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EFFECT OF METABOLIC ACIDOSIS ON PHOSPHATE TRANSPORT BY THE RENAL BRUSH-BORDER MEMBRANE

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Metabolic acidosis produces a phosphaturia which is independent of parathyroid hormone or dietary phosphorus intake. To study the underlying mechanism, inorganic phosphate (P_i) and glucose transport were studied in brush-border membrane vesicles prepared from the renal cortex of parathyroidectomized rats gavaged for three days with either 7.5 ml of 1.6% NaCl (control) or 1.5% NH_4Cl (acidosis). At killing, blood pH and plasma bicarbonate were 7.36 ± 0.01 and 21.8 ± 0.8 mequiv./l, respectively, in control and 7.12 ± 0.03 ($P < 0.01$) and 11.1 ± 1.2 ($P < 0.01$) in acidotic rats. Serum P_i was similar in both groups, while 24 h urine P_i excretion was higher in the acidotic group ($P < 0.01$). Peak sodium-dependent uptake of P_i, measured after 1.5 min of incubation, was higher in controls than acidotic rats (4442 ± 464 vs. 2412 ± 259 pmol/mg protein, $P < 0.01$), whereas peak glucose uptake at 1.5 min was not significantly different between the groups. Equilibrium values for P_i and glucose uptake were similar in the two groups. K_m for P_i uptake in the control and acidotic animals were not different, 0.036 and 0.040 mM, respectively. By contrast, V_{max} was higher in controls than in the acidotic group, 3.13 vs. 1.15 nmol/mg protein per 15 s. These results suggest that metabolic acidosis directly inhibits P_i uptake by the brush border of the proximal tubule by decreasing the availability of P_i carriers of the renal brush-border membrane.

Introduction

Acute and chronic metabolic acidosis are associated with a phosphaturia which is independent of parathyroid hormone (PTH) and dietary phosphorus (P) intake [1,2]. Micropuncture studies have demonstrated that reduced phosphate (P_i) reabsorption in the proximal nephron is a critical factor in the development of the phosphaturia [3]; however, the precise mechanism whereby reduced P_i reabsorption occurs remains to be elucidated. Some studies have suggested that alterations in the luminal pH, by increasing the concentration of a less permeant species of P_i, might be important in inhibiting P_i reabsorption [2,4]. Another possibility is that metabolic acidosis might have a direct inhibitory effect on P_i transport.

The present studies were undertaken to explore further the mechanism of reduced tubular reabsorption of P_i by examining the effect of metabolic acidosis induced in vivo on P_i transport measured in vitro using renal cortical brush-border membrane vesicle. The results of these studies suggest that acidosis directly inhibits P_i transport by the brush-border membrane of the proximal tubule,

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

probably by reducing the available P_i carriers in the membrane.

Methods

Male Sprague-Dawley rats weighing 100–139 g were surgically parathyroidectomized. Adequacy of parathyroidectomy was confirmed when serum Ca^{2+} levels fell below 7.5 mg/dl. 1 week following parathyroidectomy, the rats were divided into two groups: one group (controls) was gavaged twice daily for 3 days with 7.5 ml 1.6% NaCl containing 5 g of pulverized diet (0.3% P, 1.2% Ca^{2+}); the other group (metabolic acidosis) was gavaged with the same liquid diet but 1.5% NH_4Cl was substituted for the NaCl. In some experiments, rats were made acidotic for 3 days and then continued on NH_4Cl for an additional day or switched to the NaCl solution for 1 day.

To delineate the relationship between the onset of acidosis and change in P_i transport by brush-border membrane, rats were studied at only 2 or 16 h after the initiation of NH_4Cl or saline gavage. Rats studied 2 h after gavage were given 5 ml 5% NH_4Cl rather than the 1.5% solution. Preliminary studies showed rats were acidotic within 15 min with this regimen. Rats studied at 16 h were gavaged with 7.5 ml 1.5% NH_4Cl 16 and 8 h before study.

In the 3-day experiments, a 24 h urine was obtained from each rat on the day prior to killing for measurement of P_i and creatinine. On the morning of the experiment rats were anesthetized with intraperitoneal pentobarbital, 40 mg/kg; their abdomens were opened and blood was obtained from the abdominal aorta with a heparinized syringe for measurement of pH, p_{CO_2}, P_i, Ca^{2+} and creatinine. Following blood sampling, the kidneys were rapidly removed for preparation of brush-border membrane as described below.

Analytical methods

Inorganic phosphate was measured in both plasma and urine using a Technicon Autoanalyzer. Serum Ca^{2+} was measured using atomic absorption spectrometry (Perkin Elmer model 303, Perkin Elmer, South Pasadena, CA). Creatinine in plasma and urine was measured by a Beckman Creatinine Analyzer No. 2 (Beckman Instruments, Inc., Fullerton, CA). Blood pH and p_{CO_2} were measured on blood gas analyzer IL-213 (Instrumentation Laboratory, Lexington, MA) and plasma bicarbonate was calculated utilizing the standard Henderson-Hasselbach equation using a solubility coefficient of 0.0309 and pK_a of 6.1.

Vesicle preparation

The method used to prepare brush-border membrane vesicle was modified from that of Beck and Sacktor [5]. Briefly, the renal cortex was minced and then homogenized in 320 mM sorbitol buffered with 1 mM Hepes, pH adjusted to 7.5 with Trizma base, using a Polytron Homogenizer at 27000 rpm for 20 s and 15 strokes/specimen (1 g/8 ml). $CaCl_2$ was then added to the homogenate (final Ca^{2+} concentration, 10 mM) which was stirred for 15 min and centrifuged at $1500 \times g$ for 5 min. The resultant supernate was centrifuged at $35000 \times g$ for 20 min; the pellet was resuspended in 320 mM sorbitol and recentrifuged at $35000 \times g$ for 20 min. The final pellet was then resuspended in 320 mM sorbitol buffered with 1 mM Hepes, buffered to pH 8.5 with Trizma base to give a protein concentration of approx. 2 mg/ml. All the above steps were performed at 0–4°C.

Uptake studies

Transport studies were done using standard Millipore filtration techniques [6]. Briefly, 50 μl of the vesicle suspension were added to 100 μl of uptake solution, pH 8.5, and incubated for varying lengths of time at 22°C. The final incubation medium contained 100 mM NaCl, 0.1 mM K_2HPO_4, ^{32}P (1 μCi/tube) and 121 mM sorbitol. For glucose transport, K_2HPO_4 was replaced with 0.1 mM D-[U-^{14}C]glucose (1 μCi/tube). The reaction was stopped by adding 800 μl of ice-cold 320 mM sorbitol immediately followed by filtering through a 0.45 μm millipore filter. After washing the filter with 4 ml of ice-cold 320 mM sorbitol, the radioactivity in the filter was counted in a Beckman liquid scintillation counter. Values obtained at time zero were considered to reflect nonspecific binding to the filter rather than transport and were subtracted from subsequent values. In each experiment brush-border membrane vesicle preparations were prepared from the control and experimental group and were studied in parallel.

Chemical analysis

Protein was measured by a protein-dye binding assay (BioRad Lab, Richmond, CA) [7]. Activities of various marker enzymes were assayed to identify and establish the purity of each brush-border membrane preparation [5,18]. The alkaline phosphatase activity was measured under zero-order kinetics after 10 min incubation using *p*-nitrophenylphosphate as substrate in a final volume of 1 ml at pH 9.5. Maltase was measured using the method of Dahlquist [9]. $(Na^+ + K^+)$-ATPase was measured using Tris-ATP as substrate with and without added Na^+ [10]. DNA was measured using the method of Burton [11], and succinic dehydrogenase and NADPH-cytochrome *c* reductase were measured using the methods of Pennington [12] and Sottocasa et al. [13], respectively.

With our preparation, alkaline phosphatase in brush-border membrane fractions showed an enrichment of 8.7 ± 0.7 in control rats and 9.0 ± 0.7 in acidotic rats when compared to the initial homogenate of renal cortex: these values were not significantly different. Enrichment factors for maltase were 11.1 ± 0.8 in control rats and 10.8 ± 0.6 in the acid group ($P > 0.20$). Thus, metabolic acidosis of 3 days duration did not appear to effect the preparation of the brush border membrane itself. $(Na^+ + K^+)$-ATPase, succinic dehydrogenase, NADPH-cytochrome *c* reductase and DNA were barely detectable in the brush-border membrane fractions. These results indicate a high enrichment of our brush-border membrane preparations with little contamination by basolateral membranes, mitochondria, endoplasmic reticulum or nuclear material. The fraction of alkaline phosphatase present in brush-border membrane was 22–24% of that in the total homogenate with no difference between the control and acid groups. Differences between groups was assessed using the Student's paired *t*-test [14]. Unless otherwise indicated, the difference was considered significant when a *P* value was less than 0.01.

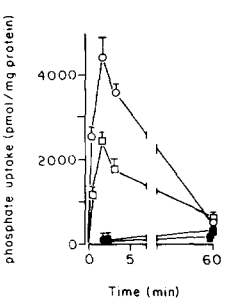

Fig. 1. (Left.) Phosphate uptake by brush-border membrane vesicles from control and acidotic rats. The vesicles were incubated at 22°C in uptake solution of 0.1 mM phosphate, 100 mM NaCl or 100 mM KCl and buffered with Tris-Hepes (pH 8.5). Values are mean ±S.E., $n = 5$. Sodium-dependent phosphate uptake was significantly decreased in the acidotic rats (□) compared to controls (○) at both 15 s and 1.5 min of incubation ($P < 0.01$). The uptake at 60 min was similar in the two groups. Phosphate uptake values obtained when vesicles were incubated in Na^+-free KCl solution (solid symbols) were decreased to similar levels in each group.

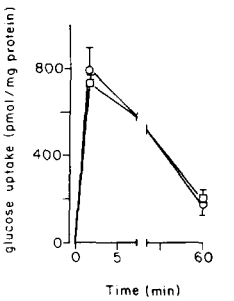

Fig. 2. (Right.) Glucose uptake by brush-border membrane vesicles from control (○) and acidotic (□) rats. The vesicles were incubated at 22°C in uptake solution of 0.1 mM glucose/100 mM NaCl and buffered with Tris-Hepes (pH 8.5). Values are mean ±S.E., $n = 5$. Sodium-dependent glucose uptake was similar in the two groups at 1.5 min of incubation.

Results

The plasma P_i, Ca^{2+}, creatinine and bicarbonate and blood pH and p_{CO_2} for the 3-day experiments are shown in Table I. Plasma Ca^{2+} and P_i were similar in the acidotic and control

TABLE I

BLOOD CHEMISTRIES OF CONTROL AND ACIDOTIC RATS AFTER 3 DAYS OF GAVAGE

Values represent mean ±S.E.; $n = 5$ with three rats per group. Blood was obtained at killing.

Groups	Ca^{2+} (mg/dl)	P (mg/dl)	Creatinine (mg/dl)	pH	p_{CO_2} (mmHg)	HCO_3^- (mequiv./l)
Control	7.64±0.32	6.25±0.41	0.4±0.05	7.36±0.01	39.4±0.9	21.8±0.8
Acidosis	8.37±0.24	5.76±0.36	0.5±0.08	7.12±0.03 [a]	33.2±2.5	11.1±1.2 [a]

[a] Significantly different from control, $P < 0.01$.

groups, while blood pH and plasma bicarbonate were significantly lower in the acidotic rats. By day 2 of the experiment, urine P_i excretion was higher in the acidotic group than controls: 12.7 ± 2.1 and 4.1 ± 1.1 mg/24 h, respectively.

The uptake of P_i by brush-border membrane vesicle in the control and acidotic groups is shown in Fig. 1. The initial part of the P_i uptake curve represents the Na^+-dependent portion and has a typical 'overshoot' pattern which is absent when NaCl is replaced with KCl [15]. As can be seen, acidosis blunted the degree of 'overshoot' but did not affect the uptake when KCl was substituted for NaCl. Moreover, the uptake of P_i at 60 min of incubation did not differ between the groups. Therefore, acidosis specifically affected the Na^+-dependent portion of the P_i uptake curve. Uptake of glucose in both groups is depicted in Fig. 2. In contrast to the observation with P_i, both early Na^+-dependent uptake of glucose and 60 min equilibrium values were similar in both groups.

As shown in Fig. 3, the uptake of P_i by the brush-border membrane vesicle displayed Michaelis-Menten characteristics with saturation occurring at approx. 0.5 mM P_i. Fig. 4 shows a Lineweaver-Burk plot of the data. The values used were calculated by subtracting P_i uptake at 15 s in the absence of an Na^+ gradient from that obtained in the presence of an Na^+ gradient. V_{max} was 3.13 nmol/mg protein per 15 s in the control group and 1.15 in the acidotic group; K_m was 0.036 mM in controls and 0.040 in the acidotic animals.

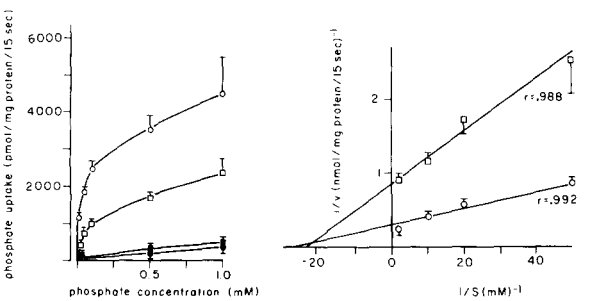

Fig. 3. (Left.) Effect of acidosis on initial velocity of phosphate uptake. Vesicles were incubated at 22°C for 15 s in an uptake solution of 100 mM NaCl (open symbol) or 100 mM KCl (closed symbols) and Tris-Hepes (pH 8.5). Values are mean ± S.E., $n = 5$. Uptake shows saturation kinetics and was lower at all P_i concentrations in the acidotic group (□) compared to the control group (○), $P < 0.01$. Uptake values were similar in each group when vesicles were incubated in KCl instead of NaCl solution.

Fig. 4. (Right.) A Lineweaver-Burk plot of initial velocity of Na^+-dependent phosphate uptake for both acidotic (□) and control (○) groups, $n = 4$. Phosphate concentration varied from 0.02 to 0.5 mM.

TABLE II

ARTERIAL pH, HCO_3 AND BRUSH-BORDER MEMBRANE UPTAKE OF PHOSPHATE AND GLUCOSE AT 2 AND 16 H AFTER THE INDUCTION OF ACIDOSIS BY NH_4Cl GAVAGE

Data are mean ± S.E., $n = 3$ with 2–4 rats per group. Vesicles were incubated at 22°C in medium containing 0.1 mM phosphate, 100 mM NaCl and Tris-Hepes (pH 8.5).

	Arterial:		Vesicle uptake (pmol/mg protein)			
	pH (units)	HCO_3^- (mequiv./l)	Phosphate		Glucose	
			1.5 min	60 min	1.5 min	60 min
2 h						
Control	7.42 ± 0.022	24.52 ± 1.21	1582.0 ± 135.3	329.0 ± 40.8	687.5 ± 138.5	228.0 ± 61.3
Acidosis	7.17 ± 0.056 [b]	12.40 ± 1.42 [b]	1604.3 ± 119.2	324.7 ± 53.6	625.0 ± 128.0	198.5 ± 63.5
16 h						
Control	7.43 ± 0.031	28.04 ± 1.96	1838.3 ± 118.1	402.7 ± 61.2	630.5 ± 116.8	271.3 ± 40.6
Acidosis	7.18 ± 0.034 [b]	15.60 ± 1.04 [b]	1471.3 ± 97.0 [a]	451.6 ± 100.3	628.0 ± 176.1	244.6 ± 51.1

[a] $P < 0.01$ compared to appropriate control.
[b] $P < 0.005$ compared to appropriate control.

In rats made acidotic for 3 days and then allowed to recover for 1 day, serum pH and HCO_3^- returned to normal within the 24-h recovery period (7.48 ± 0.025 units and 30.89 ± 1.60 mequiv./l, respectively). On the other hand, P_i uptake after 1.5 min incubation remained low in the recovery group, being 2126.2 ± 102.3 pmol/mg protein in the acidotic rats and 1997.1 ± 258.9 in recovered rats.

The specific activity of alkaline phosphatase in renal cortical homogenate decreased after 3 days of NH_4Cl gavage from 65.5 ± 6.2 nmol/min per mg protein in control animals to 51.1 ± 8.4 in acidotic rats ($P < 0.01$). Maltase activity remained unchanged, being 70.6 ± 5.7 nmol/min per mg protein in control rats and 64.9 ± 8.0 in acidotic rats.

The arterial pH and HCO_3^- and the uptake values for P_i and glucose by brush-border membrane vesicles prepared from rats studied at earlier time periods are shown in Table II. Arterial pH and HCO_3^- were both decreased with NH_4Cl gavage at 2 and 16 h while P_i uptake by brush-border membrane vesicles were lower only at 16 h; glucose uptake remained unchanged. Uptake values for P_i are lower in general in the earlier time periods than in the 3 day experiments because dietary P was changed from 0.6% to 0.3% only at the start of experiments. Thus, the rats in the more acute studies probably did not equilibrate the 0.3% P diet.

Discussion

The present results clearly show that chronic metabolic acidosis directly reduces P_i uptake by the brush-border membrane vesicle of the rat proximal tubule. This conclusion is based upon the demonstration that metabolic acidosis produced in vivo resulted in blunting of the early Na^+-dependent uptake of P_i by the brush-border membrane vesicle measured in vitro. Changes in P_i transport were preceded by the lowering of arterial pH and could be demonstrated within 16 h after NH_4Cl gavage. That the effect represented a specific action on P_i uptake rather than a general depression of Na^+-dependent transport is supported by the lack of any effect of acidosis on Na^+-dependent glucose uptake. Previous studies had suggested that metabolic acidosis might reduce P_i uptake by increasing the concentration of a less permeant P_i species in the tubular lumen [4,15]. Since in the present study, uptake by brush-border membrane vesicle of acidotic and control rats was measured at the same incubation pH, it is unlikely that changes in the ratio of divalent to monovalent P_i played any role in the in vitro depression of P_i reabsorption. Furthermore, in recovery studies, P_i uptake remained suppressed after acid-base parameters had normalized.

Kinetic analyses of the data indicate that acidosis decreased the V_{max} of P_i uptake. In the present study, initial rates were measured at 15 s of incubation, and hence the value obtained for V_{max} and K_m are probably only relative estimates of their true values. However, it appears reasonable to infer that the reduction in P_i uptake may be effected via a reduction in the availability of carriers in the brush-border membrane. These results are similar to those seen with other factors shown to affect P_i uptake by the brush-border membrane in vitro such as PTH and changes in dietary P content; i.e., changing V_{max} but not changing K_m [16,17]. The exact reason for the decrease in V_{max} is not clear. The enzyme alkaline phosphatase may play an important role in P_i transport by the brush border of the proximal tubule [8,18,19]. Indeed, in several studies, changes in P_i transport were associated with a concomitant change in alkaline phosphatase activity [8,18], and it was suggested that the alteration in P_i transport is related to the change in alkaline phosphatase activity [8,18,19]. Since in the present study both alkaline phosphatase activity and P_i transport were reduced by chronic metabolic acidosis, it is tempting to speculate that the decrease in P_i transport was related to the reduction in alkaline phosphatase activity. However, recent data demonstrating that a change in the P_i transport may occur prior to or without alterations in alkaline phosphatase activity have cast doubt on the critical role of this enzyme in P_i transport [17,20–22]. Therefore, it is unlikely that the reduced alkaline phosphatase activity found in the present study is critical to alterations in the P_i transport by the brush-border membrane vesicle caused by metabolic acidosis. The V_{max} for P_i uptake by brush-border membranes could also be decreased in acidotic rats by an increase in the

rate of dissipation of the Na^+ gradient. The lack of change in Na^+-dependent glucose uptake in acidotic rats is indirect evidence that changes in dissipation of the Na^+ gradient cannot fully explain the findings in this study. However, direct measurement of the Na^+ uptake, per se, is needed to exclude this possibility fully.

It has been recently suggested that an increase in the NAD concentration in the cytosol of proximal tubule cells may be a common factor responsible for an inhibition of P_i transport across the brush-border membrane [23]. According to this hypothesis, most of the known stimuli causing phosphaturia and depressed P_i uptake by the brush-border membrane are associated with enhanced gluconeogenesis, a metabolic process which is present in proximal tubule and which may be associated with an increased NAD in the cytosol; these stimuli include PTH, cyclic AMP and fasting [23,24]. Conversely, phosphate deprivation, a condition associated with avid renal P_i reabsorption and an enhanced P_i uptake by the brush-border membrane, is accompanied by suppression of gluconeogenesis [23]. It has been shown that NAD is a potent inhibitor of P_i uptake by the brush-border membrane when added in vitro [23]. Moreover, the in vivo administration of nicotinamide, which increases the NAD content of the kidney, causes a brisk phosphaturia and suppression of P_i uptake by the brush-border membrane vesicle [23]. If this hypothesis is correct, changes in P_i uptake of the brush-border membrane in metabolic acidosis as shown in the present study may be causally related to an enhanced gluconeogenesis, a well-known phenomenon to occur in the kidney with metabolic acidosis [25–27].

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