*Biochimica et Biophysica Acta* 815 (1985) 85–90
Elsevier



BBA 72556

# Effects of fasting compared to low phosphorus diet on the kinetics of phosphate transport by renal brush-border membranes

Stephen A. Kempson

*Department of Physiology and Biophysics, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46223 (U.S.A.)*

(Received October 26th, 1984)

Key words: Phosphate transport; $Na^+$-dependent transport; Diet; Kinetics; (Rat kidney)

**Changes in the kinetics of sodium gradient-dependent brush border $P_i$ transport in response to dietary phosphorus deprivation were analysed using initial rate conditions. In rats adapted to low phosphorus diet the apparent $V_{max}$, determined from a double-reciprocal plot, was increased 2-fold but the apparent $K_m$ was not different compared to control rats fed normal phosphorus diet. In contrast when renal adaptation to low phosphorus diet was reversed by fasting the apparent $V_{max}$ was not significantly different but the apparent $K_m$ was increased 5-fold. The results suggest that regulation of renal $P_i$ transport in vivo may occur not only through changes in the apparent $V_{max}$ of the brush border $P_i$ transport system but also, in certain circumstances, through changes in the apparent $K_m$.**

## Introduction

The sodium gradient-dependent $P_i$ transport system in the renal brush-border membrane is modified in response to the dietary P intake and to several hormones and drugs administered in vivo [1,2]. Kinetic studies are often employed in analysis of alterations in renal brush border $P_i$ transport to determine whether the changes involve the $V_{max}$ or the $K_m$ of the system. In almost all the situations analysed in this way the change in $P_i$ transport was accompanied by an increase or decrease in the $V_{max}$ without a significant change in the $K_m$. This includes the effects of low P diet [3–5], parathyroid hormone [6], growth hormone [6], nicotinamide [7], glucocorticoids [8], and thyroxine [9]. A potential problem with most of these studies is that the kinetic analyses are based on measurements of $P_i$ uptake under non-linear conditions, using incubation times of 20 s or longer. In the present study conditions were established for measurement of true initial rates of sodium gradient-dependent $P_i$ transport by rat renal brush-border membrane vesicles. Under these conditions the kinetic changes which occur during renal adaptation to low P diet, an antiphosphaturic stimulus, were re-evaluated. In the same way, reversal of this adaptation during subsequent fasting, a phosphaturic stimulus [10], was also analysed.

## Methods

Brush-border membrane vesicles were prepared from rat renal cortex by a magnesium precipitation procedure and $Mg^{2+}$ was included in all subsequent solutions [4,11]. The isolated brush-border membranes were washed and suspended in a solution of 300 mM mannitol, 1 mM $MgSO_4$, 5 mM Tris, pH adjusted to 7.4 with Hepes. The purity of the final membrane suspension was as-

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

sessed by measurement of appropriate marker enzymes and was similar to that reported in previous studies on rats [12,13]. Immediately after preparation the brush-border membrane vesicles were used for uptake studies with a rapid filtration technique [3,12–14]. Briefly, the vesicles were incubated at 20°C in a medium containing (final concentrations) 100 mM NaCl, 100 mM mannitol, 1 mM $MgSO_4$, 5 mM Tris-Hepes (pH 7.4) and 0.1 mM $K_2H\ ^{32}PO_4$ (about $3 \cdot 10^5$ counts/min per tube). The uptake was initiated by adding 0.030 ml of incubation medium to 0.015 ml of the membrane vesicle suspension, and was terminated at various times by rapid addition of ice-cold solution containing 135 mM NaCl, 10 mM sodium arsenate, 1 mM $MgSO_4$, 5 mM Tris-Hepes (pH 7.4), followed by filtration through prewetted filters. The vesicles were retained on the filters which were processed for liquid scintillation counting. All data were corrected by subtraction of the radioactivity recovered in blanks which were obtained by adding the stopping solution to the membrane vesicles prior to addition of the incubation medium.

When the incubation time was less than 10 s the following procedure was used. The 0.015 ml sample of membrane vesicles was placed at the bottom of a clear polystyrene tube. The tube was tilted to the side and the 0.030 ml of incubation medium was placed as a separate droplet on the wall of the tube. Uptake was initiated by vortexing to mix the droplets, and the incubation time was measured with a metronome set at 1 s intervals. The uptake was terminated in the usual way.

Adaptation to low P diet was monitored by housing rats individually in metabolic cages and collecting urine for 24 h. Initially all rats were fed normal P diet while urinary $P_i$ excretion was determined. The rats were then divided into two groups which did not differ in mean urinary $P_i$ excretion. One group was continued on normal P diet while the other group was switched to low P diet, and the groups were pair-fed for the next four days. Food intake was monitored and rats eating less than 5 g of food per day were excluded from the experiment to avoid a possible starvation response [10]. After the fourth day the urinary excretion of $P_i$ in low P diet rats had declined to a level which was significantly lower ($p < 0.005$, group $t$-test) compared to rats fed normal P diet. The values in low P diet rats ($n = 3$) were $1.0 \pm 0.3$ compared to $283 \pm 40$ $\mu$mol $P_i$/24 h per 100 g body weight in normal P diet rats ($n = 3$). At this point the kidneys were removed and processed for brush-border membrane preparation.

The protocol for fasting rats which were adapted to low P diet was the same as described previously [10]. The rats were fed low P diet for five days, then half of the rats were deprived of all food for the next four days while the remainder were maintained on low P diet. Brush-border membranes were prepared from these animals on day 9, at which point urinary $P_i$ excretion in the fasted rats ($n = 4$) was $500 \pm 166$ compared to $4.1 \pm 2.2$ $\mu$mol $P_i$/24 h per 100 g body weight in the fed animals ($n = 4$, $p < 0.025$).

Protein determination, enzyme assays, and other procedures were carried out as described before [10,12,13]. The low P diet containing 0.03% P was from ICN Nutritional Biochemicals (Cleveland, OH). The normal P diet was Rodent Laboratory Chow (Ralston Purina Co., St. Louis, MO) containing 0.86% P.

## Results and Discussion

In the presence of a transmembrane sodium gradient and at a $P_i$ concentration of 0.1 mM, the uptake of $P_i$ increased linearly as the amount of brush-border membrane protein increased in the range 0.01–0.09 mg. Further increases in the amount of membrane protein up to 0.35 mg were not accompanied by corresponding increases in $P_i$ uptake, which may be due to the amount of $P_i$ becoming a limiting factor. Membrane vesicles from rats fed low P diet exhibited a significantly higher rate of $P_i$ uptake ($p < 0.05$, $n = 3$) at all the protein concentrations which were tested except the lowest (0.01 mg). In the subsequent experiments the protein concentration was adjusted to within the linear range observed here.

Sodium gradient-dependent uptake of $P_i$ increased linearly with time in the range 1–7 s (Fig. 1). Deviation of $P_i$ uptake from linearity was observed after 8 s in both normal and low P diet groups, and appeared to be more pronounced in the low P diet group where the rate of $P_i$ uptake was increased at all time points compared to normal P diet rats. The non-linear uptake at times

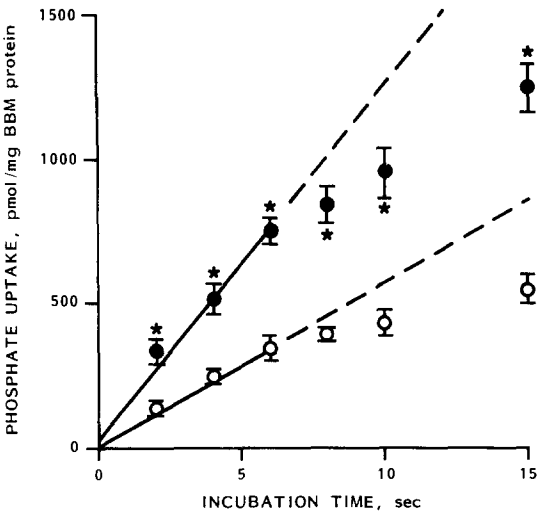


Fig. 1. Time dependence of sodium gradient-dependent $P_i$ uptake by isolated renal brush-border membrane vesicles from rats fed normal P diet (○) or low P diet (●). The lines were drawn by regression analysis of the data obtained for times in the range 2–6 s. The correlation coefficients for both lines were 0.99. The two groups were always compared in the same experiment and the data are the mean ± S.E. of three experiments, each analysed in triplicate. Each incubation tube contained 0.09 mg of membrane protein. An asterisk (*) indicates that $P_i$ uptake in the low P diet group was significantly greater ($P < 0.01$, group $t$-test) compared to the normal P diet group.

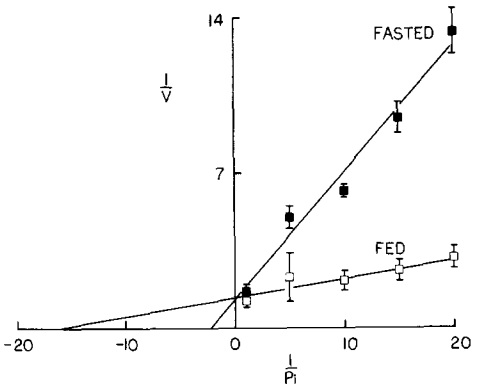


Fig. 2. Double-reciprocal plot of sodium gradient-dependent $P_i$ uptake by renal brush-border membrane vesicles from rats fed low P diet (□) and from rats fasted for four days after feeding low P diet (■). The two groups were always compared in the same experiment and the data are the mean ± S.E. of four experiments, each analysed in triplicate. The correlation coefficient for both lines was 0.99. The incubation time was 5 s and 0.09 mg of membrane protein were used. $V$ represents $P_i$ uptake expressed in nmol/mg protein per 5 s, and the $P_i$ concentration in the incubation medium is expressed as mM.

greater than 7 s may occur in part because the coupling between the flux of sodium ions and the flux of $P_i$ alters the transmembrane gradients of both ions [15,16]. Thus, for measurement of a true initial rate of transport it is necessary to use an incubation time within the linear range indicated in Fig. 1. This range of linearity is similar to that reported for $P_i$ uptake at pH 8.5 by vesicles prepared from rabbit kidney [17].

The initial rate conditions established in the preceding experiments were used for kinetic analysis of the increased rate of $P_i$ transport in low P diet rats. The kinetic constants are referred to as apparent $K_m$ and apparent $V_{max}$, because the $K_m$ and $V_{max}$ for sodium gradient-dependent solute transport may reflect not only the characteristics of solute transport but also the interaction of the solute transport system with cotransported sodium ions. Transport of $P_i$ was measured with the transmembrane gradient of sodium set at 100 mM extravesicular and 0 mM intravesicular at the start of the 5 s incubation period. Under these conditions, the increase in sodium gradient-dependent $P_i$ uptake by brush border membrane vesicles from low P diet rats was characterised by a change in the apparent $V_{max}$ which was increased 2-fold compared to normal P diet rats (Table I), as determined by a linear double-reciprocal plot. The apparent $K_m$ in these groups was not significantly different (Table I).

Since the double-reciprocal plot tends to emphasize the data points obtained at low concentrations of $P_i$ where the degree of error is likely to be greatest [18], the data from these experiments were analysed also by the linear Eadie-Hofstee plot [16,20]. This transformation of the data yielded similar findings, a significant increase in apparent $V_{max}$ in the low P diet group without a change in apparent $K_m$. As an additional check, the kinetic constants were determined from a simple least-squares fit of the untransformed data to a rectangular hyperbola [21] described by the equation $V = V_{max} \cdot P_i/(K_m + P_i)$, where $V$ is the phosphate uptake at 5 s and $P_i$ is the phosphate concentration in the incubation medium. This non-linear plot was constructed with the aid of a computer program designed by Dr. Thomas L. Crox-

TABLE I

CHANGES IN KINETICS OF INITIAL RATE OF SODIUM GRADIENT-DEPENDENT $P_i$ TRANSPORT AFTER ADAPTATION TO LOW PHOSPHORUS DIET

Normal P diet and low P diet groups were always compared in the same experiment. Transport of $P_i$ by isolated brush-border membrane vesicles was determined using an incubation time of 5 s and 0.09 mg of membrane protein per tube. The apparent $V_{max}$ (pmol/mg protein per s) and the apparent $K_m$ ($\mu$M) were determined by regression analysis of double-reciprocal plots of $P_i$ transport measured at various $P_i$ concentrations (in the range 0.05–1.00 mM) in the incubation medium [8,9,13]. These plots gave straight lines with correlation coefficients > 0.95. Data are the mean ± S.E. of three experiments, each experiment was conducted in triplicate. n.s., not significant ($p > 0.05$, group $t$-test).

| | Normal P diet | Low P diet | $p$ value |
|---|---|---|---|
| $V_{max}$ | 106 ± 19 | 209 ± 27 | < 0.05 |
| $K_m$ | 141 ± 24 | 141 ± 15 | n.s. |

ton in this department. The apparent $V_{max}$ values were 258 ± 38 in the low P diet group and 132 ± 22 pmol/mg per s in the normal diet rats ($p < 0.05$, group $t$-test). The apparent $K_m$ in the low P diet rats (183 ± 35 $\mu$M) was not significantly different from the apparent $K_m$ (155 ± 35) in the normal diet group. Thus, all analyses of the data indicate that adaptation to low P diet is accompanied by an increase in the apparent $V_{max}$ of the renal brush border $P_i$ transport system.

These findings confirm the results of the previous studies [3–5] which were based on 20-s uptake measurements, well outside the linear range (Fig. 1). This leads to the conclusion that measurement of sodium gradient-dependent $P_i$ uptake at times (e.g. 20 s) which are in the non-linear range, but which represent the early 'uphill' part of the overshoot phase, may be adequate for determining whether changes in brush border $P_i$ transport are due primarily to a change either in apparent $V_{max}$ or apparent $K_m$.

Measurement of $P_i$ uptake at short incubation times (e.g. 5 s) within the linear range probably resembles more closely the in vivo situation where, for example, the sodium gradient across the brush-border membrane does not decline but is maintained by the activity of the $(Na^+ + K^+)$-ATPase-linked pump in the basolateral membrane. Our previous studies [13] of the kinetics of brush border $P_i$ transport noted the large difference between the apparent $K_m$, determined under non-linear conditions, and the $P_i$ concentration (0.6–1.6 mM) in proximal tubular fluid. We suggested [1,13] that it was unlikely that the rate of $P_i$ transport could be regulated through changes in the apparent $K_m$ because the $P_i$ transport system would be fully saturated in vivo. In the present study, using brush-border membrane vesicles from normal P diet rats, the apparent $K_m$ for sodium gradient-dependent $P_i$ uptake at 5 s was 126 ± 18 $\mu$M (mean ± S.E., $n = 3$), and was 2–3 fold greater than the value of 55 ± 12 $\mu$M ($n = 3$) determined from the $P_i$ uptake at 30 s ($p < 0.05$, group $t$-test). This raises the possibility that under in vivo conditions the apparent $K_m$ for brush border $P_i$ transport may be much closer to the prevailing $P_i$ concentration in tubular fluid, indicating that in some situations a change in the apparent $K_m$ may serve to regulate the rate of $P_i$ transport.

A specific example of a change in brush border $P_i$ transport which is accompanied by a change in the apparent $K_m$ is provided by the effects of fasting on rats adapted to low P diet. Fasting for four days leads to a decrease in sodium gradient-dependent brush border $P_i$ transport and an increase in urinary $P_i$ excretion [10]. The kinetics of this change in $P_i$ transport were analysed in the present study using a double-reciprocal plot, and the principal change was an increase in the apparent $K_m$ of the brush border $P_i$ transport system (Fig. 2). In the fasted group the apparent $K_m$ was increased more than 5-fold compared to the group fed low P diet, while the apparent $V_{max}$ was not significantly different (Table II).

Transformation of the same data to an Eadie-Hofstee plot confirmed these findings, as did the computer-assisted plot of the untransformed data. The latter yielded the following values for the kinetic constants. The apparent $K_m$ in the fasted rats was 698 ± 82 $\mu$M compared to 180 ± 40 $\mu$M in the fed group ($p < 0.005$, group $t$-test). The apparent $V_{max}$ in the fasted rats was 219 ± 38, not significantly different from the value of 226 ± 58 pmol/mg per s in the fed group. Although the $K_m$ of the brush border $P_i$ transport system may be altered in vitro in response to changes in pH and

TABLE II

CHANGES IN KINETICS OF INITIAL RATE OF SODIUM GRADIENT-DEPENDENT $P_i$ TRANSPORT AFTER FASTING

All rats were fed low P diet for five days, then the fasted group were deprived of all food for the next four days while the fed group were maintained on low P diet. Sodium gradient-dependent $P_i$ transport by isolated brush-border membrane vesicles was determined using an incubation time of 5 s and 0.09 mg of membrane protein. Other details as in Table I. Data are the mean ± S.E. of four experiments, each analysed in triplicate. Fed and fasted groups were always compared in the same experiment. n.s., not significant ($p > 0.05$, group $t$-test).

| | Fed | Fasted | $p$-value |
|---|---|---|---|
| $V_{max}$ | 199 ± 52 | 233 ± 71 | n.s. |
| $K_m$ | 130 ± 21 | 708 ± 194 | < 0.025 |

temperature [19,20], this is one of the first reports of a maneuver which induces a change in the apparent $K_m$ in vivo without affecting the apparent $V_{max}$.

It should be pointed out that the changes in brush border transport of $P_i$ which occur during adaptation to low P diet and during subsequent fasting represent a specific change in the membrane $P_i$ transport system. Low P diet and fasting do not change $P_i$ uptake at equilibrium [5,10,12] suggesting that the changes in initial rates of sodium gradient-dependent $P_i$ uptake are not due to differences in the intravesicular volume. Furthermore, these maneuvers do not alter the sodium gradient-dependent transport of other solutes such as D-glucose and L-proline [5,10,12].

In summary, using conditions for measurement of initial rates of sodium gradient-dependent $P_i$ transport, it was demonstrated that the increased renal $P_i$ transport in rats fed low P diet may be due, in part, to an increase in the apparent $V_{max}$ of the brush border $P_i$ transport system. In contrast, it is likely that an increase in the apparent $K_m$ of the $P_i$ transport system mediates, in part, the decrease in renal $P_i$ transport which occurs when low P diet rats are fasted. The increase in the apparent $V_{max}$ of brush border $P_i$ transport during adaptation to low P diet could occur either by an increase in the number of $P_i$ transporters, as suggested by the importance of protein synthesis [22], or through an increase in the efficiency of existing $P_i$ transporters [5]. An additional possibility which could account for altered kinetic constants is a specific change in the interaction of the $P_i$ transporters with the cotransported sodium ions.

We conclude that renal brush-border membrane $P_i$ transport can be regulated in vivo not only through changes in the apparent $V_{max}$ but also, in certain situations, through changes in the apparent $K_m$. The cellular mechanisms which mediate these changes remain to be determined.

## Acknowledgements

These studies were supported by grant AM-32148 from the National Institutes of Health. I thank Drs. William McD. Armstrong, Thomas P. Dousa, and Thomas L. Croxton for helpful discussions, and Pamela Widener for expert technical assistance.

## References

1 Dousa, T.P. and Kempson, S.A. (1982) Miner. Electrolyte Metab. 7, 113–121
2 Bonjour, J.P. and Caverzasio, J. (1984) Rev. Physiol. Biochem. 100, 161–214
3 Murer, H., Evers, C., Stoll, R. and Kinne, R. (1978) Current Problems in Clinical Biochemistry (Guder, W.G. and Schmidt, U., eds.), Vol. 8, pp. 455–462, Hans Huber, Bern
4 Barrett, P.Q., Gertner, J.M. and Rasmussen, H. (1980) Am. J. Physiol. 239, F352–F359
5 Cheng, L., Liang, C.T. and Sacktor, B. (1983) Am. J. Physiol. 245, F175–F180
6 Hammerman, M.R., Karl, I.E. and Hruska, K.A. (1980) Biochim. Biophys. Acta 603, 322–335
7 Turner, S.T., Kempson, S.A., Braun, J. and Dousa, T.P. (1982) Clin. Res. 30, 542A
8 Turner, S.T., Kiebzak, G.M. and Dousa, T.P. (1982) Am. J. Physiol. 243, C227–C236
9 Espinosa, R.E., Keller, M.J., Yusufi, A.N.K. and Dousa, T.P. (1984) Am. J. Physiol. 246, F133–F139
10 Kempson, S.A., Shah, S.V., Werness, P.G., Berndt, T., Lee, P.H., Smith, L.H., Knox, F.G. and Dousa, T.P. (1980) Kidney Int. 18, 36–47
11 Aronson, P.S. (1978) J. Membrane Biol. 42, 81–98
12 Kempson, S.A. and Dousa, T.P. (1979) Life Sci. 24, 881–888
13 Kempson, S.A., Berndt, T.J., Turner, S.T., Zimmerman, D., Knox, F.G. and Dousa, T.P. (1983) Am. J. Physiol. 244, R216–R223
14 Beck, J.C. and Sacktor, B. (1978) J. Biol. Chem. 253, 5531–5535
15 Hopfer, U. (1978) Am. J. Physiol. 234, F89–F96
16 Turner, R.J. and Moran, A. (1982) Am. J. Physiol. 242, F406–F414

17 Lang, R.P., Yanagawa, N., Nord, E.P., Sakhrani, L., Lee, S.H. and Fine, L.G. (1983) Am. J. Physiol. 245, F263–F271
18 Fersht, A. (1985) Enzyme Structure and Mechanism, 2nd Edn. p. 107, Freeman, New York
19 Burckhardt, G., Stern, H. and Murer, H. (1981) Pfluegers Arch. 390, 191–197
20 Brunette, M.G., Beliveau, R. and Chan, M. (1984) Can. J Physiol. Pharmacol. 62, 229–234
21 Dowd, J.E. and Riggs, D.S. (1965) J. Biol. Chem. 240 863–869
22 Shah, S.V., Kempson, S.A., Northrup, T.E. and Dousa, T.P (1979) J. Clin. Invest. 64, 955–966