Pflügers Arch (1987) 409:333–336

Pflügers Archiv
European Journal of Physiology

Adaptation of phosphate transport to low phosphate diet in renal and intestinal brush border membrane vesicles: influence of sodium and pH

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Abstract. The possible role of changes in the sodium (Na) affinity of the carrier for inorganic phosphate (P_i) in the adaptation of P_i transport to low P_i diet was examined in both renal and intestinal brush border membranes vesicles (BBMV) obtained from the same animal. This role was assessed by measuring the Na concentration resulting in half maximal activation of P_i transport ($K_{0.5\,Na}$) in renal and intestinal BBMV prepared from animals adapted to either low (LPD) or high (HPD) phosphorus diet for 7 days. The $K_{0.5\,Na}$ was not modified by dietary P_i, in both renal and intestinal BBMV. LPD increased maximal P_i transport from 1794.8 ± 198.0 to 2964.0 ± 362.0 in renal and from 28.2 ± 3.4 to 80.5 ± 7.2 pmol/mg 10 s in intestinal BBMV. For both LPD and HPD lowering pH from 7.4 to 6 dramatically increased $K_{0.5\,Na}$ in renal and intestinal BBMV. As compared to pH 7.4, it was enhanced by approximately 200% in both renal and intestinal membranes. This change of Na affinity with acidic pH prevented the expression of P_i transport adaptation at 100 mM Na concentration. However, at saturating Na concentrations (500 mM for renal, 300 mM for intestinal membranes), P_i transport adaptation was equally expressed at pH 6 and 7.4 in both types of membranes. Hill coefficient analysis indicates a 2:1 stoichiometry of Na to P_i in renal and intestinal membranes isolated from high or low P_i diet animals. This ratio was not modified by changes of the medium pH.

Key words: Renal and intestinal P_i transport – Brush border membrane – Low P_i diet – Na – pH

Introduction

The capacity of the kidney to reabsorb inorganic phosphate (P_i) increases with dietary P_i restriction (Bonjour and Fleisch 1980). The initial and may be the rate limiting step in this adaptive response is a stimulation of the Na-dependent P_i transport (NaP_iT) across the luminal membrane of the proximal tubule (Hoffmann et al. 1976; Bonjour et al. 1980; Hammerman et al. 1980). Recent studies in both chicken (Quamme 1985) and rats (Caverzasio et al. 1985a) have documented that dietary P_i restriction also enhanced the NaP_iT by intestinal brush border membrane vesicles (BBMV). The intrinsic mechanism of these adaptive responses observed in both renal and intestinal BBMV is unknown. Alterations in NaP_iT in BBMV can be explained either by changes in the number of carriers or in the affinity of the substrates for the transporters. Moreover, it could also result from changes in the Na gradient.

In neonatal pig renal BBMV, preequilibrated with Na^+, a stimulation of the P_i uptake induced by low P_i diet was observed which could not be accounted for by an increased affinity for P_i (Barrett et al. 1980). This finding would support the view that the adaptation to low P_i diet is concomitant to an increase in the number of Na^+-phosphate carriers (Barrett et al. 1980). However, in rabbit renal BBMV the effect of dietary P_i on P_i uptake was absent when the BBMV were preequilibrated with Na^+ (Cheng et al. 1983). This finding would rather favor hypothesis that adaptation to low P_i diet involves an alteration in the rate of translocation of the Na^+-phosphate carrier by the Na^+ gradient (Cheng et al. 1983). Furthermore, in rabbit BBMV the adaptive response to low P_i diet was not observed when the P_i uptake was determined at an acidic pH (Cheng et al. 1983). This observation was interpreted as indicating that dietary adaptation may be concomitant to an alteration in the pH dependence of the transport system itself (Cheng et al. 1983).

The present study examines whether alterations in Na-affinity of the P_i carrier plays a role in the stimulation of P_i transport induced by dietary P_i reduction in both renal and intestinal brush border membranes. Since extravesicular pH has been shown to modify the Na-affinity of the P_i transport system (Brunette et al. 1984a, Burckhardt et al; Sacktor and Cheng 1981), we investigated the influence of pH on the adaptation of P_i transport at different extravesicular Na concentrations. Finally the opportunity to isolate renal and intestinal BBMV from the same animal led us to compare P_i transport characteristics of these two apical membranes.

Methods

Male Wistar rats raised on a commercial chow (Altromin 1314, Altrogge, Lage Lippe, FRG) containing 1.2% phosphorus (P), 1.1% Ca and about 280 I.U./100 g vitamin D3 were used. When weighing 160–180 g they were fed a semisynthetic vitamin D poor diet (Sodi 34/1, Kliba, Switzerland). 10 I.U. of vitamin D3 dissolved in 0.1 ml of vegetable oil was added on the daily ration. During the first 7 experimental days all animals received a diet containing 0.8% P and 1.1% Ca. This high P_i diet was prepared by

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addition of calcium gluconate and sodium phosphate to the semisynthetic diet containing 0.2 g/100 g Ca and 0.2 g/100 g P. Then, animals were either maintained on this high P_i diet (HPD) or switched to a low P_i diet (LPD 0.2% P and 1.1% Ca) for 8 days before the preparation of renal and intestinal brush border membrane vesicles (BBMV).

Brush border membrane isolation and transport studies. Renal cortex and intestinal jejunum segment from 10 cm distal to pylorus up to 30 cm long were obtained from the same animal. Renal brush border vesicles were isolated by the method described by Evers et al. (1979), with a minor modification, calcium chloride being substituted by magnesium chloride in the solution used for membrane precipitation. Intestinal brush border vesicles were isolated as reported previously (Danisi et al. 1984), except that EGTA was omitted and Hepes added to the buffer solutions.

Purified renal brush border vesicles were suspended in a final membrane buffer containing 500 mM choline chloride and 20 mM Hepes/Tris (pH 7.4) or 20 mM Mes/Tris (pH 6.0). The final membrane buffer for intestinal brush border vesicles contained 600 mM mannitol, 20 mM Hepes/Tris (pH 7.4) or Mes/Tris pH 6.0, 5 mM KN_3, 1 mM $CaCl_2$ and 1 mM $MgCl_2$. Uptake of labelled substrate was measured in triplicate by the millipore filtration technique (Hopfer et al. 1973). The initial rate of P_i uptake was measured after 10 s incubation time using a short time reaction uptake measurement apparatus (Innovativ-Labor AG, Zürich, Switzerland).

Preliminary experiments indicated that at this incubation time period of 10 s the P_i uptake by both renal and intestinal BBMV was still linearly related to incubation time. P_i uptake in renal brush border vesicles was studied in a medium containing 0.1 mM $H_3{}^{32}PO_4$, 20 mM Hepes/Tris (pH 7.4) or 20 mM Mes/Tris (pH 6.0) and NaCl or KCl ranging from 0–500 mM. In intestinal brush border vesicles it was measured in 0.1 mM $H_3{}^{32}PO_4$, 5 mM KN_3 (added to prevent bacterial growth), 20 mM Hepes/Tris (pH 7.4), or Mes/Tris (pH 6.0), and NaCl or KCl from 0–300 mM. The uptake experiment was started by adding to a test tube in separate spots 5 µl of membrane suspension (approx. 50 µg protein) and 45 µl incubation medium. The uptake reaction apparatus initiated the reaction by shaking the tube and thus mixing both droplets. It stopped the transport process by injecting approximately 1 ml of ice-cold stop solution containing for the renal studies 500 mM NaCl, 20 mM Hepes/Tris (pH 7.4) and 5 mM Na_2HASO_4 and for the intestinal studies 320 mM NaCl, 10 mM Tris/HCl pH 7.4, 5 mM NaN_3, 5 mM KH_2PO_4 and 1 mM $MgCl_2$. The membranes were immediately harvested on a cellulose nitrate filter (pore size 0.65 µm: Sartorius, Göttingen, FRG) and washed once with 5 ml ice-cold buffer. The amount of labelled substrates remaining on the filters was determined by standard liquid scintillation counting. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard after precipitation of the membrane with ice-cold 10% (wt/vol) trichloro-acetic acid and complete solubilization with NaOH. Chemical reagent of high purity were obtained from Merck (Darmstadt, FRG). Radioactive isotopes were purchased from New England Nuclear (Boston, MA, USA).

Statistical analysis. All results are expressed as means ± SEM. Significance of differences were calculated by the two-side Student's *t*-test.

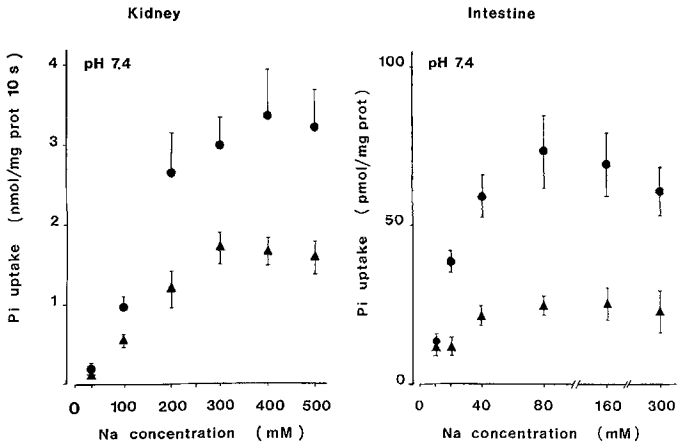

Fig. 1. P_i uptake measured at various extravesicular Na concentrations in BBMV isolated from renal cortex and jejunum of rats fed a high P_i diet (▲) $n = 6$ or a low P_i diet (●) $n = 5$ (renal) and 6 (intestinal). The intra- and extravesicular buffer solutions were adjusted to pH 7.4

Results

The influence of various extravesicular Na concentrations on the P_i uptake measured at pH 7.4 and at an incubation time of 10 s in renal and intestinal BBMV is shown in Fig. 1. Increasing the Na gradients enhanced the P_i uptake up to a certain level in both types of membranes. This pattern was observed in BBMV isolated from animals fed either on high or on low P_i diet. The stimulation induced by low P_i feeding was expressed over a wide range of extravesicular Na concentrations. This effect was maintained under saturated Na-gradient conditions, i.e. beyond 300 and 80 mmol/l NaCl for the renal and intestinal BBMV respectively. Kinetic analysis indicated that the Na affinity constant for the P_i transport system, as estimated by the Na-concentration corresponding to half maximal P_i transport rate ($K_{0.5\,Na}$), was not altered by the dietary P_i condition (Table 1). However, this parameter was about 7 times higher in renal (149.1 ± 10.4 mmol/l) as compared to intestinal (20.2 ± 1.7 mmol/l) BBMV isolated from HPD fed animals. The LPD-induced increase in the P_i uptake was solely due to a higher V_{max} of the Na-activated P_i transport system (Table 1). Using the same batches of isolated membranes, the P_i transport was also studied in a buffer solution adjusted to pH 6.0 instead of to pH 7.4. The results are illustrated in Fig. 2. The relation between the P_i transport rate the the external Na concentration was shifted to the right as compared to that obtained at pH 7.4 (Fig. 1). This shift indicates an important pH-induced alterations in the interaction of Na with the P_i transport carrier, confirming previous observations (Brunette et al. 1984a; Burckhardt et al. 1981; Sacktor et al. 1981). In renal membranes isolated from animals fed the high P_i diet $K_{0.5\,Na}$ for the P_i transport system was increased by more than 100% at pH 6.0 (430.5 ± 51.2 mmol/l, $n = 6$) as compared to pH 7.4 (149.1 ± 10.4 mmol/l, $n = 6$; $p < 0.001$). In intestinal membranes, $K_{0.5\,Na}$ was about 300% larger at pH 6.0 (62.8 ± 14.2 mmol/l, $n = 6$) than at pH 7.4 (20.2 ± 1.7 mmol/l, $n = 6$; $p < 0.001$). The expression of the LPD-induced increase in P_i utake was also observed at acidic pH, but became only apparent at higher extravesicular Na concentrations than at pH 7.4 (Fig. 2). At pH 6.0, an external Na-gradient concentration of 500 mM

Table 1. Na-dependency of P_i uptake at pH 7.4

	Kidney		Intestine	
	HPD $n = 6$	LPD $n = 5$	HPD $n = 6$	LPD $n = 6$
V_{max} (pmol P_i/mg prot/10 s)	1794.8 ± 198.0	2964.0 ± 362.0*	28.2 ± 3.4	80.5 ± 7.2***
$K_{0.5\,Na}$ (mmol/l)	149.1 ± 10.4	144.4 ± 3.4	20.2 ± 1.7	20.8 ± 1.2
Hill coefficient	2.2 ± 0.1	2.5 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Na-dependency of P_i uptake at pH 6.0				
	Kidney		Intestine	
	HPD $n = 6$	LPD $n = 5$	HPD $n = 6$	LPD $n = 6$
V_{max} (pmol P_i/mg prot/10 s)	1086.2 ± 179.3	–	16.7 ± 3.2	35.4 ± 3.1***
$K_{0.5\,Na}$ (mmol/l)	430.5 ± 51.2^{+++}	–	62.8 ± 14.2^{+}	89.3 ± 7.2^{+++}
Hill coefficient	2.0 ± 0.2	–	1.5 ± 0.1	1.8 ± 0.1

Kinetic analysis of P_i uptake changes with various external Na concentrations in brush border vesicles isolated from rats fed either a high (HPD) or a low phosphorus diet (LPD). The same membrane vesicles were used to study these P_i uptake changes at pH 7.4 and pH 6.0. The V_{max} and the $K_{0.5\,Na}$ were determined by double reciprocal plot analysis. Hill coefficient from slope of Hill plot regression line.
*$p < 0.05$; ***$p < 0.005$; as compared to data obtained in brush border vesicles isolated from animals fed the high P_i diet.
$^{+}p < 0.02$; $^{+++}p < 0.001$; as compared to results obtained at pH 7.4

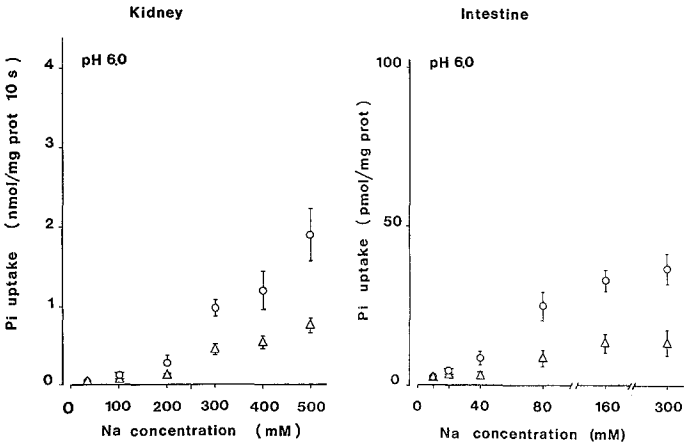

Fig. 2. P_i uptake measured at various extravesicular Na concentrations in BBMV isolated from renal cortex and jejunum of rats fed a high P_i diet (△) $n = 6$ or a low P_i diet (○) $n = 5$ (renal) and 6 (intestinal). The intra- and extravesicular buffer solutions were adjusted to pH 6.0

was not sufficient to saturate the P_i transport process in renal membranes. In intestinal membranes, saturation was reached at a Na-gradient of about 160 mmol/l (Fig. 2). The calculation of the Hill coefficient indicates a 2:1 stoichiometry of sodium to phosphate for all experimental conditions. These data confirm previous observations that renal and intestinal Na-dependent P_i transport system require two sodium ions for the translocation of one P_i (Hoffmann et al. 1976); Danisi et al. 1984). This ratio was not modified by the medium pH.

Discussion

The results of this study indicate that the apparent affinity of Na for the P_i transport system, estimated by determining the half maximal activation by Na ($K_{0.5\,Na}$), was not altered in BBMV isolated from renal cortex and intestine of rats adapted to low as compared to high P_i diet. Previous studies in renal (Cheng et al. 1983; Brunette et al. 1984b; Levine et al. 1984; Murer et al. 1980) and more recently in intestinal BBMV (Quamme 1985; Caverzasio et al. 1985a) also suggested that the P_i affinity was not, or at least only slightly, altered by P_i deprivation. Therefore, the adaptive change in the activity of the Na-dependent P_i transport to variations in the dietary P_i supply does not probably result from increased affinity of the P_i transport system for P_i and/or Na. Thus, the adaptation in the rate of P_i transport must be due to other modifications of the P_i carrier, such as for instance covalent modification(s) of one or several constitutive protein(s) of the luminal membrane. Another explanation would be the insertion of newly synthetized P_i carriers in the apical membrane. This hypothesis is supported by recent in vitro studies on the change of P_i transport with lowering environmental P_i concentration (Caverzasio et al. 1985b). In this report, it was shown that the adaptive response to low P_i medium was located in isolated apical membrane and was mostly dependent of the de novo synthesis of protein.

As previously reported for renal BBMV (Burckhardt et al. 1981), a reduction in Na affinity for the P_i transport system by acidifying the medium dramatically decreased the P_i uptake. The present investigation indicates further that this response can be demonstrated in both renal and intestinal BBMV and also, is present to the same extent in membranes isolated from animals fed either on high or on low P_i diet. Calculation of Hill coefficients also indicates that in both renal and intestinal BBMV the 2:1 stoichiometry for Na:P_i is not modified by the medium pH. In previous studies in rat (Berner et al. 1976) and rabbit (Danisi et al. 1984) intestinal BBMV, lowering the extravesicular pH from 7.6 to 6.0 was found to increase the Na-dependent P_i uptake. The reason for the difference between the previous observations and our present data is not clear. It could be related to intravesicular pH. In our study both the intra- and extra-

vesicular pH were identical. In contrast to previous reports (Berner et al. 1976; Danisi et al. 1984), the internal pH was maintained at a fixed value while the external pH was varied. Whether the presence or the absence of a pH gradient across the BBMV might explain the difference remains to be clarified.

The data presented in Fig. 2 indicate that at acidic pH, the expression of increased P_i uptake in renal membranes with low P_i diet was not present at extravesicular Na concentrations lower than 200 mM. The full expression of the adaptive response was only present at 500 mM Na. At pH 6.0 a saturation in the P_i transport process was not attained at this very high extravesicular Na concentration. This observation indicates that even though the affinity of Na is not responsible for the adaptive change in P_i uptake with low P_i diet, it remains an important factor for its full expression, especially when the environmental pH of the brush border membrane is relatively low. Further, our data suggest that the previously reported absence of adaptation of P_i transport at pH 6.5 in rabbit BBMV could be related to the relatively low Na-gradient driving force (100 mM) imposed across the luminal membrane (Cheng et al. 1983). In the present study, at this extravesicular Na concentration, no significant change of P_i uptake was observed between high and low P_i diet (Fig. 2). Finally the data presented in this study allow comparison between the P_i transport characteristics of rat renal and intestinal brush border membranes. The capacity of renal BBMV to transport P_i is about two order of magnitude higher than that of intestinal membranes when compared at saturating extravesicular Na concentrations. The apparent Na affinity for the transporter, however, was about 10 times higher in intestinal than in renal BBMV. It is interesting to note that the influence of Na, dietary P_i and environmental pH were similarly expressed in both types of membranes. These observations may suggest some similarities in the biological constituents and mechanisms of regulation for the two P_i transport systems.

In conclusion, the apparent affinity of Na for the P_i transport system is not modified in renal and intestinal brush border membranes adapted to low P_i diet. Therefore, modifications of the binding sites for P_i and Na are not important elements in this adaptive change of P_i transport. The environmental pH of the brush border membrane, however, plays an important role since it modifies the binding characteristics of Na which in turn determines the driving forces required for the full expression of the adaptive P_i transport response.

Acknowledgements. We are indebted to Pascale Chabert for her excellent technical help. We also acknowledge Marie-Christine Brandt and Brigitte Mathys for their secretarial work.

This work was supported by the Swiss National Science Foundation Grants No 3.954-1.85 and 3.182-0.85. This study was presented at the 7th international workshop on phosphate and other minerals, 1985, Marseille, France.

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Received November 19, 1986/Accepted February 13, 1987