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INTERACTIONS BETWEEN Na^+-DEPENDENT UPTAKE OF D-GLUCOSE, PHOSPHATE AND L-ALANINE IN RAT RENAL BRUSH BORDER MEMBRANE VESICLES

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D-Glucose decreases phosphate reabsorption in rat proximal tubule. It is also postulated that some amino acids interact with phosphate reabsorption. To investigate the mechanism of these interactions, phosphate, D-glucose and L-alanine transport kinetics were measured in brush border membrane vesicles isolated from superficial rat kidney cortex by the calcium precipitation technique. At pH 7.4, Na^+-dependent phosphate transport was inhibited in the presence of either D-glucose (39 mM) or L-alanine (2.4 mM). In this model, with D-glucose or with L-alanine the V value of the phosphate uptake was decreased, whereas the apparent K_m for the phosphate uptake was not affected. However, some inhibition of phosphate transport was observed in the presence of L-glucose, D-alanine or D-glucose after phlorizin preincubation. A 30% Na^+-dependent L-alanine (0.1 mM) transport inhibition was observed in the presence of 5 mM phosphate. D-Glucose (1 mM) was also inhibited by 20% when 5 mM phosphate was added to incubation medium. According to several authors, in our model, D-glucose decreased the L-alanine transport and vice versa. Moreover, when the membrane potential was abolished, a clear inhibition of D-glucose by L-alanine persisted. These multiple interactions could be explained by the accelerated dissipation of the Na^+ gradient insofar as the rate of the Na^+ uptake was increased with D-glucose, L-alanine or phosphate and since the absence of variations in membrane potential did not suppress these inhibitions.

Introduction

In a series of studies performed on the dog, it has been established that the infusion of D-glucose induces a decrease in renal maximal tubular reabsorption of inorganic phosphate [1–3]. Recently, it has been shown in the case of the rat that this inhibitory effect of glucose is located in the proximal tubule [4]. It was also demonstrated that some neutral amino acids interact with renal phosphate reabsorption [5,6]. On the other hand, the intestinal glucose transport system is also altered by neutral amino acids [7–10] and vice versa [11,12]. In 1960, Crane [13] formulated the theory that organic solute transport is coupled to ion active transport. In recent years, a sodium gradient dependency has been reported for intestinal and renal transport of glucose [14–16], amino acids [17–19] and phosphate [20,21] from studies using isolated plasma membrane fractions. The aim of the present work was to investigate the role of the Na^+ gradient in the interactions between D-glucose, phosphate and L-alanine transports. Accordingly phosphate, D-glucose and L-alanine transport kinetics were measured in brush border membrane vesicles isolated from superficial rat kidney cortex.

A preliminary report of this work was presented in abstract form to the 2nd International Congress on Cell Biology, Berlin, 1980.

Materials and Methods

Preparation of brush border membrane vesicles

Rat renal brush border membranes were prepared

according to the calcium chloride precipitation method [22]. Kidneys from 6–10 adult male Sprague-Dawley rats were quickly removed, washed in ice-cold 0.01 M mannitol buffered with 2 mM Tris-HCl, pH 7.1, then decapsulated. The external cortex were sliced and suspended in the same buffer to obtain a ratio of 1 g tissue to 10 ml medium. This preparation was homogenized using a polytron (type 10 ST, Kinematica GmbH, Lucerne, Switzerland) at the minimum speed by pulsing twice for 1 min each time.

To this homogenate was added 1 M $CaCl_2$ solution to the final concentration of 10 mM; the mixture was stirred in an ice-bath for 15 min, diluted with mannitol buffer containing 10 mM $CaCl_2$ (1 : 1) and centrifuged at $500 \times g$ for 12 min in an RC5B Sorvall refrigerated centrifuge. The supernatant was carefully decanted and kept on ice. The pellet was re-suspended in the mannitol buffer (the same volume used to suspend cortices) and submitted to a new homogenization with the polytron at the minimum speed but for only 1 min. This second homogenate was treated as was the first. But the second pellet was discarded and both supernatants were centrifuged at $22\,000 \times g$ for 12 min. The pelleted material was treated as described by Evers [23] for the pellet II except that the supernatant 3 was centrifuged at $30\,000 \times g$. The quality of the final brush border membrane vesicle preparation was evaluated by enzyme markers; specific activity of alkaline phosphatase was 1.26 ± 0.07 μmol transformed substrate/mg protein per min at 37°C, representing an enrichment relative to the cortex homogenate of 12.12 ± 0.44 $n = 25$. Specific activity of maltase was 11.63 ± 0.78 μmol/mg protein per min at 25°C; the enrichment was 14.6 ± 0.6 ($n = 18$).

Transport measurements

Uptake of phosphate, D-glucose and L-alanine by the brush border membrane vesicles were measured by the Millipore filtration technique [14]. The intravesicular medium contained 100 mM mannitol and 20 mM Hepes-Tris (pH 7.4). The incubation solution was prepared from the intravesicular medium by addition of 100 mM NaCl and the radioactive transported substrate. This Na^+ gradient condition was used by numerous authors [20,18,30,31]. In some experiments the NaCl concentration was only 12.5 mM. In this study, 10 μl vesicle suspension (70–120 μg proteins) was incubated in 50 μl incubation medium at room temperature. After 15 s, 20 μl of these vesicles were diluted in 1 ml ice-cold stop solution (100 mM mannitol, 150 mM NaCl, 20 mM Hepes-Tris, (pH 7.4), 0.1 mM phlorizin or 10 mM arsenate) for glucose or phosphate uptake, respectively, and unlabeled substrate at 20-times the radioactive substrate concentration). The membranes were immediately collected on a Millipore filter (HAWP 025, 0.45 μM) and the filters were washed with 5 ml of the same ice-cold stop solution. All incubations were carried out in triplicate. For the time-course experiments, 30 μl vesicle suspension were mixed in 120 μl incubation medium. Each time, 20 μl of these suspensions were filtered as above. In some cases, 15 μl highly concentrated vesicle suspension (25 mg protein/ml) were mixed with 300 μl incubation medium.

Chemicals

D-[^{14}C]Glucose, D-[^{3}H]glucose and L-[^{3}H]alanine, were obtained from the Commissariat à l'Energie Atomique, Saclay, France; [^{32}P]orthophosphate and ^{22}NaCl were from the Radiochemical Centre, Amersham, U.K. Phloridzin was purchased from Serva Feinbiochemica, Heidelberg, F.R.G. All solutions were filtered before use.

Results

Phosphate uptake inhibition by D-glucose

The uptake of 0.5 mM phosphate by brush border membrane vesicles during incubation for different lengths of time is illustrated in Fig. 1. The presence of a Na^+ gradient induced a marked stimulation of the phosphate uptake. The initial rate was 5.24 ± 0.63 nmol/mg protein per 15 s. The accumulation of the phosphate in the brush border membrane vesicles was maximal at 30 s. Afterwards, the amount of phosphate in the vesicles decreased and at 90 min the intravesicular content reached an equilibrium value. In the presence of 39 mM glucose (this concentration is about 15-times the apparent K_m value for the D-glucose transport in our system (2.4 ± 0.5 mM, $n = 4$)), the initial rate of phosphate uptake was reduced at 3.57 ± 0.18 nmol/mg protein per 15 s. The intravesicular phosphate content remained lower until the final level of the phosphate uptake was reached. The equilibrium value was identical to those obtained in

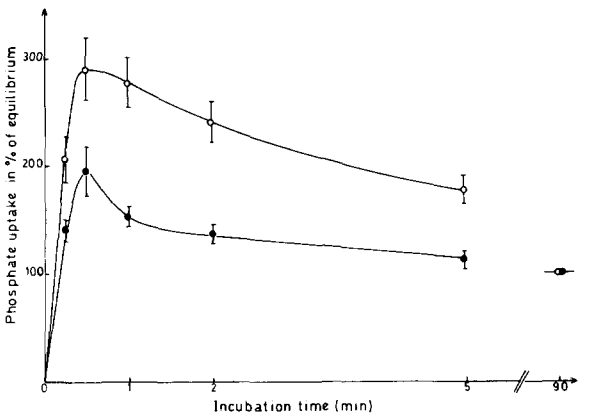

Fig. 1. Uptake of phosphate by brush border membrane vesicles. The vesicles contained 100 mM mannitol, 20 mM Hepes-Tris (pH 7.4) and were incubated in a medium containing in addition 0.5 mM [^{32}P] phosphate, 100 mM NaCl and 39 mM mannitol (control) (○) or 39 mM D-glucose (●). Values are expressed as percentages of the phosphate taken up by vesicles after 90 min of incubation. Values are means of four experiments ± S.E.

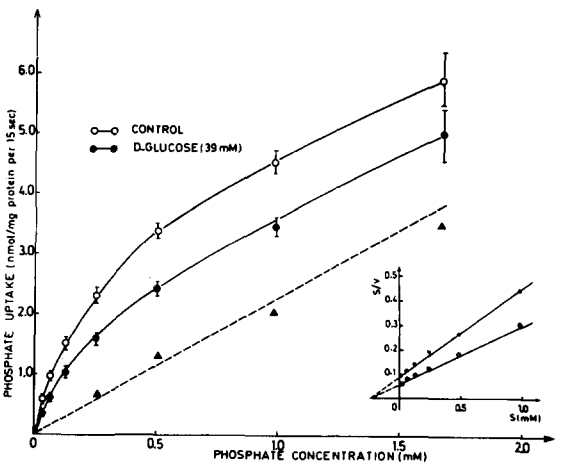

Fig. 2. Influence of phosphate concentration and D-glucose on phosphate uptake by brush border membrane vesicles. The incubation medium contained 100 mM mannitol, 20 mM Hepes-Tris, 100 mM NaCl, 39 mM mannitol (control) (○), 39 mM D-glucose (●). To measure the Na^+-independent uptake, the incubation medium contained only 300 mM mannitol, 20 mM Hepes-Tris (pH = 7.4) and 39 mM D-glucose (dotted line, ▲- - - - -▲). The inset shows data plotted according to Hanes C.S. after removal of the Na^+ independent movement component. The results are the means of five experiments ± S.E. The slope = $1/V$; the intersection with abscissa = $-K_m$.

the control experiments: 2.53 ± 0.07 vs. 280 ± 0.11 nmol/mg protein (n = 4).

The kinetics of the phosphate uptake have been measured from 0.01 to 4 mM in the presence or in the absence of D-glucose at a concentration of 39 mM. The Na^+-independent influx component was estimated either by computer analysis or by experiment. In this later case the phosphate uptake was determined in an incubation medium containing 300 mM mannitol and no sodium. After removal of this component, the data were plotted (Fig. 2) according to Hanes C.S. representation: $[S]/V$ against [S] [24]. The apparent K_m value was not affected by the presence of D-glucose; the K_m values were 0.19 ± 0.04 mM in the absence of D-glucose and 0.21 ± 0.07 mM with D-glucose (n = 5). However, V was reduced to around 70% (3.24 ± 0.43 vs. 2.30 ± 0.51 nmol/mg protein per 15 s).

This inhibition was specific for D-glucose. The addition of 39 mM L-glucose did not decrease the phosphate uptake (Fig. 3A). Moreover, in the presence of 0.1 mM phlorizin, this transport was not affected by 39 mM D-glucose (Fig. 3B).

Different concentrations of D-glucose were tested. A significant inhibition of phosphate uptake was observed with 6 mM sugar and was maximal at 16 mM.

Phosphate uptake inhibition by L-alanine

D-Glucose uptake and phosphate uptake are sodium-dependent and the inhibition of phosphate uptake by D-glucose could be related to this sodium dependency. To check this possibility we have measured the uptake of phosphate in the presence of L-alanine, which is co-transported with Na^+ and stimulated by the sodium gradient.

The phosphate transport by brush border membrane vesicles was also inhibited by L-alanine (2.4 mM). This concentration of L-alanine was 10-times the value of the apparent K_m measured for the L-alanine uptake in our system (K_m = 0.22 ± 0.08 mM, n = 3). The time course of the 0.5 mM phosphate uptake shows that the phosphate intravesicular content was lower in the presence than in the absence of L-alanine (2.4 mM). At 60 min the phosphate concentration in the vesicles was the same in both cases (Fig. 4): control, 1.74 ± 0.32 nmol/mg protein; with L-alanine, 1.78 ± 0.52 nmol/mg protein. The kinetic study was performed over a wide range of phosphate

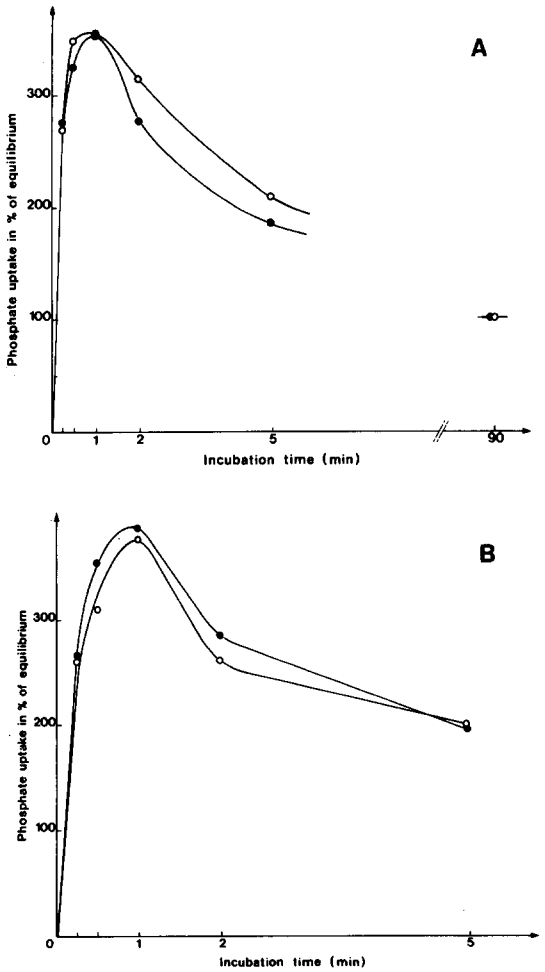

Fig. 3. The time course of the uptake of phosphate by brush border membrane vesicles in the standard incubation medium. A, with 39 mM mannitol (control) (○) and with 39 mM L-glucose (●); B, in the presence of 0.1 mM phlorizin and with 39 mM mannitol (control) (○) and with 39 mM D-glucose (●).

concentrations, from 0.01 to 4 mM with or without 2.4 mM L-alanine in the glucose. Fig. 5 shows that the apparent K_m value was not affected by the presence of L-alanine (0.17 ± 0.05 mM and 0.19 ± 0.5 mM with and without L-alanine, respectively). However, the V was reduced from 4.86 ± 0.44 to 3.54 ± 0.51 nmol/mg protein per 15 s (n = 4) (Fig. 5).

This inhibition of phosphate transport was specific for L-alanine: 2.4 mM D-alanine did not modify the phosphate uptake (Fig. 6). Inhibition by L-alanine was observed at a concentration as low as 0.2 mM and was maximal at 1.5 mM.

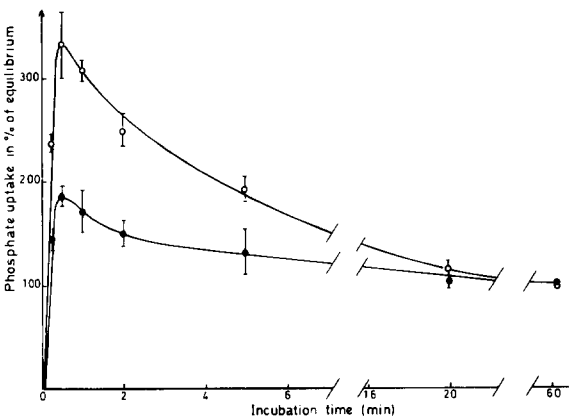

Fig. 4. Uptake of phosphate by brush border membrane vesicles in the standard incubation medium in the presence (●) or in the absence (control) (○) of 2.4 mM L-alanine. Values are expressed as percentages of the phosphate taken up by vesicles after 60 min of incubation. Values are means of two experiments ± S.E.

D-Glucose uptake inhibition

Since phosphate uptake was clearly inhibited by D-glucose, it was of interest to check whether D-glucose uptake could be modified by phosphate.

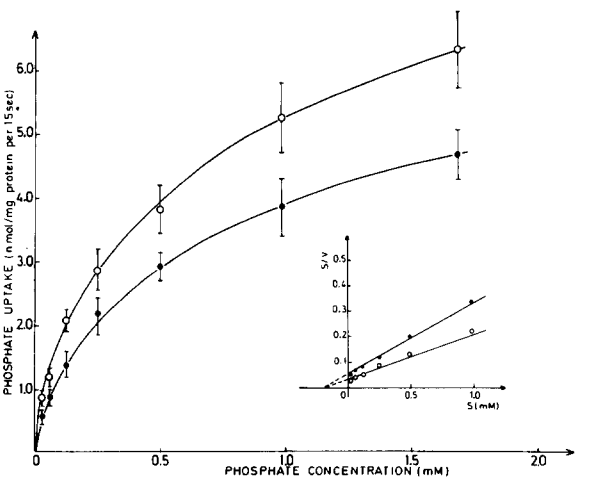

Fig. 5. Influence of phosphate concentration and L-alanine on phosphate uptake by brush border membrane vesicles in the standard incubation medium with (●) or without (control) (○) 2.4 mM L-alanine. The inset represents data plotted according to Hanes C.S. after removal of the Na^+-independent phosphate influx components. The results are the means of four experiments. The slope = $1/V$, the intersection with abscissa = $-K_m$.

The addition of phosphate to a final concentration of 5 mM in the incubation medium induced a significant decrease in the initial rate of 1 mM D-glucose uptake. According to several authors [25,7] the glucose uptake is also inhibited by L-alanine. Under our experimental conditions, 5 mM L-alanine reduced the initial rate of glucose uptake by 22.5% (Table IA).

L-Alanine uptake inhibition

The initial rate of the L-alanine uptake was decreased by 15 mM D-glucose and by 5 mM phosphate. These two kinds of inhibition were similar in magnitude: around 35% (Table IB).

Influence of the Na^+ accompanying anion

Phosphate, D-glucose and L-alanine uptake were measured in the presence of an NaCl gradient or an Na_2SO_4 gradient. The initial rates of 1 mM D-glucose and 0.1 mM L-alanine uptake were reduced to 52.2% ± 3.1 ($n = 5$) and 51.4% ± 3.4 ($n = 2$), respectively, if the Na^+ accompanying anion were SO_4^{2-}. However, in the presence of SO_4^{2-}, the initial rate of 0.5 mM phosphate uptake was not modified or perhaps slightly increased (112.8% ± 2.2, $n = 2$).

Effect of the membrane potential on the L-alanine inhibition of the phosphate and D-glucose uptake

The inhibition of the initial rate of phosphate and glucose influx by 2.5 mM L-alanine was determined in the absence or in the presence of a membrane potential. In these experiments, 100 mM KCl and valinomycin (about 10 μg/mg protein) were added to the intravesicular and the incubation medium, providing a very high permeability to K^+ and a complete dissipation of the $\Delta\psi$. In these conditions, in the presence of only an Na^+ chemical gradient, the uptake of phosphate and of D-glucose was always inhibited by L-alanine (Table II).

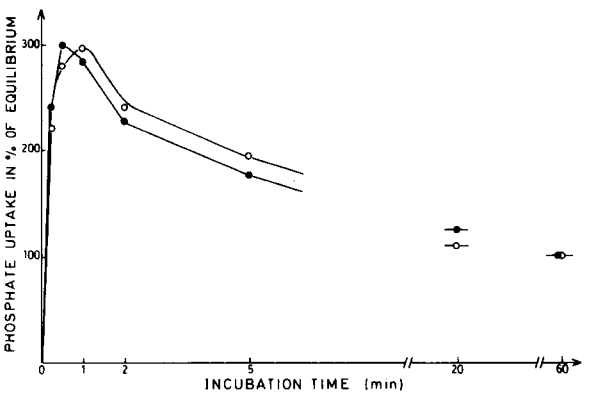

Fig. 6. The time course of the uptake of phosphate by brush border membrane vesicles in the standard incubation medium with (•) or without (control) (○) 2.4 mM D-alanine.

Na^+ uptake

The uptake of Na^+ was measured in the absence of transported substrates in the presence of 5 mM phosphate, 2.4 mM L-alanine or 20 mM D-glucose, i.e., at the inhibitory concentrations. The results show that by 15 s there was an increment of the rate of Na^+ uptake when Na^+-coupled transport was present (Table III). In addition to the basal Na^+ influx (52.3 ± 4.1 nmol/mg protein per 15 s, due to unspecific movements), about 16 nmol/mg protein was accumulated in the vesicles at 15 s in the presence of

TABLE I

INTERACTIONS BETWEEN D-GLUCOSE AND L-ALANINE: INFLUENCE OF PHOSPHATE ON THEIR UPTAKE RATES

Values are means ± S.E.; n = number of experiments.

Inhibitor	n	Control		With-inhibitor	Percentage of control
A. 1 mM D-glucose uptake (nmol/mg protein per 15 s)					
L-Alanine (5 mM)	4	2.32 ± 0.34	$P < 0.025$	1.81 ± 0.27	77.5 ± 3.9
Phosphates (5 mM)	5	2.79 ± 0.37	$P < 0.005$	2.17 ± 0.33	72.2 ± 2.9
B. 0.2 mM L-Alanine uptake (pmol/mg protein 15 s)					
D-Glucose (10 mM)	4	349.2 ± 61.3	$P < 0.05$	216.1 ± 41.0	63.6 ± 8.5
Phosphates (5 mM)	4	359.6 ± 90.9	$P < 0.05$	233.2 ± 57.8	66.2 ± 3.8

TABLE II

INFLUENCE OF THE MEMBRANE POTENTIAL ($\Delta\psi$) ON THE L-ALANINE INHIBITION OF THE PHOSPHATE AND D-GLUCOSE UPTAKE

The results are expressed in percentages of the substrate uptake at 15 s in the presence of an NaCl gradient. $-\Delta\psi$ (without membrane potential i.e., KCl (100 mM) and valinomycin (10 μg/mg protein) inside and outside. $+\Delta\psi$: with membrane potential, in the absence of KCl and valinomycin. Values are means ± S.E.; n = number of experiments. n.s., not significant.

	Relative rate of substrate uptake (%)			
	D-Glucose (1 mM)		Phosphates (0.5 mM)	
	$+\Delta\psi$	$-\Delta\psi$	$+\Delta\psi$	$-\Delta\psi$
Control	100	100	100	100
+L-Alanine (2.4 mM)	78.4 ± 3.9 n.s. (n = 3)	80.1 ± 2.1 (n = 3)	77.8 ± 4.2 n.s. (n = 2)	83.1 ± 7.0 (n = 2)

D-glucose or L-alanine and 24 nmol/mg protein in the presence of phosphate (n = 4).

The time-course experiment was carried out with a reduced NaCl gradient (12.5 mM in the incubation medium). Fig. 7 shows that in the presence of either 10 mM D-glucose or 5 mM L-alanine, the sodium uptake increase was very high at 15 s, and persisted until 2 min, then the equilibrium value was reached at around 20 min.

Effect of D-glucose, L-alanine and arsenate preloading on the phosphate uptake

As suggested by the kinetic analyses, the phosphate uptake inhibition by D-glucose or L-alanine, is non-competitive. The mechanism of the D-glucose and L-alanine effect on phosphate transport was defined by phosphate countertransport experiments. The vesicles were equilibrated in 100 mM NaCl and preloaded with 5 mM D-glucose, 5 mM L-alanine or 5 mM arsenate. Table IV shows that only those vesicles preincubated with the competitive inhibitor [20] arsenate showed any accelerated uptake of phosphate.

TABLE III

INFLUENCE OF D-GLUCOSE, L-ALANINE AND PHOSPHATES ON Na^+ UPTAKE

The Na^+ uptake was measured at 15 s with or without transported substrate. 100% represents the value of Na^+ uptake without substrate. Values are means ± S.E.; n = number of experiments.

	Relative rate of sodium uptake (%)
+D-Glucose (20 mM)	130.7 ± 5.0 (n = 4)
+L-Alanine (2.4 mM)	132.6 ± 1.0 (n = 3)
+Phosphates (5 mM)	144.8 ± 27.7 (n = 3)

Discussion

In the present study, carried out in a cell-free system derived from renal brush border vesicles, we con-

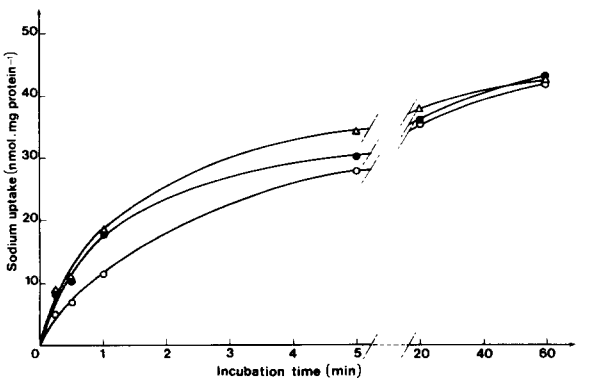

Fig. 7. Time course of the Na^+ uptake by brush border membrane vesicles. The incubation medium was 100 mM mannitol, 12.5 mM NaCl, 20 mM Hepes-Tris (pH 7.4) and 10 mM mannitol (○, control) or 10 mM D-glucose (●) or 5 mM L-alanine (△).

TABLE IV

EFFECT OF PRELOADING VESICLES WITH D-GLUCOSE, L-ALANINE AND ARSENATE ON PHOSPHATE UPTAKE

Initial rates of phosphate uptake (0.15 mM) are expressed in pmol/mg protein/15 s. Vesicles were preloaded with 100 mM mannitol, 100 mM NaCl, 20 mM Hepes-Tris (pH 7.4) and 5 mM mannitol, D-glucose, L-alanine or arsenate. The incubation medium was composed of 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.4) and [^{32}P]phosphate (0.15 mM). Gramicidin D (around 10 μg/mg protein) was added just before the uptake measurement. Experiments were carried out simultaneously in triplicate from each vesicle preparation.

Expt.	Preincubation medium			
	Mannitol (5 mM)	D-Glucose (5 mM)	L-Alanine (5 mM)	Arsenate (5 mM)
190381	360.5	384.4	352.4	785.3
020481	230.8	271.7	269.8	393.5

firm many findings on the phosphate transport in the intact kidney [1–5]. Moreover, the use of this kind of system allowed us to clarify the mechanism of the inhibition of phosphate transport by D-glucose, inhibition suggested by the data of Hoffman et al. [10]. Kinetic analysis revealed that D-glucose reduced the V of phosphate uptake without significant modification of the K_m value. As observed with D-glucose, the neutral amino acid L-alanine decreased the phosphate influx, but only by a modification of the V value. Thus D-glucose and L-alanine were non-competitive inhibitors of the phosphate transport system. The fact that L-glucose and D-alanine do not alter the rate of the phosphate uptake indicates that the inhibitions of phosphate influx are specific to D-glucose and L-alanine, which are the Na^+-cotransported enantiomers [15,25]. Moreover, when the vesicules were preincubated with phlorizin, D-glucose did not modified phosphate transport, indicating that it is the translocation of the D-glucose and not its presence in the incubation medium which is responsible for the inhibition. Finally, these observations and the lack of effect of D-glucose or L-alanine preloading on phosphate uptake suggest that phosphate, D-glucose and L-alanine molecules do not share the same binding site on the carrier in the brush border membrane. Two hypotheses could be put forward to explain the phosphate uptake inhibition: a modification of membrane potential and/or a variation of the electrochemical sodium gradient induced by D-glucose or L-alanine. It has been demonstrated that the D-glucose and L-alanine transport processes are electrogenic [25,26]. This is very likely the case in our experimental conditions, since the presence of an Na_2SO_4 gradient extravesicular intravesicular (which tends to make the membrane less negative on the inside) decreased strongly (from about 50%) the D-glucose and L-alanine uptake. In contrast, the Na^+-gradient-dependent uptake of phosphate was not affected (or slightly increased) when the less permeant anion was substituted for Cl^- in the salt gradient.

This later result strongly suggests that the phosphate uptake was electroneutral (at least at pH 7.4), i.e., this cotransport accross the microvillus membrane was not associated with the net transfer of electrical charge. Moreover, it appeared that the inhibition of phosphate uptake by L-alanine was independent of the presence of a membrane potential. In view of these results, it is reasonable to assume that in the present experiments the inhibition of Na^+-dependent phosphate uptake by D-glucose and L-alanine is not due to a modification of the membrane potential. Another way to explain the inhibition of phosphate uptake could be by transport-dependent localized changes of the Na^+ concentration in the vicinity of the brush border membrane. When D-glucose or L-alanine was added to the incubation medium, these molecules, as was the case for phosphate, were cotransported with Na^+, thus involving an 30–40% increase of the initial rate of the sodium uptake. In this case, there is a more rapid dissipation of the Na^+ gradient, reducing the energy utilized for the increase of phosphate in brush border membrane vesicles.

The interaction between D-glucose transport and amino acid transport (which is confirmed in our system) has given rise to several hypotheses [11,27–29]. The more attractive describes the transport interaction being brought about by an electrical coupling [7] which is partially the driving force for this active transport. But our findings show that a clear inhibition of D-glucose by L-alanine persists in the absence of variations of the membrane potential. Probably in these experiments the more rapid dissipation of the Na^+ gradient could be implicated. On the other hand, the observation of the L-alanine or D-glucose trans-

port inhibition by phosphate could be explained by the same mechanism, since the phosphate uptake is non-electrogenic under our conditions at pH 7.4 and of course the addition of phosphate to the incubation medium did not modify the membrane potential. Finally, some variations of the membrane potential were not absolutely required to explain the observed interactions between transport of L-alanine, D-glucose and phosphate. The more rapid dissipation of the sodium gradient could be involved in all cases observed.

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