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PHOSPHATE TRANSPORT IN BRUSH-BORDER MEMBRANES FROM CONTROL AND RACHITIC PIG KIDNEY AND SMALL INTESTINE

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SUMMARY

1. Na–P_i co-transport was analysed using renal cortical and small intestinal brush-border membrane vesicles which were isolated from control (normal, heterozygotes) and rachitic piglets (homozygotes).

2. A kinetic analysis of Na-dependent initial linear uptake of P_i was performed using vesicles obtained from control animals. The results suggest similar kinetic properties for the renal and small intestinal co-transport system. (i) A sigmoidal dependence on Na concentration of P_i uptake suggests the involvement of more than one Na ion in the co-transport. (ii) Increasing Na concentration leads to an increase in the apparent affinity of the transport system for P_i and has minimal effect on the apparent V_{max} (maximum velocity of uptake). (iii) Increasing pH leads to an increase in P_i transport rate.

3. The kinetic characteristics of the Na–P_i co-transport system in vesicles obtained from rachitic animals were similar to those in controls. The apparent V_{max}, but not the apparent K_m (Michaelis constant) for Na and P_i, is reduced in intestinal and renal brush-border membranes isolated from rachitic animals as compared to control animals. Injection of vitamin D_3, three days prior to killing of rachitic litter-mates, increased the Na–P_i uptake rate in the brush-border membrane vesicles isolated from these piglets.

4. It is concluded that intestinal and renal brush-border membranes from piglets contain a similar Na–P_i co-transport system and that in vitamin-D-dependent rickets the number of operating transport units is reduced in both membranes.

INTRODUCTION

In 1969, Plonait described an inherited form of vitamin-D-dependent rickets in piglets which was transferred to offspring by an autosomal recessive gene (Meyer & Plonait, 1968; Plonait, 1969). The characteristics of the disease are similar to those

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first described by Prader, Illig & Heierli (1961) for primary vitamin-D-resistant rickets in humans. Shortly after birth, homozygote pigs develop traits of rickets such as hypocalcaemia, hypophosphataemia, and an increased blood level of alkaline phosphatase. In sick animals, plasma levels of parathyroid hormone and 25(OH)-vitamin-D_3 are increased while the concentration of 1,25$(OH)_2$-vitamin-D_3 is decreased. Daily administration of 1,25$(OH)_2$-vitamin-D_3 (1–4 μg/kg body weight) or of 10000 units of 25(OH)-vitamin-D_3 result in clinical healing. These results suggest a defective renal 1-hydroxylase as the primary defect of this animal model (Harmeyer & Plonait, 1967; Meyer & Plonait, 1968; Plonait, 1969; Harmeyer, Martens & Vogelsang, 1975; Wilke, Harmeyer, von Grabe, Hehrmann & Hesch, 1979; Winkler, Schreiner & Harmeyer, 1986).

Intestinal inorganic phosphate (P_i) transport is reduced in the presence of low levels of 1,25$(OH)_2$-vitamin-D_3 (Bickle, Morrissey, Zolock & Rasmussen, 1981; Murer & Hildmann, 1981; Murer & Burckhardt, 1983), and renal P_i transport is reduced when low 1,25$(OH)_2$-vitamin-D_3 levels are associated with high circulating parathyroid hormone levels (Murer & Burckhardt, 1983; Bonjour & Caverzasio, 1984; Gmaj & Murer, 1986).

It has been shown previously that altered renal and intestinal P_i transport *in vivo* is often paralleled by altered Na–P_i co-transport at the level of isolated brush-border vesicles (Bickle *et al.* 1981; Murer & Hildmann, 1981; Murer & Burckhardt, 1983; Bonjour & Caverzasio, 1984; Gmaj & Murer, 1986). The purpose of the present study was to evaluate the Na–P_i co-transport capability at the level of the intestinal and renal brush-border membrane in rachitic animals and in control (normal) litter-mates. Furthermore, a comparison was made between the 'basic' characteristics of the Na–P_i co-transport systems of intestinal and renal brush-border membranes isolated from the same animals and analysed under the same experimental conditions.

METHODS

Animals. Piglets with autosomal recessive vitamin-D-dependent rickets were used for the study (Harmeyer & Plonait, 1967; Meyer & Plonait, 1968; Plonait, 1969; Harmeyer *et al.* 1975; Wilke *et al.* 1979; Winkler *et al.* 1986). The homozygote animals developed severe symptoms of rickets within 3 months after birth, while heterozygote litter-mates developed normally. For the study with renal cortical brush-border membranes we have used a total of nine animals obtained from three different litters. To test for the effect of vitamin D_3 on Na–P_i co-transport, two female homozygote animals from an additional litter were killed; one of the animals was injected intramuscularly with 6·25 mg vitamin D_3 3 days prior to killing. For small intestinal brush-border membrane vesicles preparation six animals obtained from two litters were used.

Controls (normal) and rachitic animals from the same litter were killed on the same day at an age between 6½ and 9 weeks. At this age control animals reached a body weight of 9·0 ± 1·4 kg (S.D.) and rachitic animals a body weight of 5·7 ± 1·7 kg. On the day of killing, serum calcium was between 2·2 and 2·3 mM in homozygotes and between 2·65 and 2·93 mM in heterozygotes. Serum alkaline phosphatase was between 1206 and 2921 u/l in homozygotes and between 309 and 318 u/l in heterozygotes. The animals were fed *ad lib.* a diet containing (w/w) 18 % protein, 1 % L-lysine, 3·5 % crude fat, 4·5 % roughage, 6·5 % crude ash, 0·8 % calcium, 0·6 % phosphorus, 0·2 % sodium. Except for one litter (see above), the animals were not treated with vitamin D_3 or vitamin D_3 metabolites.

Membrane isolation. After killing by stunning followed by opening of the jugular vein, the kidneys were removed and 1 g pieces of superficial cortex were immediately frozen in liquid nitrogen. Small pieces of intestine were obtained 1·8 m distal from the pylorus and rinsed in 0·9 % NaCl, and 5 g specimens were immediately frozen in liquid nitrogen. The tissue was stored in liquid nitrogen for up to 12 months without significant loss in transport capacity (data not shown).

For membrane isolation, pieces of tissue were thawed at 4 °C in a buffer containing 300 mM-mannitol and 20 mM-HEPES which was adjusted with Tris to pH 7·4. After thawing, the tissue was minced with scissors. Renal membrane preparation was started with 2 g of frozen cortex and intestinal membrane preparations with 5 g of frozen intestine. For renal preparations, the divalent cation (Mg^{2+}) precipitation method described in detail by Biber, Stieger, Haase & Murer (1981) was followed precisely. For small intestinal membrane preparations, epithelial cell fragments were removed from the underlying muscle layers by vibration for 3 min (Chemap E, Chemap AG, Männedorf, Switzerland). The intestinal cell suspension was filtered through a Buchner funnel and vesicles were isolated from the filtrate with divalent cation (Mg^{2+}) precipitation as described in detail by Binder & Murer (1986).

Aminopeptidase M (EC 3.4.11.2) and alkaline phosphatase (EC 3.1.3.1) were assayed as brush-border enzyme markers and determined as described by Berner & Kinne (1976) and by Haase, Schaefer, Murer & Kinne (1978). The enrichment of aminopeptidase M was between 15- and 20-fold for renal and intestinal brush-border membranes. The enrichment factor for alkaline phosphatase, measured only in renal membranes, was 13-fold. There was no difference in the enrichment factors and in the absolute enzyme activity values between the two groups of animals, except for a slightly lower content of aminopeptidase M in the group of rachitic animals (data not shown).

Protein was determined according to the method of Bradford (1976) using γ-globulin as standard.

Transport studies. In order to pre-load the vesicles with the desired buffer (see legends), the final membrane pellet was homogenized in 30 ml of buffer with a glass–Teflon potter homogenizer (10 strokes at about 3000 rev/min). Membranes were collected by centrifugation and resuspended in the desired buffer by passing twenty times through a fine needle (0·5 × 23 mm) fitted to a 1 ml syringe. After resuspension, the vesicles were kept at room temperature for at least 1 h. For uptake studies a semi-automatic set-up was used as described by Kessler, Tannenbaum & Tannenbaum (1978) and purchased from Innovativ AG, Adliswil (Switzerland). The composition of the incubation media is given in the legends of the Tables and Figures. All uptake studies were performed at room temperature. After 'stopping' the incubation by the injection of ice-cold stop solution (see legends), the membrane suspension was immediately filtered through a 0·65 μm pore size cellulose nitrate filter (Fa. Sartorius, Göttingen). The radioactivity retained on the filter was determined by liquid scintillation counting.

Materials. Radioisotopes were purchased from New England Nuclear Co. (Boston, MA, U.S.A.). All chemicals purchased were of analytical grade purity.

Presentation of data. Uptake was always measured under conditions of initial linear flux (Fig. 1), and was expressed, except for Fig. 1, as pmol/(mg protein . s), i.e. uptake within the linear phase of uptake has been divided by the incubation time. In some cases, longer time points were used in order to increase experimental precision under conditions with slow uptake rates. From each uptake value, a blank was subtracted which was obtained by adding stop solution without previous mixing of vesicle suspension and incubation medium. Each value was determined in quadruplicate; in general, the quadruplicates were within 10%. Each type of experiment (Figs. 1–3) was performed at least three times with qualitatively identical results. The experiments describing the basic transport properties (Figs. 1–3) have been performed with membrane vesicles isolated from heterozygote (control) animals.

RESULTS

Basic properties of P_i transport in renal and small intestinal brush-border membranes

Fig. 1 shows the time course of Na (●) or potassium (○) dependent P_i uptake. As shown, an inwardly directed gradient of NaCl accelerates P_i influx, as compared to an inwardly directed KCl gradient. In the presence of a KCl gradient, virtually no uptake of P_i can be observed within the first 10–20 s of incubation. Linearity of uptake in the presence of a NaCl gradient is observed for at least 16 s (intestinal membranes) or 5 s (renal membranes). Although the entire time course of uptake was not monitored, an 'overshooting' uptake of P_i energized by the Na gradient is observed, i.e. the values obtained after 10 s of incubation are higher than the equilibrium value. In order to remain within the linear phase of uptake, for the kinetic

characterization of the P_i transport system (see below), incubation periods longer than 4 s were never used. After prolonged incubation of 3 h, an equilibrium is apparently reached, as levels of P_i in the presence of Na are equal to those in the presence of K. The observation of Fig. 1 is best explained by Na–P_i co-transport.

In further studies we have analysed a few of the kinetic properties of the Na–P_i co-transport in order to (a) compare the renal and the intestinal transport systems and (b) to compare P_i transport in contol (heterozygote) and in rachitic (homozygote) animals.

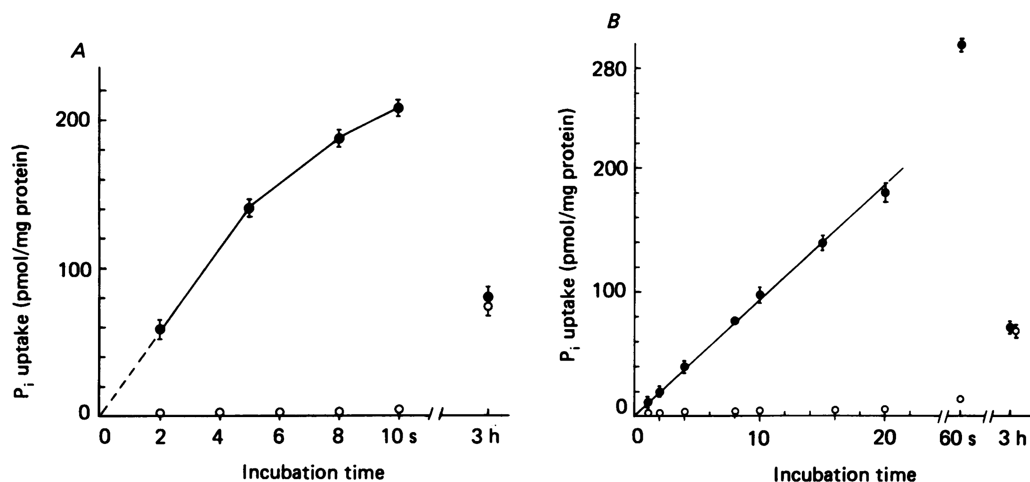

Fig. 1. Initial linear uptake of P_i by renal (*A*) and intestinal (*B*) brush-border vesicles from normal animals. Brush-border membrane vesicles were suspended in a medium containing 400 mM-mannitol and 20 mM-HEPES–Tris. The incubation medium contained 200 mM-NaCl (●) or 200 mM-KCl (○), 0·2 mM-$KH_2{}^{32}PO_4$ and 20 mM-HEPES adjusted with Tris to pH 7·4. The uptake was initiated by mixing 10 μl of membranes with 10 μl of incubation medium and stopped by quenching with ice-cold stop solution containing 150 mM-mannitol, 150 mM-KCl, 5 mM-Tris–HCl at pH 7·4. This is a typical experiment performed in quadruplicate on membranes isolated from heterozygote animals. Values are means ± S.D..

Fig. 2 shows the effect of different Na concentrations on initial linear influx of P_i. For both renal and intestinal brush-border membranes, a sigmoidal relationship between P_i uptake and Na concentration was observed. This indicates the involvement of more than one Na ion in the activation of the transport processes. The Hill coefficients calculated from four separate experiments were 1·86 ± 0·21 for renal membranes, and 2·12 ± 0·13 for intestinal membranes (data not shown). When the data of Fig. 2 were analysed using a Woolf–Augistinsson–Hofstee transformation of the Lineweaver–Burk equation (Segel, 1976), the best fit in linear regression analysis was obtained with the Na concentration expressed as $[Na]^2$. In this manner, we obtained apparent Michaelis constant (K_m) values for Na interaction as given in Table 1.

The change in P_i uptake as a function of P_i concentration, was analysed under two conditions (Table 2) either at saturating (300 mM) or at intermediate (60 mM) Na concentrations. At saturating Na concentration (300 mM), the apparent K_m value for

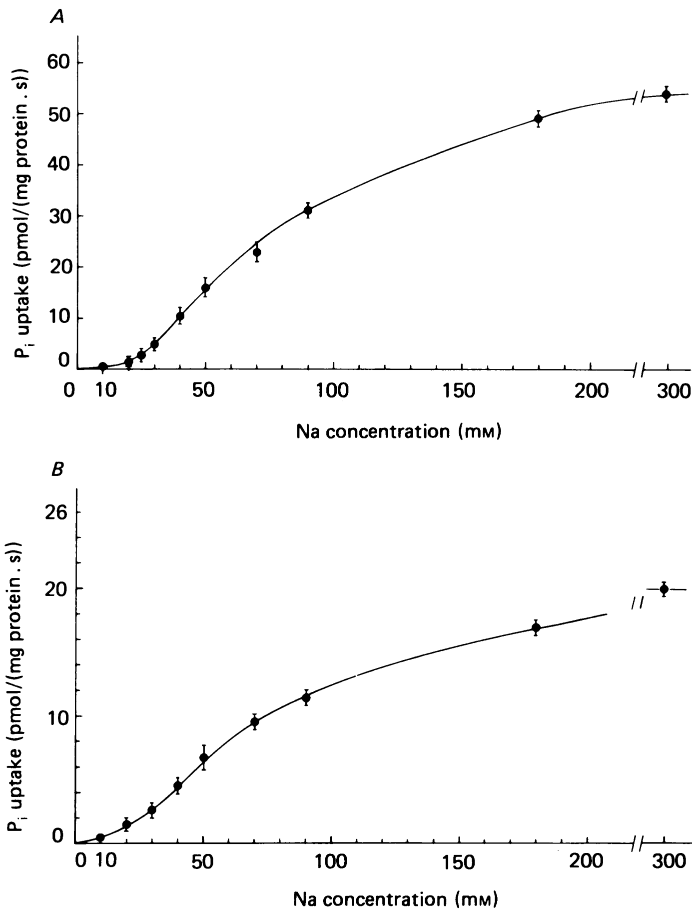

Fig. 2. Influence of different Na concentrations on initial linear uptake of P_i by renal (*A*) and intestinal (*B*) brush-border vesicles. Membranes were suspended in 600 mM-choline chloride, 20 mM-HEPES adjusted with Tris to pH 7·4 and 50 mM-KCl. The incubation medium contained 49·8 mM-KCl, 20 mM-HEPES–Tris at pH 7·4 (intestine) or pH 7·0 (kidney), 0·2 mM-$KH_2{}^{32}PO_4$ and NaCl–choline chloride at different concentrations ratios yielding a total concentration of 600 mM. Membranes and incubation medium contained valinomycin at a concentration of 10 μg/l ml. The uptake was started by mixing 10 μl of membranes with 10 μl of incubation medium and stopped by quenching with an ice-cold solution containing 500 mM-choline chloride, 200 mM-NaCl, 5 mM-KH_2PO_4 and 5 mM-Tris–HCl at pH 7·4. For Na concentration of 25 mM and lower the incubation time was 4 s; for Na concentration higher than 25 mM the incubation period was 2 s. This is a typical experiment performed in quadruplicate on membranes isolated from heterozygote animals. Values are means ± S.D. The values have been corrected for uptake of P_i in the complete absence of Na.

P_i interaction was approximately 0·2 mM in both membrane preparations. Lowering the Na concentration led to a decrease in affinity for P_i in the two membranes. The apparent maximum velocity of uptake (V_{max}) value was several time higher in renal membranes as compared to intestinal membranes. Alteration of Na concentration had no influence on the apparent V_{max}.

Finally, we have analysed the pH dependence of the initial linear uptake of P_i (Fig. 3). In agreement with earlier observations on rat and rabbit renal brush-border membrane vesicles, we found in the pig renal membranes an increase in transport rate with an increase in pH (Cheng & Sacktor, 1981; Sacktor & Cheng, 1981; Amstutz, Mohrmann, Gmaj & Murer, 1985). In contrast with earlier observations on rats and rabbits, in pig intestinal brush-border vesicles an increase in transport with increase

TABLE 1. Kinetic analysis of P_i flux in relation to medium Na concentration in Na–P_i co-transport by renal and intestinal brush-border membranes

	Renal membranes		Intestinal membranes	
	Apparent K_m (mM)	Apparent V_{max} (pmol/(mg . s))	Apparent K_m (mM)	Apparent V_{max} (pmol/(mg . s))
Control	112·6 (G_2)	74·4 (G_2)	83·3 (G_4)	21·0 (G_4)
	128·1 (G_3)	71·6 (G_3)	89·4 (G_3	19·2 (G_3)
Rickets	115·6 (K_3)	30·1 (K_3)	103·4 (K_5)	14·6 (K_5)
	128·5 (K_4)	26·9 (K_4)	96·0 (K_4)	13·2 (K_4)

The apparent K_m and V_{max} values were calculated by linear regression analysis of initial linear uptake (see Figs. 1 and 2) and on the basis of the Woolf–Augustinsson–Hofstee plot (v (P_i uptake extrapolated to 1 s of incubation) *versus* $v/[Na]^2$; r^2 (coefficient of determination in multiple linear regression analysis) was always higher than 0·9). The experiment with renal membranes was performed at a pH of 7·0; the experiment with intestinal membranes at a pH of 7·4. P_i concentration was 0·1 mM. Each value was obtained from an independent experiment performed in quadruplicate. The code in parentheses refers to the animal used for the particular experiment. The litter-mates were three pairs of G_2 and K_3, G_3 and K_4, G_4 and K_5.

in pH was observed (Berner, Kinne & Murer, 1976; Danisi, Murer & Straub, 1984). In earlier studies on rabbit intestinal brush-border membrane vesicles, P_i transport (Danisi *et al.* 1984) was observed to have a different pH sensitivity at different Na concentrations. Therefore, we measured the pH dependence of pig intestinal P_i transport at a non-saturating Na concentration. Again, we observed an increase in transport with increase in pH (Fig. 3*B*).

Difference in P_i transport between control (normal) and rachitic animals

In initial experiments we have analysed the basic properties of Na–P_i co-transport, similar to the experiments given in Figs. 1–3, and found no difference in the qualitative properties between homozygote (rachitic) and heterozygote (control) animals (data not shown). In further experiments, the kinetics of Na interaction (Table 1) and the kinetics of P_i interaction (Table 2) were analysed, and compared to values from control animals in at least two separate pairs of homozygote and heterozygote animals.

For renal membranes we did not observe a difference in the apparent K_m for Na. The V_{max} was less than half for the rachitic animals, compared to the control group (Table 1). Similarly, in intestinal membranes the value for the apparent V_{max} was markedly reduced in rachitic animals without reduction in the value for the apparent K_m for Na. In P_i saturation experiments (Table 2), a reduction in the apparent V_{max} was found in renal and intestinal membranes obtained from rachitic animals, compared to the control group. Additional experiments performed on intestinal

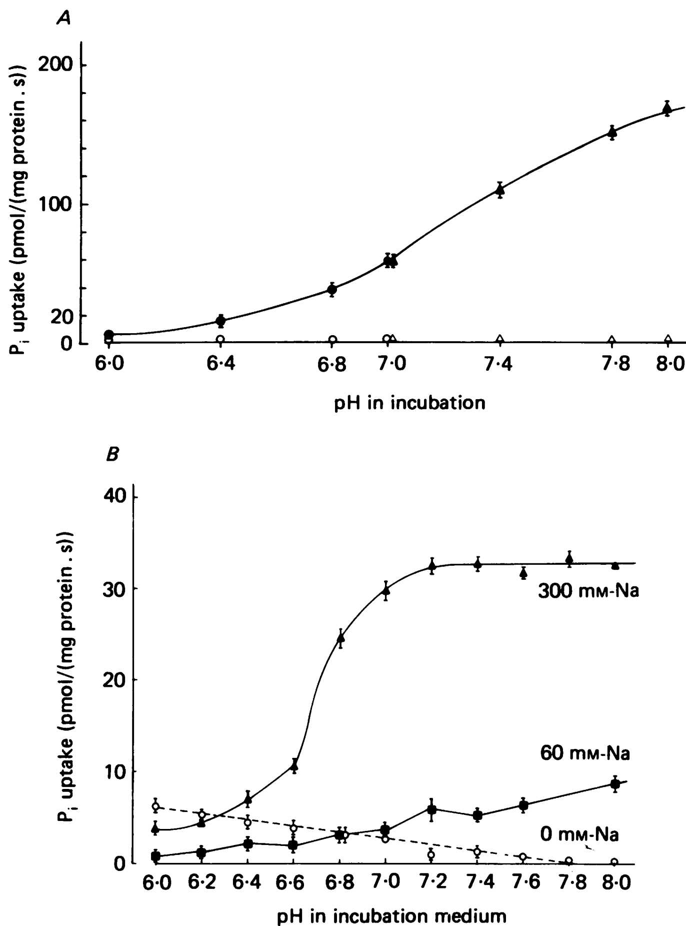

Fig. 3. The influence of medium pH on initial linear uptake of P_i by renal (*A*) and intestinal (*B*) brush-border vesicles. *A*, renal brush-border membranes have been pre-loaded with 400 mM-mannitol, 1 mM-HEPES adjusted with Tris to pH 7·4. The incubation medium contained 200 mM-NaCl (●, ▲) or 200 mM-KCl (○, △), 20 mM-2-[*N*-morpholino]ethanesulphonic acid (MES) adjusted with Tris to either pH 6·0, 6·4, 6·8 or 7·0 (●, ○) or 20 mM-HEPES adjusted with Tris to pH 7·0, 7·4, 7·8 or 8·0 (▲, △) and 0·2 mM-$KH_2{}^{32}PO_4$. The incubation was initiated by mixing 10 μl of membrane suspension with 10 μl of incubation media. The incubation was terminated by quenching with an ice-cold solution containing 200 mM-mannitol, 100 mM-NaCl, 5 mM-$KH_2{}^{32}PO_4$ and 5 mM-Tris–HCl at pH 7·4. *B*, intestinal brush-border membranes were pre-loaded with 600 mM-choline chloride, 50 mM-KCl, 2 mM-HEPES adjusted with Tris to a pH of 7·5. The incubation media contained 30 mM-MES–Tris for the pH values between 6·8 and 6·0 or 30 mM-HEPES–Tris for the pH values between 6·8 and 8·0, 50 mM-KCl, 0·2 mM-$KH_2{}^{32}PO_4$ and either 600 mM (▲), 120 mM (■) or no NaCl (○) with an iso-osmotic replacement of NaCl by choline chloride. The uptake was initiated by mixing 10 μl of membrane suspension with 10 μl of incubation media and was quenched by injection of an ice-cold solution containing 500 mM-choline chloride, 200 mM-NaCl, 5 mM-$KH_2{}^{32}PO_4$ and 5 mM-Tris–HCl at pH 7·4. Net Na-dependent uptake is shown (▲, ■). Membrane suspension and incubation media contained valinomycin at a concentration of 10 μl/ml. Typical experiments performed in quadruplicates on membranes isolated from heterozygote animals are given. Values represent means ± S.D..

membranes show that in rachitic animals the apparent P_i affinity of the P_i transport system also decreases with declining Na concentrations. For the renal and intestinal brush-border membranes, these data suggest a reduction in V_{max}, i.e. in number or activity of operating transport systems without a change in the affinity of the transport system for Na and P_i.

TABLE 2. Kinetic analysis of P_i flux in relation to medium P_i concentration in Na–P_i co-transport by renal and intestinal brush-border membranes

	Na Concentration (mM)	Renal membranes		Intestinal membranes	
		Apparent K_m (μM)	Apparent V_{max} (pmol/(mg.s))	Apparent K_m (μM)	Apparent V_{max} (pmol/(mg.s))
Control	300	236 (G_1)	211 (G_1)	195 (G_5)	28·6 (G_5)
	300	241 (G_3)	216 (G_3)	213 (G_3)	26·6 (G_3)
	60	830 (G_1)	188 (G_1)	526 (G_5)	23·3 (G_5)
	60	1010 (G_3)	222 (G_3)	583 (G_3)	26·4 (G_3)
Rickets	300	242 (K_2)	117 (K_2)	195 (K_6)	14·1 (K_6)
	300	262 (K_4)	121 (K_4)	184 (K_4)	13·6 (K_4)
	60	n.d.	n.d.	489 (K_6)	11·1 (K_6)
	60	n.d.	n.d.	553 (K_4)	14·1 (K_4)

The apparent K_m and V_{max} values were calculated by linear regression analysis of initial linear uptake values and on the basis of the Woolf–Augustinsson–Hofstee plot (v versus $v/[P_i]$; r^2 was always higher than 0·9), n.d. = not determined. The P_i concentrations were 0·05, 0·1, 0·2, 0·4, 0·8, 1·5 and 3·0 mM and incubation time was 2 s for 300 mM-Na and 4 s for 60 mM-Na. Membranes were suspended in 400 mM-mannitol, 400 mM-choline chloride, 50 mM-KCl and 20 mM-HEPES adjusted with Tris to a pH of 7·4. The incubation medium contained KCl and $KH_2{}^{32}PO_4$ at various concentrations, 20 mM-HEPES–Tris at pH 7·4 and either 600 mM-NaCl, 120 mM-NaCl plus 480 mM-choline chloride or 600 mM-choline chloride. KCl and $KH_2{}^{32}PO_4$ had together a total concentration of 50 mM. The incubation was started by mixing 10 μl of membrane suspension with 10 μl of incubation media. The uptake was stopped by quenching with an ice-cold stop solution containing 300 mM-choline chloride, 200 mM-NaCl, 400 mM-mannitol, 5 mM-KH_2PO_4 and 5 mM-Tris HCl at pH 7·4. Incubation media and membranes contained valinomycin at a concentration of 10 μl/ml. Each value was obtained from an independent experiment performed in quadruplicate. The code in parentheses refers to the animal used for that particular experiment. The three pairs of litter-mates were G_1 and K_1, G_3 and K_4, G_5 and K_6.

Na-dependent D-glucose uptake

We have also analysed Na-dependent D-glucose influx at a substrate concentration of 0·1 mM, under conditions identical to those given in Fig. 1 for P_i uptake. The Na-dependent glucose uptake by renal membranes from heterozygote (controls) was 9 ± 2 pmol/(mg.s) ($n = 4$) while for rachitic animals a value of $11{\cdot}5 \pm 2$ pmol/(mg.s) ($n = 4$) was obtained. Na-dependent D-glucose uptake by intestinal membranes was 11 ± 1 pmol/(mg.s) ($n = 4$) for heterozygote, and 10 ± 1 pmol/(mg.s) ($n = 4$) for the homozygote animals. These limited experiments suggest that our observation of reduced P_i transport rates in rachitic animals is not related to indirect effects, e.g. vesicle fragility.

Influence of treatment with vitamin D_3 on P_i transport into pig kidney brush-border membranes

In one litter, the effect of treatment with vitamin D_3 on initial uptake of P_i into pig renal brush-border vesicles was also tested (Fig. 4). Two homozygote piglets from

the same litter were killed at an age of 9 weeks. One piglet received 6·25 mg vitamin D_3 3 days before killing. As illustrated in Fig. 4, renal P_i transport was markedly augmented after treatment with a single injection of vitamin D_3. This agrees with the concept of a direct or indirect (see Discussion) vitamin D dependency of reduced P_i transport in this genetic 'defect'.

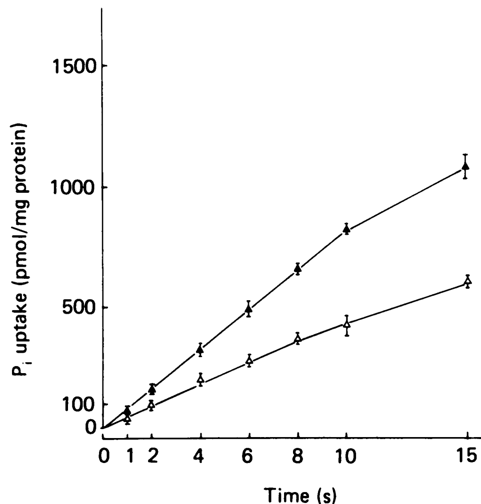

Fig. 4. Effect of treatment of rachitic pigs with vitamin D_3 on initial P_i uptake by isolated renal brush-border vesicles. The experimental conditions are identical to those given in the legend to Fig. 1. △ represent the values from an untreated, rachitic vitamin-D-deficient animal; ▲ represent the values from the litter-mate treated 3 days before killing with 6·25 mg (intramuscular) vitamin D_3.

DISCUSSION

Analysis of the basic characteristics of the Na–P_i co-transport in pig renal and intestinal brush-border membranes reveals almost identical properties for the two membranes, with the exception of higher transport rates in renal as compared to intestinal membranes. A study on Na activation (Fig. 2 and Table 1) indicates an interaction of two Na ions with the P_i co-transport system. The slight difference in the K_m for Na between renal and intestinal membranes should not be given too much emphasis. When more experiments are performed, a difference in affinity would almost certainly be small and of negligible physiological significance. Furthermore, the lower affinity of the renal transport system can also be partially due to the lower pH in the incubation medium (Amstutz *et al.* 1985). Interaction of the transport system with Na leads to an increase in the apparent affinity for P_i (Table 2), which is an agreement with Na being the first ligand in sequential interaction of the substrates with the transport system.

Co-transport of Na and P_i has been described for small intestinal and renal brush-border vesicle preparations obtained from various mammals including rats, rabbits, pigs, mice, dogs and humans (for review see: Murer & Hildmann, 1981; Bickle *et al.* 1981; Murer & Buckhardt, 1983; Bonjour & Caverzasio, 1984; Gmaj & Murer, 1986). Only a limited comparison of basic properties of Na–P_i co-transport between

species is possible, since detailed data of the kinetic characteristics is missing in most studies. The characteristics of pig renal Na–P_i co-transport compares favourably with the properties of Na–P_i co-transport in rat and/or rabbit preparations. Qualitatively the same properties were obtained with respect to pH dependence, Na interaction and P_i interaction (Cheng & Sacktor, 1981; Sacktor & Cheng, 1981; Amstutz *et al.* 1985). The data for the pig intestinal Na–P_i co-transport system are at variance with those obtained previously in studies on rat and rabbit brush-border vesicles (Berner *et al.* 1976; Danisi *et al.* 1984). In both rabbit and rat intestinal brush-border membrane preparations, Na–P_i co-transport rate increased by lowering pH, whereas in pig membrane preparations it was increased by increasing pH. In rabbit brush-border vesicles, no effect of increasing Na concentrations on the interaction of the transport system with P_i was observed (Danisi *et al.* 1984), whereas in pig membrane preparations an increased Na concentration resulted in an increased affinity for P_i. The previous study on rat and rabbit brush-border vesicles led to the suggestion that the renal transport mechanism is different from the intestinal. The present study reveals similar characteristics of the renal and intestinal transport system in pigs.

Evidence for a regulatory control of transepithelial P_i transport has been demonstrated at the membrane level for the intestinal and renal Na–P_i co-transport for different physiological situations (for review see: Murer & Hildmann, 1981; Bickle *et al.* 1981; Murer & Burckhardt, 1983; Bonjour & Caverzasio, 1984; Gmaj & Murer, 1986). The present study extends these earlier observations to an animal model with a form of autosomal recessive vitamin-D-dependent rickets. For renal and intestinal membrane preparations we found a reduction in the apparent V_{max} of the Na–P_i co-transport system without a change in the other characteristics of the transport system. With respect to a control of intestinal Na–P_i co-transport by $1,25(OH)_2$-vitamin-D_3, our data are in full agreement with previous studies on various species, where an increased P_i transport was consistently found with increased levels of $1,25(OH)_2$-vitamin-D_3 (for review see: Bickle *et al.* 1981; Murer & Hildmann, 1981; Murer & Burckhardt, 1983). At present, it is under debate whether the renal Na–P_i co-transport is also under direct control of $1,25(OH)_2$-vitamin-D_3 (Bonjour & Caverzasio, 1984). From our data we cannot conclude whether the renal decrease in Na–P_i co-transport in the rachitic group is related to an increased parathyroid hormone level, a decreased $1,25(OH)_2$-vitamin-D_3 level, or even other 'humoral' factors including serum P_i levels and growth-related differences (Bonjour & Caverzasio, 1984). It should be noted that the homozygote (rachitic) animals have a decreased serum P_i level, increased parathyroid hormone level and are also retarded in growth. Further experiments with this animal model involving parathyroidectomy may be useful in evaluating the direct and/or indirect effects of $1,25(OH)_2$-vitamin-D_3 on renal P_i transport.

In summary, the present study describes the basic characteristics of Na–P_i co-transport in pig renal and intestinal brush-border vesicles. It was found that in pigs, the renal and intestinal Na–P_i co-transport systems show very similar properties. In addition, it was shown that in vitamin-D-dependent rickets, both the renal and intestinal transport mechanisms are reduced in activity without a change in other qualitative and kinetic characteristics.

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