Biochem. J. (1979) **180**, 465–470
Printed in Great Britain

Effect of Dietary Phosphate Intake on Phosphate Transport by Isolated Rat Renal Brush-Border Vesicles

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(*Received* 16 *October* 1978)

Renal brush-border membrane vesicles isolated from rats kept for 6–8 weeks on a low-phosphate diet (0.15% of dry matter) showed a markedly faster Na^+-dependent phosphate uptake than did membrane vesicles isolated from animals kept on a high-phosphate diet (2% of dry matter). Phosphate-uptake rate by brush-border membrane vesicles isolated from animals on a low-phosphate diet remained significantly increased after acute parathyroidectomy. Dietary adaptation was also observed in animals that had been parathyroidectomized before exposure to the different diets. In animals on the low-phosphate diet parathyrin administration inhibited phosphate uptake by brush-border vesicles only if the animals were repleted with P_i (5ml of 20mM-NaH_2PO_4) 1h before being killed. After acute phosphate loading and parathyrin administration the difference in the transport rate between the two dietary groups remained statistically significant. The results suggest that the adaptation of proximal-tubule phosphate transport to dietary intake of phosphate is reflected in the Na^+/phosphate co-transport system located in the luminal membrane of the proximal-tubule cell. Since the dietary effects on phosphate transport by brush-border membranes are only partially reversed by acute changes in parathyrin concentration and are also observed in chronically parathyroidectomized animals, the adaptation of the Na^+/phosphate co-transport system to dietary phosphate intake seems to involve an additional mechanism independent of parathyrin.

Studies using micropuncture techniques *in vivo* and isolated membrane vesicles *in vitro* have shown that renal proximal-tubule phosphate transport is Na^+-dependent, involving a co-transport of Na^+ and P_i across the brush-border membrane (Baumann *et al.*, 1975; Hoffmann *et al.*, 1976). Furthermore micropuncture and clearance studies have revealed that proximal-tubular phosphate transport is subject to regulation by various factors. Thus it is known that parathyrin inhibits proximal-tubule phosphate reabsorption, whereas adaptation of animals to a low-phosphate diet stimulates phosphate reabsorption (Agus *et al.*, 1971, 1973; Ullrich *et al.*, 1977; Tröhler *et al.*, 1976; Steele & De Luca, 1976).

In studies with isolated renal brush-border membrane vesicles we recently demonstrated that the inhibition of proximal-tubule phosphate reabsorption by parathyrin administration is accompanied by a decreased rate of Na^+-dependent uptake of P_i into the brush-border vesicles (Evers *et al.*, 1978*a*).

The results of the experiments presented here show that Na^+-dependent phosphate uptake by renal brush-border membrane vesicles isolated from rats adapted to a low-phosphate diet is higher than the phosphate uptake by brush-border vesicles isolated from animals kept on a high-phosphate diet, indicating that alterations in the rate of phosphate translocation across the brush-border membrane might be responsible for the observed alterations in transepithelial phosphate transport. Furthermore, the studies presented here suggest that the Na^+/phosphate co-transport by the renal brush-border membrane is not only regulated by parathyrin but, in addition, independently from the parathyrin system, by the dietary intake of phosphate. Preliminary reports were given at the Spring Meeting of the German Physiological Society, Göttingen, 1978 (Stoll *et al.*, 1978*a*) and at the Swiss Biochemical Society Meeting, Davos, 1978 (Stoll *et al.*, 1978*b*).

Methods and Materials

Preparation of animals

Male Wistar rats were used throughout this study. The animals were kept on a high- (2% of dry matter) or a low- (0.15% of dry matter) phosphate diet for 6–8 weeks starting at an age of 8 weeks. Altromin diet (C 1034; Altrogge, Lage, Lippe, W. Germany) was used as a basis and was enriched with different amounts of sodium phosphate. Tap water was offered *ad libitum*. Parathyroidectomy was carried out by electrocoagulation. Controls were sham-operated. For acute parathyroidectomy the operation was

carried out 5 h before the animals were killed, whereas for chronic parathyroidectomy the operation was carried out 1 week before offering the different diets to the animals. To monitor the success of the operation, the calcium concentration in the plasma was measured. A decrease in serum calcium to 80% compared with the control before operation was considered as evidence for a successful parathyroidectomy. For the studies on the effect of parathryin, the hormone (Hormonchemie, München, Germany; 50 U.S.P. units/rat) was dissolved in 5 ml of 20 mM-NaCl or 5 ml of 20 mM-NaH_2PO_4 and injected intraperitoneally 1 h before the animals were killed. In the experiments of Evers *et al.* (1978*a*) we demonstrated that parathyrin at a dose of 30 U.S.P. units/rat elicited a maximal inhibitory effect on Na^+-dependent phosphate uptake. Therefore the use of 50 U.S.P. units of hormone/rat in the present experiments can be assumed to have produced maximal parathyrin-mediated inhibition.

Brush-border membrane isolation and transport studies

Brush-border membrane vesicles were isolated from four animals by the calcium-precipitation method as described in detail in Evers *et al.* (1978*b*).

Uptake of labelled substrates by the isolated membrane vesicles was measured in triplicate by a Millipore filtration technique as described previously (Hoffmann *et al.*, 1976); a quantity of vesicles corresponding to approx. 15 μg of protein was used for each assay. Na^+-dependent solute uptake was studied in 100 μl of medium containing 100 mM-mannitol, 20 mM-Hepes [4-2(-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/Tris (pH 7.4), 100 mM-NaSCN and 0.1 mM-$KH_2{}^{32}PO_4$ (3 μCi) or 1 mM-D-[^{3}H]glucose (5 μCi). Tracer permeability for mannitol was studied by adding D-[^{3}H]mannitol (5 μCi) to the ^{32}P-containing medium.

Biochemical characterization

Protein was determined, after precipitation of the membranes with ice-cold 10% (w/v) trichloroacetic acid, by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard. Alkaline phosphatase was determined as decribed by Berner & Kinne (1976) by using an LKB reaction-rate analyser 8600.

Materials

All chemical reagents used were of the highest chemical purity available. Radioactive isotopes were purchased from New England Nuclear (Boston, MA, U.S.A.).

Results and Discussion

Transport properties of brush-border membranes isolated from rats fed on a low- or high-phosphate diet

Fig. 1 shows a typical time course of phosphate uptake, D-glucose uptake and mannitol uptake in the presence of a 100 mM-NaSCN gradient directed to the vesicle inside, by renal brush-border vesicles isolated from animals fed on low- or high-phosphate diets. In both vesicle preparations, for phosphate and D-glucose a rapid initial uptake is observed, which leads to a transient intravesicular accumulation of solute. Membrane vesicles isolated from animals on the low-phosphate diet, however, show a higher initial rate of phosphate uptake than do membrane vesicles isolated from animals on a high-phosphate diet. On the contrary, the uptakes of D-glucose and D-mannitol (the latter being used as an indicator for the diffusional permeability of the membrane), as well as the equilibrium values for phosphate and D-glucose, were identical under all the different experimental conditions (Table 1). The latter findings indicate that the different rate of phosphate transport observed after the two different dietary regimens is caused by an alteration of the Na^+/phosphate co-

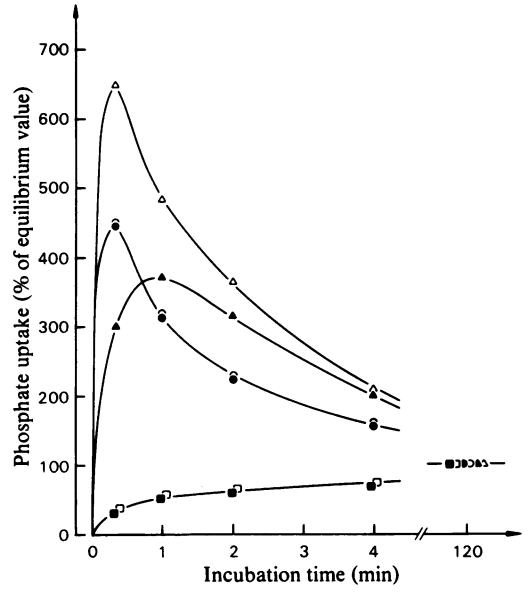

Fig. 1. *Uptake of P_i, D-glucose and tracer D-mannitol by renal brush-border vesicles isolated from rats on low- and high-phosphate diets*

▲, △, Phosphate uptake; ●, ○, glucose uptake; ■, □, tracer mannitol uptake; the open symbols represent solute uptake by the low-phosphate-diet group, the closed symbols that by the high-phosphate-diet group.

Table 1. *Uptake of P_i by renal brush-border membrane vesicles isolated from rats with different parathyrin or dietary status*

Abbreviations used: PTH, parathyrin; PTX, parathyroidectomy; sham, sham-operated; low-P, low-phosphate diet (0.15% of dry matter); high-P, high-phosphate diet (2.0% of dry matter). For details of experimental conditions, see the Methods and Materials section. Results are expressed as means ± S.E.M. % of equilibrium = (uptake after 20s/uptake after 120min) × 100.

Status of the animals	No. of experiments	No. of animals/ experiment	Phosphate uptake after 20s (% of equilibrium)	Phosphate uptake after 120min (pmol/mg of protein)	D-Glucose uptake after 20s (% of equilibrium)	D-Glucose uptake after 120min (pmol/mg of protein)
Low-P, sham	10	4	623 ± 37	166.8 ± 6.0	508 ± 32	171.5 ± 5.7
High-P, sham	10	4	282 ± 22	167.4 ± 6.9	502 ± 18	172.1 ± 5.9
Low-P, acute PTX	4	4	545 ± 41	170.1 ± 4.5	496 ± 14	165.4 ± 6.3
Low-P, NaCl	5	4	599 ± 29	170.9 ± 4.3	510 ± 28	180.4 ± 6.1
Low-P, NaCl, PTH	5	4	626 ± 47	164.5 ± 7.1	491 ± 35	165.7 ± 4.2
Low-P, NaH_2PO_4	4	4	624 ± 42	169.9 ± 8.2	518 ± 23	170.4 ± 7.1
Low-P, NaH_2PO_4, PTH	4	4	484 ± 21	175.7 ± 9.1	507 ± 26	180.1 ± 5.0
High-P, acute PTX	4	4	345 ± 28	168.5 ± 3.2	504 ± 32	164.3 ± 3.5
High-P, NaH_2PO_4	4	4	275 ± 22	175.2 ± 7.1	512 ± 19	182.5 ± 7.5
High-P, NaH_2PO_4, PTH	4	4	261 ± 32	167.3 ± 6.9	510 ± 26	169.7 ± 8.1

transport system and not by a change of other, more general, properties of the membrane vesicles, such as Na^+ permeability or surface/volume ratio. If those had been affected, the Na^+-dependent D-glucose transport and mannitol uptake would also be expected to be different in the two groups of animals (Evers *et al.*, 1978*a*,*b*).

Although in all experiments presented here the complete time course for the uptake of the solutes was investigated, in the following only the results obtained for the rapid initial phase (uptake after 20s) will be reported (Table 1). That such measurements indeed reflect properties of the brush-border membrane quite well is demonstrated by the close parallelism of results obtained in micropuncture studies *in vivo* and with membrane vesicles *in vitro* (Murer *et al.*, 1978).

On the basis of the known function of parathyrin in phosphate homoeostasis of the organism one has to assume that animals adapted to a high-phosphate diet have high parathyrin concentrations, whereas animals adapted to a low-phosphate diet have low parathyrin concentrations. Recently, we were able to demonstrate that administration of parathyrin 1h before the animals, which were kept on a normal phosphate diet, were killed, decreases Na^+-dependent transport of P_i in isolated brush-border membrane vesicles by about 30% (Evers *et al.*, 1978*a*). Therefore it was decided to investigate whether the difference in Na^+-dependent transport of P_i observed in the animals with different dietary phosphate intakes could be due to the difference in the parathyrin concentration in these animals.

Effect of acute parathyroidectomy on the transport properties of brush-border membranes isolated from animals on high- and low-phosphate diets

In the sham-operated animals a highly significant increase ($P<0.001$) in the rate of phosphate uptake is observed after they are fed on low-phosphate diet as compared with the high-phosphate diet. A smaller, but still statistically highly significant ($P<0.001$), difference between the two groups remains after acute parathyroidectomy (Table 1). Comparing the transport rates for P_i within the same dietary group but of animals with different parathyroid status, a stimulatory effect of parathyroidectomy on the phosphate uptake can be observed in the animals on the high-phosphate diet ($P<0.05$) but not in those on the low-phosphate diet. These results suggest that the low phosphate transport in brush-border vesicles isolated from animals on the high-phosphate diet partly represents a parathyrin-mediated decrease in the transport capacity of the Na^+/phosphate co-transport system, as a result of the high parathyrin concentration in these animals. However, as indicated by the difference ($P<0.001$) between the two dietary

groups in acutely parathyroidectomized animals, the transport system seems to also be regulated via a parathyrin-independent mechanism. The lack of stimulation of phosphate uptake by brush-border vesicles after parathyroidectomy in the group on the low-phosphate diet is difficult to explain. One possible explanation would be that the transport system is already maximally stimulated by the low-phosphate diet and that, owing to the already very low parathyrin concentration, no further stimulation through parathyroidectomy is possible.

Effect of acute parathyrin administration on the transport properties of brush-border membranes isolated from animals on high- and low-phosphate diets

It is known from studies with dogs and rats that in animals on a low-phosphate diet parathyrin cannot elicit its phosphaturic action (Steele *et al.*, 1976). In dogs, this resistance to parathyrin can be overcome by an acute phosphate load (Sutton *et al.*, 1978). In our experiments performed with rats on a low-phosphate diet corresponding results were found (Table 1). In the control group of rats on a low-phosphate diet parathyrin injected intraperitoneally 1 h before death at a dose of 50 U.S.P. units/animal had no effect. If, however, sodium phosphate was injected together with parathyrin, the hormone produced an inhibition ($P<0.05$) of Na^+-dependent phosphate uptake by brush-border vesicles. The extent of inhibition was similar to that observed in earlier studies with animals that had been fed with a normal standard diet (1% P_i) (Evers *et al.*, 1978*a*). Phosphate injection alone did not alter the phosphate transport by brush-border vesicles if compared with a control group that received 0.9% NaCl to act as a control for possible volume expansion.

Therefore for a comparison of the effect of parathyrin on phosphate transport in brush-border vesicles isolated from animals on low- and high-phosphate diets, all animals received sodium phosphate together with the parathyrin. As can be seen from Table 1 a significant difference ($P<0.01$) between the high- and low-phosphate-diet group is still present after phosphate loading and parathyrin administration. This means that the drastic increase in phosphate transport in the low-phosphate-diet group might only be partly caused by the lack of parathyrin in this group. The hormone-dependent effect is represented by the decrease observed in the low-phosphate-diet group after hormone administration. Apparently in our experimental conditions parathyrin is not able to lower further the transport rate in the high-phosphate-diet group. This might be explained by the high parathyrin concentrations in these animals causing already maximal hormone-induced inhibition of the phosphate/Na^+ co-transport system in the brush border.

The experiments described thus far demonstrate that only part of the difference found in phosphate uptake of the brush border in animals on high- and low-phosphate diets can be explained by the difference in the parathyrin concentration. There remains a significant difference between animals on a high-phosphate diet and those on a low-phosphate diet, even when the supposedly high concentration of parathyrin in the high-phosphate-diet group is decreased by acute parathyroidectomy and the supposedly low concentration of parathyrin in the low-phosphate-diet group is increased by hormone administration (Table 1: 345 ± 28 against 484 ± 21, uptake after 20 s as percentage of equilibrium value; $P<0.01$).

Effect of chronic parathyroidectomy on the adaptation of the Na^+/phosphate co-transport system to low- and high-phosphate diets

If the hypothesis were correct that the Na^+/phosphate co-transport system in the renal brush-border membrane is influenced in a non-parathyrin-mediated manner by phosphate in the diet, then adaptation of the transport system to the diet should also be observed in chronically parathyroidectomized rats. There is indeed a highly significant difference ($P<0.001$) between the phosphate transport of brush-border vesicles isolated from animals on low- and high-phosphate diets that have been parathyroidectomized before the different dietary regimens were started (Fig. 2). Again no difference in the Na^+-dependent D-glucose transport by the vesicles was observed.

Assuming that the parathyrin concentration in the chronically parathyroidectomized rats is low and that residual parathyrin formation is negligible, these data also demonstrate a non-parathyrin-mediated effect of phosphate in the diet on the Na^+/phosphate co-transport system in the brush-border membrane.

Effect of low- and high-phosphate diets on the activity of alkaline phosphatase in renal cortex homogenate and in isolated brush-border membranes

Melani & Guerritorre (1967) and Kempson *et al.* (1978) observed a parallelism in the amount of alkaline phosphatase present in kidney homogenates and the renal phosphate transport. On the basis of these data they suggested that alkaline phosphatase might be involved in renal phosphate transport. In our experiments (Table 2), there is only a small difference (less than 20%) in the specific activity of alkaline phosphatase in the homogenates or in brush-border membranes isolated from animals on a low-phosphate diet. Thus, under our experimental conditions, only small changes in alkaline phosphatase, but drastic differences in the phosphate-transport properties, of

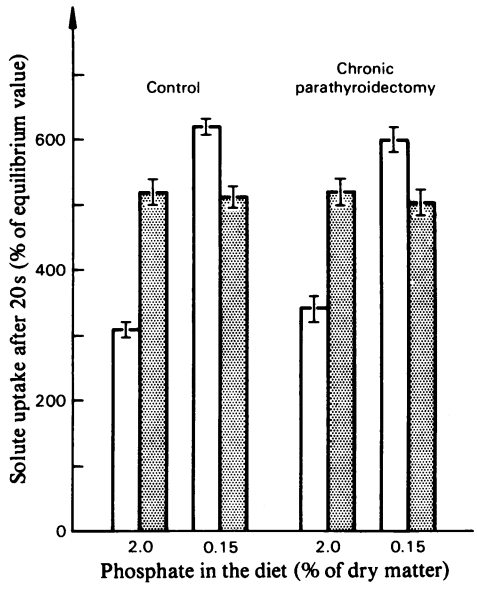

Fig. 2. *Effect of chronic parathyroidectomy on phosphate (□) and D-glucose (▒) uptake by renal brush-border vesicles isolated from rats on low- and high-phosphate diets*

The equilibrium uptake amounted to: 168.7 ± 5.9 pmol of P_i/mg of protein and 178.3 ± 4.9 pmol of D-glucose/mg of protein in sham-operated animals on the high-phosphate diet; 174.9 ± 6.4 pmol of P_i/mg of protein and 168.7 ± 7.2 pmol of D-glucose/mg of protein in sham-operated animals on the low-phosphate diet; 167.6 ± 8.5 pmol of P_i/mg of protein and 173.8 ± 6.1 pmol of D-glucose/mg of protein in chronic parathyroidectomized animals on the high-phosphate diet; 172.8 ± 7.4 pmol of P_i/mg of protein and 166.4 ± 5.9 pmol of D-glucose/mg of protein in chronic parathyroidectomized animals on the low-phosphate diet. The values are means for four determinations, and the error bars indicate ± S.E.M.

Table 2. *Specific activities of alkaline phosphatase in renal cortical homogenate and in isolated brush-border membranes*

The values are expressed in munits/mg of protein and represent means ± S.D. for four experiments One unit is 1 μmol of *p*-nitrophenol released/min at 37°C. Abbreviations are as defined in Table 1.

Experimental group	Homogenate	Brush-border membranes
Low-P, sham	1523 ± 159	16342 ± 2056
High-P, sham	1498 ± 101	13256 ± 2408
Low-P, acute PTX	1741 ± 129	16337 ± 1635
High-P, acute PTX	1594 ± 254	14185 ± 1864
Low-P, NaH_2PO_4, PTH	1458 ± 95	14713 ± 1951
Low-P, NaH_2PO_4	1504 ± 216	14325 ± 3063
Low-P, NaCl	1521 ± 103	14056 ± 1686
Low-P, PTH	1325 ± 100	12675 ± 2650
High-P, NaH_2PO_4, PTH	1575 ± 132	16250 ± 895

the brush-border membrane between the various experimental groups were observed. These findings make it unlikely that alkaline phosphatase is directly involved in the transport of P_i across the brush-border membrane.

Conclusion

The results presented above indicate that in the rat renal proximal tubule the adaptation of phosphate transport to dietary phosphate intake is accompanied by parallel changes in the Na^+/phosphate co-transport system in the brush-border membrane. Therefore the adaptation of the Na^+/phosphate co-transport system seems to be brought about by at least two different mechanisms, one mediated by parathyrin and the other induced by the diet independently of the hormone. The biochemical basis for these mechanisms is not known hitherto. The parathyrin- (and cyclic AMP-) mediated regulation has been postulated to involve phosphorylation of the transport system or of an associated regulatory protein, since cyclic AMP-dependent protein kinases have been found in brush-border membranes (Kinne *et al.*, 1975), and cyclic AMP-dependent phosphorylation of cell proteins has been demonstrated in rabbit kidney-cortex slices (Ausiello *et al.*, 1976). The functional role of the phosphorylated protein has, however, not been identified. The molecular basis underlying the parathyrin-independent mechanism of adaptation to low-phosphate diet is even more obscure. During phosphate depletion a variety of hormonal, biochemical and chemical changes occur in the whole animal as well as in the renal cell (Kreusser & Ritz, 1978). Their effect on the Na^+/phosphate co-transport system in the brush-border membrane is difficult to evaluate at the moment. Interestingly, however, phosphate transport in the proximal tubule is increased, whereas glucose and bicarbonate transport are decreased after phosphate depletion. This divergent behaviour might point to a specific regulation of the Na^+/phosphate co-transport system independent from the alteration in cellular metabolism, perhaps by induction or de-repression of synthesis of new Na^+/phosphate co-transport systems. An alternative possibility would be that there might be two different Na^+/phosphate co-transport systems present in the brush-border membrane, one regulated by parathyrin, the other affected by dietary phosphate intake. Identification and isolation of the transport system(s) should help to answer these unsolved questions.

With respect to the identification of the transport system, our data do not support the hypothesis that the enzyme alkaline phosphatase is involved in the phosphate transport across the brush border. The initial rate of phosphate uptake by brush-border

membranes isolated from animals on the low-phosphate diet is approximately 2-fold higher than the rate of uptake by those on the high-phosphate diet, yet the activity of alkaline phosphatase is only slightly increased.

From a physiological point of view our data support the hypothesis that regulation of transepithelial phosphate transport in the proximal tubule under a variety of conditions takes place at the luminal entry step for phosphate. The conditions tested so far are: parathyrin administration, parathyroidectomy, dietary phosphate intake and genetically determined hypophosphataemia (C. R. Scriver, personal communication). Thereby the degree of stimulation or inhibition of transport observed in the membranes *in vitro* is quantitatively similar to the changes observed in the transepithelial transport in the proximal tubule *in vivo*. This agreement leads to the conclusion that the alteration of the luminal transport system is the predominant if not exclusive cause of the altered phosphate reabsorption.

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