can be modulated by dietary phosphate. Although the apparent K_m was not altered, V_{max} appeared to be increased under standard uptake conditions. Moreover, statistically significant diet-induced increase in V_{max} of the low-affinity process was apparent in experiments conducted in the presence of 10 mM or 20 mM PFA. Under these conditions, contribution of the high-affinity system to the phosphate transport process is negligible. Our findings are consistent with micropuncture studies which suggested that the proximal convoluted tubule may be less sensitive to phosphate deprivation than the proximal straight tubule [23]. Our data also support an earlier demonstration that phosphate restriction appears to influence V_{max} of a low-affinity, high-capacity system in brush-border membranes isolated from superficial nephrons of rat kidney [9].

In the present study, we also examined the effect of the X-linked *Hyp* mutation on brush border membrane phosphate transport kinetics. The X-linked *Hyp* mouse is a murine homologue of X-linked hypophosphatemia, the most common form of inherited rickets in man [24]. A previous study reported a 50% reduction in V_{max} of the putative high-affinity transport process in brush-border membranes derived from *Hyp* mice [18]. However, that study measured phosphate transport over a narrow range of concentrations and did not examine the low-affinity transport system. In the present study, we examined both phosphate transport processes independently and demonstrated directly that the *Hyp* mutation affects only the high-affinity system, leaving the low-affinity system intact. These results are physiologically relevant since they indicate that hypophosphatemia and ensuing rachitic bone disease associated with the *Hyp* mutation can be attributed entirely to a defect in the high-affinity, low-capacity brush-border membrane phosphate transport process. The underlying mechanism for impaired renal brush-border membrane phosphate transport in *Hyp* mice is not understood. It is not clear whether the 50% decrease in V_{max} of the high-affinity transport process reflects a decrease in the number of phosphate transport units or a decrease in the functional activity of existing carriers within the brush-border membrane of the mutant strain. Moreover, it has not yet been established whether the mutant *Hyp* gene encodes the phosphate transporter per se or a regulator of the renal phosphate transport process.

The present study also addresses the issue of renal adaptation to phosphate restriction by X-linked *Hyp* mice. Although increased phosphate transport was reported in brush-border membranes of phosphate-deprived *Hyp* mice [13], in vivo studies [25] as well as experiments using cultured renal tubular cells [26] failed to show a normal adaptive response in the mutant strain. It is clear from our present kinetic data that phosphate-deprivation of *Hyp* mice elicits a significant increase in V_{max} of the high-affinity phosphate transport process and that the magnitude of the response is not less than that seen in phosphate-deprived normal mice.

The basis for the discrepancy between the present *in vitro* data and the previous *in vivo* and cultured cell data is not clear.

It is of interest that both dietary phosphate and the X-linked *Hyp* mutation exert effects on the V_{max} of the high-affinity phosphate transport system, albeit in opposite directions. These results emphasize the relative importance of the high-affinity phosphate transport process in the regulation of phosphate homeostasis. This conclusion is supported by experiments localizing the site of action of PTH, a major regulator of renal phosphate transport, in isolated nephron segments prepared from rabbit kidney [27]. The study clearly demonstrated that it was the low-capacity (high-affinity) phosphate transport system of the proximal straight tubule that was markedly inhibited by PTH whereas the high-capacity (low-affinity) phosphate transport system of the proximal convoluted tubule was insenstiive to the action of the hormone [27].

The molecular mechanisms involved in the regulation of renal brush-border membrane phosphate transport have not yet been elucidated. Studies in cultured kidney cells suggested that two mechanisms participate in the adaptive response to low phosphate in the growth medium: (1) a rapid response involving recruitment or activation of existing transporters and (2) a slower response involving new protein synthesis [28]. In another study, the adaptive increase in phosphate transport was attributed to the decrease in brush-border membrane cholesterol and subsequent increase in membrane fluidity elicited by phosphate deprivation [29]. Finally, other reports have implicated protein kinase A-dependent phosphorylation [30] and ADP-ribosylation [31] of as yet unidentified brush-border membrane proteins in the regulation of renal phosphate transport. Clearly, until methods are available to purify the phosphate transport protein(s) of renal brush-border membranes, thereby permitting the preparation of antibody and cDNA probes, the molecular mechanisms whereby dietary phosphate and the *Hyp* mutation alter phosphate transport function will remain obscure.

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