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Effect of parathyroid hormone and dietary phosphate on phosphate transport in renal outer cortical and outer medullary brush-border membrane vesicles

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Two distinctive sodium-dependent phosphate transport systems have been identified in early and late proximal tubules; a high-capacity process located only in outer cortical tissue, and a high affinity present in both outer cortical and outer medullary brush-border membranes (K_m 0.1–0.25 mM). A third, sodium-independent, pH gradient-stimulated system (V_{max} 4.7 ± 0.3 nmol · mg^{-1} · min^{-1}, K_m 0.15 ± 0.002 mM) is present in the outer medulla, but absent in outer cortex. Brush-border vesicles were prepared from outer cortical and outer medullary tissue of pigs maintained on low (< 0.05%), normal (0.4%), or high (4%) phosphate diets. Sodium-dependent phosphate uptake of the high-capacity system decreased (V_{max}, 9.4 to 2.2 nmol · mg^{-1} · min^{-1}) from low to high phosphate diet, whereas uptake rates decreased about 50% in the high-affinity system. There were no changes in the respective K_m values. The pH gradient-stimulated uptake also decreased (V_{max}, 6.9 to 3.0 nmol · mg^{-1} · min^{-1}) with no change in mean K_m value (0.15 ± 0.001 mM) with dietary manipulation. Administration of 1 U parathyroid hormone prior to study resulted in a decrease in sodium-dependent uptake by 40–50% and in pH-dependent uptake (36%) with no change in the respective K_m values. In conclusion, the antecedent dietary phosphate intake and parathyroid hormone administration appropriately alters phosphate uptake across the brush-border membrane of all three systems, sodium-dependent and pH gradient-stimulated phosphate transport.

Introduction

The majority of the phosphate filtered through the glomerular membrane is reabsorbed in the proximal tubule [1,2]. Substantial evidence has been given for heterogeneity of phosphate transport along the length of the proximal tubule [3–6]. In the pig, there appears to be three distinctive transport processes; first, a high-capacity, low-affinity (apparent K_m 4.0 mM) sodium-dependent phosphate transporter located in brush-border membrane vesicles isolated from the outer cortex of the kidney [6]. Second, a low-capacity, high-affinity (apparent K_m 0.1 mM) sodium-dependent transporter located throughout the length of the proximal tubule as reflected by its presence in both outer cortical and outer medullary brush-border membrane vesicles. Finally, evidence has been given for a facilitative phosphate transport which is sodium-independent and present only in brush-border membrane vesicles purified from outer medullary tissue [7]. This transport pathway may reflect hydroxyl-phosphate exchange or proton-phosphate symport.

The principal regulatory factors which influence renal phosphate handling involve control by circulating levels of parathyroid hormone and intrinsic adjustments related to the antecedent dietary phosphate intake [1,2]. Accordingly, parathyroid hormone leads to enhanced urinary phosphate excretion by diminishing proximal phosphate reabsorption [5,8–10]. Proximal absorption may also be altered by the availability of phosphate such that the renal cells adapt appropriately, either up-regulating or down-regulating phosphate transport in response to dietary phosphate deficit or surfeit, respectively [5–7,10–18]. These regulatory influences have clearly been shown to act, in part, at the brush-border membrane [16,19–28].

The purpose of the present studies was to evaluate the effects of parathyroid hormone and dietary phosphate on the various modes of transport across outer cortical and outer medullary brush-border membranes. The data indicate that all transport systems, located in

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the outer cortex and outer medulla, are influenced similarly by parathyroid hormone and dietary phosphate and suggest that all processes act in concert to regulate phosphate homeostasis.

Materials and Methods

Weanling pigs (15–25 kg) were maintained for 5–10 days on a grain diet containing 0.3–0.4% phosphorus. The animals had free access to water and were provided with food ad libitum up to experimentation. The influence of dietary phosphate was evaluated in three groups of animals: high, about 4%; normal, 0.4%; and low, < 0.05% total dietary inorganic phosphate. Animals were maintained on these diets for 3–7 days prior to experimentation. Following anaesthesia, urine and blood samples were collected and the kidneys of these animals were harvested for vesicle preparation. In those animals which received parathyroid hormone, the pigs were anesthetized with pentobarbital sodium and subsequently infused with 1–34 synthetic bovine parathyroid hormone (Sigma, St. Louis, MO) at rates sufficient to deliver one unit/hour for 2 h. During this time, blood and urine were sampled via catheters placed in the jugular vein and ureters, respectively. At the end of 2 h, the kidneys were perfused with ice-cold saline and removed for preparation of brush-border membrane vesicles.

Vesicle preparation. Brush-border membrane vesicles were prepared from outer cortical and outer medullary tissue of pig kidneys. Tissue slices < 0.5 mm thick were cut off the outer surface of the kidneys with a sharp scalpel blade. Brush-border membrane vesicles prepared from homogenates of these slices are from outer cortical nephrons and are presumably composed of principally S_1 and S_2 segments of the proximal tubule. The kidneys were then sectioned transversally, and 0.5–2-mm thick slices were removed from the outer strip of the outer medullary region. Vesicles prepared from these slices were termed late proximal tubule and were principally composed of S_3 segments. Because of the size of the kidney, there was no problem encountered in defining the regions or harvesting sufficient tissue. Cortical and medullary slices were obtained from each kidney and handled on a paired basis throughout the preparative stages and transport studies.

The kidney slices were suspended in 10 mM tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer containing 300 mM mannitol and 10 mM $MgCl_2$ (pH 7.1, final concentration) in a dilution of 1 g/40 ml. The tissue was homogenized on ice for 10 min at full speed with a VirTis homogenizer. Brush-border membranes were enriched and purified by differential centrifugation according to previously reported methodologies [6]. The homogenate was centrifuged for 10 min at 8000 × *g*, and the supernatant from this spin for 20 min at 21 000 × *g*. The pellet was then resuspended in 5–10 ml buffer, and the above low-speed and high-speed centrifugations were repeated. The final pellet was resuspended in the buffer, given below. The vesicle suspension was passed twice through a 25 gauge needle and then stored on ice for about 0.5–1.0 h before use. All uptake assays were completed within 5–6 h of vesicle preparation. Aliquots of homogenate and final vesicle suspension were rapidly frozen and stored for enzyme analyses.

Enzyme assays. The purity of the final vesicle suspensions was assessed by assaying the activity of enzyme associated with the brush-border and basolateral membranes and with the cytosol and mitochondria. Alkaline phosphatase was measured at pH 10.5 in a buffer containing 90 mM glycine, 1 mM $MgCl_2$, 0.1 mM $ZnSO_4$, and 6 mM *p*-nitrophenyl phosphate as substrate. Gammaglutamyl transpeptidase was determined using a commercially available kit (Sigma, St. Louis, MO). Na^+,K^+-ATPase and succinate dehydrogenase were determined as previously reported [24]. Protein was determined by the method of Lowry after treatment of the membranes with 5% sodium dodecyl sulphate (w/v); dilutions of bovine serum albumin were used as standards.

Uptake measurements. Membranes were typically suspended in a buffer containing 10 mM Tris-*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Tris-Hepes) (pH 7.5); 50 mM KSCN, and 200 mM mannitol with a protein concentration of 5–8 mg/ml. Intravesicular electrolyte composition was altered by preincubating the membranes in hypertonic solutions of the desired composition. To assess sodium-dependent uptake, incubation medium contained 10 mM Tris-Hepes (pH 7.5), 50 mM KSCN plus 100 mM NaSCN or 100 mM KSCN and variable amounts of K_2HPO_4. The determination of pH-gradient stimulated phosphate uptake was performed in vesicles prepared with the above buffer, but with an intravesicule pH 8.0. Incubation solution contained 10 mM Tris-Mes, (pH 6.5), 50 mM KSCN and 100 mM mannitol. All concentrations and pH values given here and in the legends to the figures are final values. The phosphate stop solution contained 10 mM Tris-Hepes, 140 mM NaCl, 10 mM Na_2AsO_4, and 0.5 mM NaH_2PO_4 buffered to a pH value of 7.5.

Phosphate uptakes were measured by using tracer [^{32}P]phosphoric acid generally at concentrations of 2–4 μCi/ml. All solutions were filtered through a 0.22-μm Millepore filter before use. The procedure for uptake measurements was typically started by adding 50 μl of incubation medium to 10 μl of membrane suspension kept at 21° C. Vesicle suspension and incubation solutions were added to a 12 × 75 mm plastic test tubule, and the reaction was started by mixing with a Vortex Fisher Scientific). The reaction was terminated at the set time by delivering ice-cold stop solution into the test tube. The mixture was then filtered on a Millepore filter

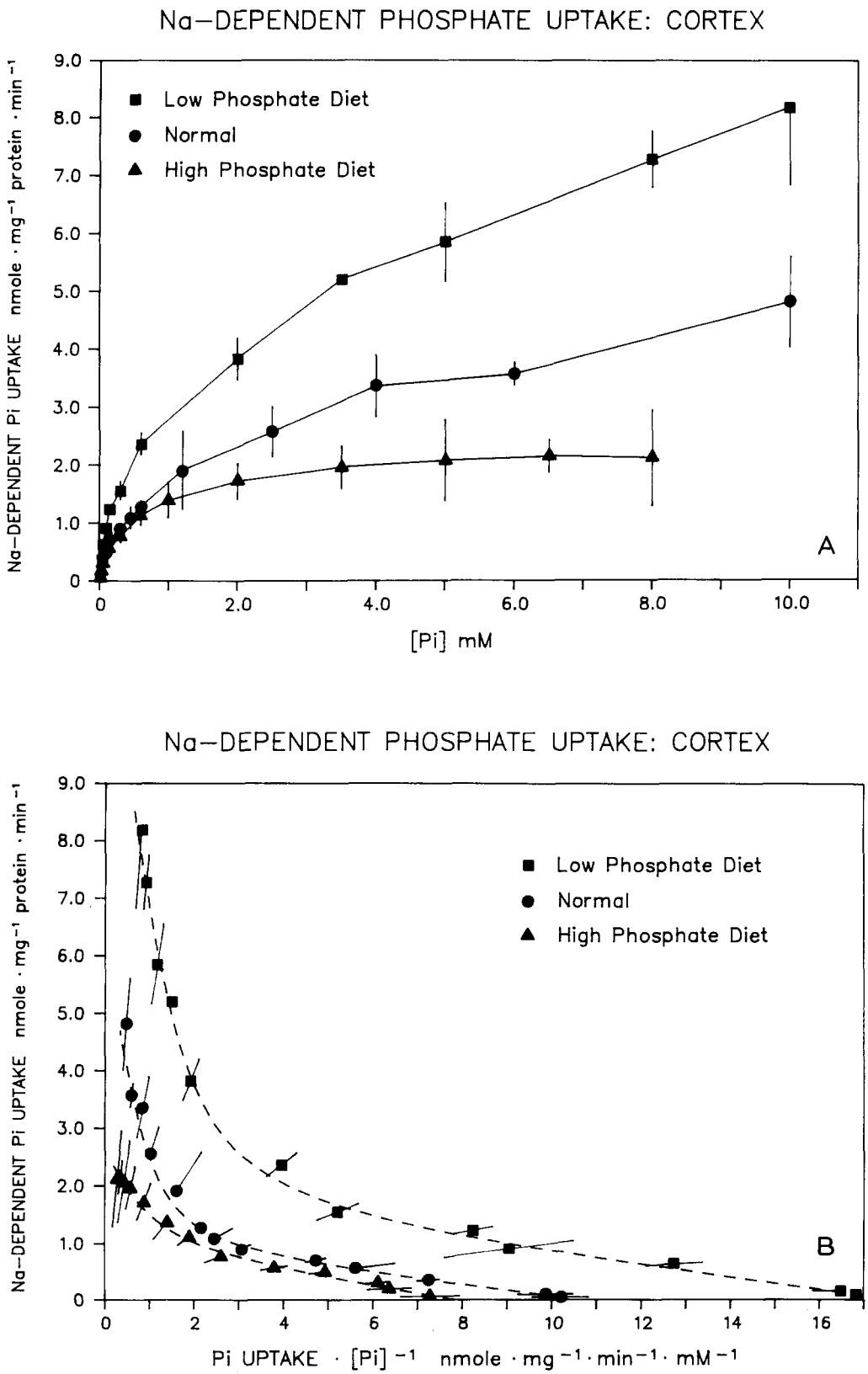

Fig. 1. Effect of dietary phosphate and parathyroid hormone on sodium-dependent phosphate transport in brush-border membrane vesicles isolated from the outer cortex. Transport was performed at pH 7.5; $pH_{in} = pH_{out}$, in the presence or absence of an external sodium concentration. Sodium-dependent transport was determined by subtracting the uptake in the absence of sodium with uptake rates measured in the presence of sodium. Intravesicular composition was (in mM): KSCN, 50; mannitol, 200; buffer, 10; and the incubation solution was: mannitol, 100; NaSCN, 100 or KSCN, 100, and buffer, 10. Panel A illustrates the dependence of uptake on the external phosphate concentration and panel B an Eadie-Hofstee transformation of these uptake data. Values are means ± S.D. for five or six separate determinations.

(HAWP 0.45 μm) and washed twice with 5 ml of cold stop solution. The filters were dried and then dissolved in Filter Count (Packard Instruments), and the radioactivity was counted by liquid scintillation techniques.

A Woolf-Augustinsson-Hofstee transformation was used to calculate the kinetic parameters with the aid of nonlinear least-squares best fit for saturation isotherm assay data assuming one or two independent sites as appropriate (Lundon Software, Cleveland, OH). This program weights the data according to the inverse of the variance of observation points for each concentration [7] and the errors are standard deviations of the mean values unless otherwise stated.

Results

Blood and urine were collected for electrolyte analysis and creatinine determination prior to harvesting the kidneys in each group of animals. Table I summarizes the mean electrolyte values for pigs maintained on normal, high and low phosphate diets and animals administered parathyroid hormone. There were no significant differences in plasma or urinary sodium, potassium or magnesium concentrations among the various groups. Plasma inorganic phosphorous was significantly elevated in pigs maintained on high dietary phosphate and plasma calcium was significantly increased in those fed a low phosphate diet. Urinary phosphate excretion was significantly increased 2–3-fold with either elevated dietary phosphate or following to parathyroid hormone infusion. Urinary phosphate was significantly diminished in those pigs maintained for 5–7 days on diets containing little inorganic phosphorous.

Fig. 1 illustrates representative saturation-dependent studies performed in the presence and absence of a sodium gradient in pigs maintained on high, normal and low phosphate diets. Table II summarizes the effect of adaptation to either a high and low phosphate diet on the apparent kinetic parameters of sodium-dependent transport. First, there was an increase in the maximal transport rate (V_{max}) in those pigs on a low phosphate diet, the V_{max} increased from 5.52 ± 0.94 to 9.44 ± 1.45 nmol · mg^{-1} protein · min^{-1}. Animals on a high phosphate diet showed a decrease in the apparent V_{max} value of the high-capacity system, 2.22 ± 0.63 nmol · mg^{-1} protein · min^{-1}. There was no significant alteration in the apparent K_m value, 4.42 ± 0.43 mM. Second, there were no apparent changes in the kinetic parameters of the high-affinity system with dietary phosphate, the mean V_{max} was 0.89 ± 0.09 nmol · mg^{-1} protein · min^{-1} and the K_m, 0.09 ± 0.02 mM. However, the high-affinity sodium-dependent system present in

TABLE I

Serum and urine electrolyte determinations prior to vesicle preparation

Values are means ± S.E. and (n) number of animals. * indicates significant difference ($P < 0.05$) from normal.

	Plasma		Urine	
	Ca	P_i	Ca/Cr	P_i/Cr
Dietary phosphate				
Normal (12)	2.41 ± 0.03	2.28 ± 0.13	0.026 ± 0.004	1.04 ± 0.09
High (6)	1.99 ± 0.22	2.81 ± 0.13 *	0.010 ± 0.004	2.62 ± 0.41 *
Low (8)	2.96 ± 0.16 *	2.00 ± 0.10	1.50 ± 0.38 *	0.20 ± 0.13 *
Parathyroid hormone (6)	2.39 ± 0.11	2.13 ± 0.13	0.46 ± 0.16	2.28 ± 0.44 *

TABLE II

Effect of dietary phosphate and parathyroid hormone on sodium-dependent phosphate transport

Values are means ± S.E., (n) is the number of preparations (animals) and * indicates significant difference ($P < 0.05$) from normal.

Outer cortex		K_m (mM)	V_{max} (nmol · mg^{-1} protein · min^{-1})
Dietary phosphate			
Normal (12)	low affinity	4.42 ± 0.43	5.52 ± 0.94
	high affinity	0.09 ± 0.02	0.89 ± 0.09
High (4)	low affinity	4.51 ± 1.46	2.22 ± 0.62 *
	high affinity	0.12 ± 0.01	0.93 ± 0.15
Low (4)	low affinity	4.65 ± 0.90	9.44 ± 1.45 *
	high affinity	0.10 ± 0.01	1.75 ± 0.48
Parathyroid hormone (5)	low affinity	3.73 ± 2.38	2.30 ± 0.48 *
	high affinity	0.14 ± 0.02	1.01 ± 0.14
Outer medulla		K_m (mM)	V_{max} (nmol · mg^{-1} protein · min^{-1})
Dietary phosphate			
Normal (3)		0.25 ± 0.02	3.25 ± 0.14
High (3)		0.27 ± 0.02	2.20 ± 0.10 *
Low (3)		0.25 ± 0.02	4.12 ± 0.27 *
Parathyroid hormone (3)		0.26 ± 0.02	1.145 ± 0.06 *

TABLE III

Effect of dietary phosphate and parathyroid hormone on pH gradient-stimulated phosphate transport in vesicles isolated from the porcine outer medulla

Values are means ± S.E., (n) is the number of membrane preparations (animals) and * indicates significantly differences ($P < 0.05$) from normal.

	K_m (mM)	V_{max} (nmol · mg^{-1} protein · min^{-1})
Dietary phosphate		
Normal (4)	0.15 ± 0.02	4.71 ± 0.34
High (4)	0.16 ± 0.01	3.02 ± 0.40 *
Low (3)	0.15 ± 0.01	6.93 ± 0.69 *
Parathyroid hormone (3)	0.16 ± 0.01	2.97 ± 0.27 *

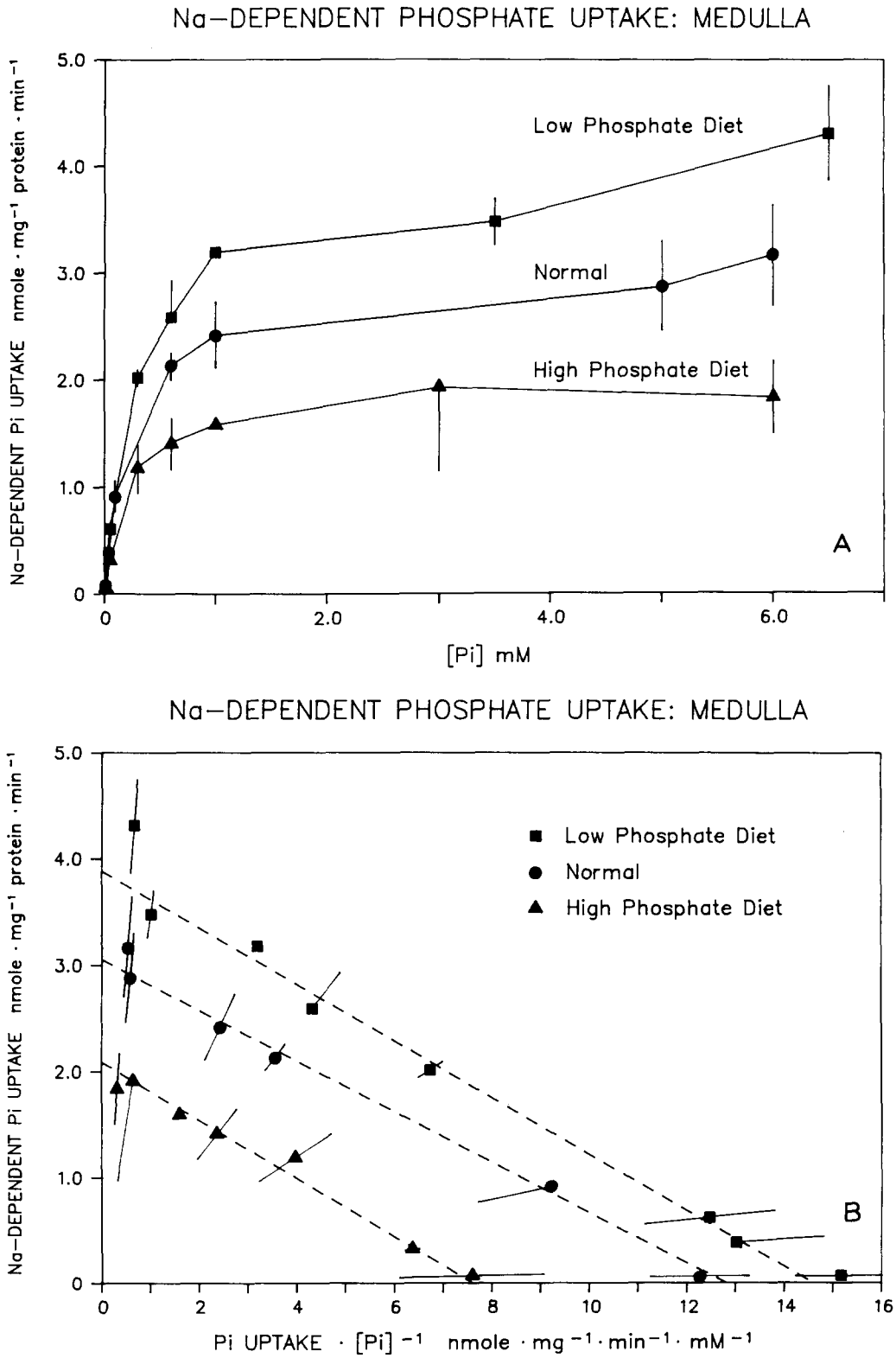

Fig. 2. Effect of dietary phosphate and parathyroid hormone on sodium-dependent phosphate transport in bursh-border membrane vesicles isolated from the outer medulla. Vesicles were prepared from outer medullary tissue and transport studied as given in legend to Fig. 1. Panel A illustrates the dependence of uptake on external phosphate concentration and panel B the kinetic transformation of these data. Values are means ± S.D. for five or six separate determinations for one representative study from each dietary group.

the outer medullary brush-border membrane vesicles (Fig. 2) was appropriately increased in animals on low dietary phosphate (from normal of 3.25 ± 0.14 to 4.12 ± 0.27 nmol · mg^{-1} protein · min^{-1}) and decreased in pigs on a high dietary phosphate (2.20 ± 0.10 nmol · mg^{-1} protein · min^{-1}). The apparent affinity (K_m, 0.25

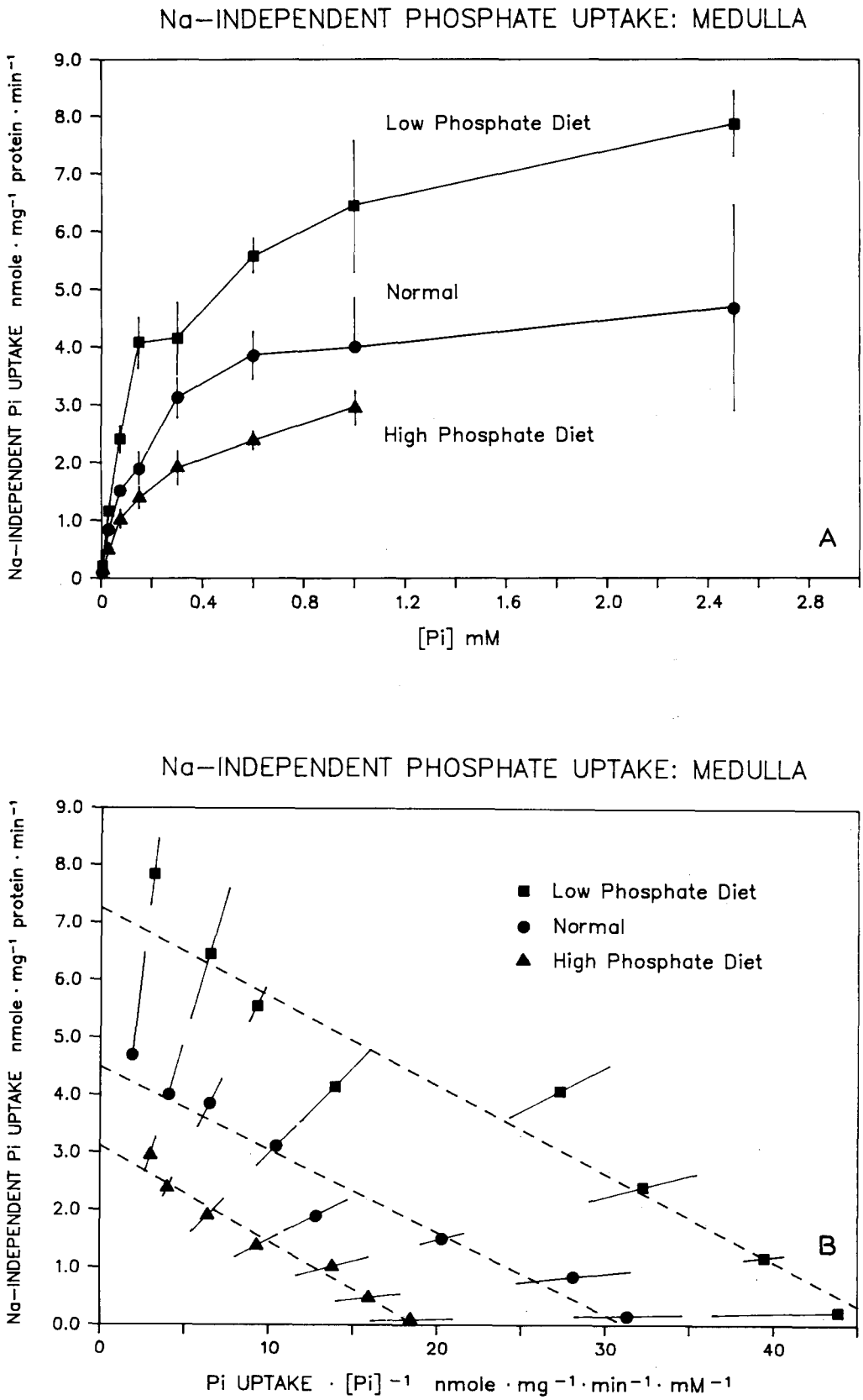

Fig. 3. Effect of dietary phosphate and parathyroid hormone on pH gradient-stimulated phosphate uptake in brush-border membrane vesicles from the outer medulla. Transport was performed with a transmembrane pH gradient, $pH_{out} = 6.5$ and $pH_{in} = 8.0$. pH gradient-stimulated uptake was determined as the difference of transport rates in the absence of a pH gradient ($pH_{in} = 8.0$, $pH_{out} = 8.0$) and in the presence of a pH gradient ($pH_{in} = 8.0$, $pH_{out} = 6.5$). Panel A illustrates the dependence of uptake on external phosphate concentration and panel B the kinetic transformation of this data. Values are means ± S.D. for five or six separate determinations performed on one representative study from each dietary group.

$\pm$ 0.02 mM) of this system was not affected by the antecedent dietary phosphate intake.

Fig. 3 illustrates the effect of dietary phosphate on pH gradient-stimulated phosphate uptake in the outer medulla and Table III summarizes the total findings. Saturations studies were performed in the absence of sodium but with a transmembrane pH gradient. pH gradient-stimulated phosphate uptake was enhanced in vesicles isolated from the animals maintained on low dietary phosphate, 6.93 ± 0.69 nmol $\cdot$ mg^{-1} protein $\cdot$ min^{-1}, and diminished, 3.02 ± 0.40, in those vesicles prepared from pigs with a high phosphate consumption.

Tables II and III summarize the effects of administration of pharmacological amounts of parathyroid hormone on phosphate transport in the outer cortex and outer medulla. As with adaptation of phosphate transport with dietary phosphate manipulation, the apparent K_m remained unchanged. However, parathyroid hormone decreased sodium-dependent phosphate transport in the outer cortex and outer medulla. The high-capacity system decreased from 5.52 ± 0.94 to 0.46 nmol $\cdot$ mg^{-1} protein $\cdot$ min^{-1} with no detectable change in the high-affinity system, 0.89 ± 0.09 before parathyroid hormone infusion and 1.01 ± 0.14 nmol $\cdot$ mg^{-1} protein $\cdot$ min^{-1} after administration. Parathyroid hormone decreased sodium-dependent phosphate transport in the outer medullary brush-border membrane vesicles from 3.25 ± 0.14 to 1.14 ± 0.06 nmol $\cdot$ mg^{-1} protein $\cdot$ min^{-1}.

Pharmacological amounts of parathyroid hormone inhibited pH gradient-stimulated phosphate uptake by about 50%; 4.71 ± 0.34 nmol $\cdot$ mg^{-1} protein $\cdot$ min^{-1} in control animals and 2.97 ± 0.27 in those receiving the hormone. The K_m value was not altered by parathyroid hormone (Table III).

Discussion

It is clearly established that the two principal factors controlling renal phosphate absorption are parathyroid hormone and the intrinsic adaptive response to the ambient phosphate [1,2]. These two influences, acting in concert, maintain phosphate homeostasis. The present studies were performed to determine the effect of each of these influences on sodium-dependent and sodium-independent phosphate transport across the brush-border membrane of early and late proximal tubules. These findings indicate that the two means of sodium-dependent phosphate transport in the outer cortex and outer medulla and the pH gradient-stimulated phosphate uptake in the outer medulla are influenced by both these regulatory mechanisms. Moreover, all transport systems are quantitatively altered by similar amounts, being up-regulated with low dietary phosphate and down-regulated with either high antecedent dietary phosphate or parathyroid hormone.

Studies using brush-border membrane vesicles have shown that parathyroid hormone and dietary phosphate alter phosphate transport across the luminal membrane [15–19]. These studies were performed on vesicles isolated from total kidney cortex. Moreover, Turner and Dousa showed that sodium-phosphate uptake was greater in superficial cortical brush-border membrane vesicles than juxtamedullary cortical vesicles of the dog kidney. Low dietary phosphate leads to a greater increase in transport in superficial than juxtamedullary vesicles [28]. Only one transport system was reported in each of these tissue regions. Brunette and colleagues investigated the effect of dietary phosphate and parathyroid hormone on phosphate uptake by vesicles prepared from superficial and deep cortical nephrons of the rat kidney [20,29]. They observed two kinetically distinctive sodium-dependent transporters in the superficial nephrons and one in the deep tissue. The K_m values were 1.6 and 0.07 mM in superficial cortical vesicles and 0.06 mM in deep, juxtamedullary vesicles. Interestingly, the apparent V_{max} value was greater for vesicles prepared from deep tissue (higher affinity) than superficial membranes. Dietary phosphate influenced phosphate uptake to a greater extent in the deep than in the superficial nephron [20]. Parathyroid hormone, on the other hand, influenced both populations of nephrons. Although there were quantitative differences between the two populations in the latter studies [20], both mechanisms of sodium-dependent phosphate transport were altered by dietary phosphate intake and by the alteration in circulating parathyroid hormone levels. Both of the above studies examined cortical tissue [20,28] whereas the present experiments were performed on membranes isolated either from the outer cortex or outer medulla according to the techniques described by Turner and Moran [30]. Nevertheless, these previous studies [20,28] and the present observations suggest that parathyroid hormone and dietary phosphate influence phosphate transport mainly by altering the rate, V_{max}, rather than the K_m value. In support of this notion, Barrett et al. reported changes in the V_{max} with no alteration in the apparent K_m value with alterations in dietary phosphate in pig brush-border membranes [19]. The latter studies [19] were performed on the total renal cortex and did not distinguish between the various transport systems. These studies (Ref. 19 and present observations) indicate that phosphate reabsorption is controlled by altering the transport rates of phosphate along the whole length of the proximal tubule. The magnitude of changes in porcine brush-border vesicles are qualitatively similar for all modes of phosphate transport indicating that all transplant systems are involved with control of phosphate homeostasis in the pig.

The concentration of phosphate in the glomerular filtrate is in the order of 2–3 mM and the tubule fluid

phosphate normally falls to 1.0–1.5 mM along the length of the proximal tubule. Accordingly, it is appropriate that the early proximal tubule possess a transporter with an apparent affinity of 4.0 mM. Thus, the high-capacity, low-affinity system plays the predominate role in phosphate reabsorption. The high-affinity systems (0.1 mM) would be saturated at these concentrations. It is only in those cases where luminal phosphate falls to low levels, 0.2–0.3 mM, where the high-affinity systems are relatively more important. Nevertheless, phosphate reabsorption is determined by the transport capacity, V_{max}, which may be functioning well below the tubule fluid phosphate concentration. A similar story has unfolded for other substrates such as glucose and amino acids [5,30–35]. For example, Turner and colleagues have reported the presence of two sodium-dependent D-glucose transporters in brush-border membranes of the rabbit kidney [30,35]. The outer cortical system possesses an apparent affinity of 6 mM and the outer medullary membranes have a K_m of 0.35 mM. As the plasma glucose concentration is 5–6 mM, the outer cortical system absorbs most of the filtered glucose with smaller amounts reabsorbed further down the tubule by the high-affinity system. It would appear that this is a common reabsorption pattern for many solutes [31,32,35].

In a previous report [7], we provided evidence for facilitative uptake of phosphate into vesicles prepared from porcine outer medullary tissue which was present in the absence of sodium. This transport was stimulated by a pH gradient ($pH_{in} > pH_{out}$), saturable, and inhibited by stilbenes such as DIDS. These observations support the notion of sodium-independent, pH gradient-stimulated phosphate uptake across the outer medullary brush-border membrane. This phenomenon was not observed in vesicles prepared from the outer cortex [7]. Although it is unknown at the present time if this represents a unique transporter in the late proximal tubule or distal tubular segments, it is apparent that facilitative uptake is influenced by similar factors which affect sodium-dependent phosphate uptake. Further studies are warranted to determine if this plays a unique role in renal phosphate homeostasis.

The present findings allow us to speculate on the role of sodium-dependent phosphate transport versus sodium-independent, pH gradient-stimulated phosphate transport. The following assumes equal importance between the high-capacity, low-affinity (apparent K_m 4.42 mM) system and the low-capacity, high-affinity (apparent K_m 0.09 mM) transporter. This is not strictly true, as mentioned above, the filtered phosphate concentration is in the order of 2.5 mM; accordingly, the high capacity system with a K_m of 4.42 mM will reclaim the predominant portion of filtered phosphate. Nevertheless, with this caveat, the total capacity of the sodium-dependent processes is in the order of 8.8 nmole $\cdot mg^{-1}$ protein $\cdot min^{-1}$ and the sodium-independent route, about 4.7 nmol $\cdot mg^{-1}$ protein $\cdot min^{-1}$. The proximal convoluted tubule is about 10 cm in length and the pars recta, 5 cm long. Accordingly, sodium-dependent phosphate systems would reclaim about 90–95% of the filtered phosphate and sodium-independent, pH gradient-stimulated transporter absorbs only about 5–10% of the filtered phosphate. This number could be much lower as we have ignored the greater brush-border surface area of the proximal convoluted tubule relative to the pars recta and have ignored the above caveat in our calculations, i.e. filtered phosphate concentration is normally much above the K_m of the high-affinity processes. As the transport rate for all processes change appropriately with either dietary phosphate or parathyroid hormone infusion, the contribution of pH gradient-stimulated phosphate transport component plays only a small role in overall renal phosphate handling.

In summary, the various modes of phosphate transport across the porcine brush-border membrane located along the length of the proximal tubule, early and late segments, is affected by parathyroid hormone and altered by the level of antecedent phosphate consumption. Moreover, the changes in each of the routes for phosphate transport are similar in magnitude in response to each of these regulatory influences. It remains to be determined whehter this is true of animal species other than the pig.

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