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Sodium-dependent glucose transport by cultured proximal tubule cells

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The cotransport of sodium ion and α-methyl glucose, a non-metabolized hexose, was studied in rabbit proximal tubule cells cultured in defined medium. The rate of uptake of α-methyl glucose shows saturation kinetics, in which K_m, but not V_{max}, is dependent upon the Na^+ concentration in the medium. The transport system was found to be of the high-affinity type, characteristic of the straight portion of the proximal tubule. Analysis of the rates of initial uptake within the context of a generalized cotransport model, suggests that two Na^+ ions are bound in the activation of the hexose transport. The steady-state level of accumulation of α-methyl glucose also depends upon sodium concentration, consistent with the initial rate findings. The uptake of α-methyl glucose is inhibited by other sugars with the relative potencies of D-glucose > α-methyl glucose > D-galactose = 3-*O*methylglucose. L-Glucose, D-fructose, and D-mannose show no inhibition. Phlorizin inhibits the α-methyl glucose uptake with a K_i of $9 \cdot 10^{-6}$ M. Ouabain (10^{-3} M) decreases the steady-state α-methyl glucose accumulation by 60%. In the absence of sodium, the accumulation of α-methyl glucose is 7-fold less than at 142 mM Na^+, reaching a level comparable to the sodium-independent accumulation of 3-*O*-methyl-D-glucose. These findings are similar to those observed in the proximal tubule of the intact kidney.

Introduction

Sodium-dependent glucose transport is a function found only in well differentiated epithelial tissue, such as that of the intestine and kidney. In the kidney, only the proximal portion of the nephron shows this transport, and thus it can be used as a functional marker for proximal tubule cells. In the study reported here, we have examined the kinetic properties of the Na^+-dependent uptake of α-methyl glucose in rabbit proximal tubule cells cultured and maintained in hormone-supplemented defined medium.

Previous studies of cultured rabbit proximal tubule cells have shown that this preparation maintains properties unique to the proximal segment of the nephron in vivo. In particular, these cell cultures exhibit a high level of activity of the brush-border enzymes alkaline phosphatase, γ-glutamyl transpeptidase, and leucine aminopeptidase [1]. Immunofluorescence techniques have demonstrated the presence of angiotensin converting enzyme in the plasma membranes of these cells [2]. This antigen is localized in brush border of the renal proximal tubule cells [3]. Further-

Abbreviations: PTH, parathyroid hormone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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more, in accord with findings in proximal tubule cells in vivo, the cultured preparation exhibits PTH-sensitive cAMP synthesis with no response to arginine vasopressin, or calcitonin [1]. Finally, Waqar has reported a Na^+-dependent phosphate transport in these cultured cells; in the intact kidney, this transport is confined to the proximal tubule [4].

Our investigation of Na^+-dependent uptake in the rabbit proximal tubule culture demonstrates the existence of a high-affinity type transport system, such as that found in the straight portion of the proximal tubule. The observed dependency of the initial uptake rate of α-methyl glucose upon sodium concentration indicates that Na^+ levels affect the apparent K_m, but not the V_{max} of the hexose transport system. The data suggests a stoichiometry of two for Na^+ in the process by which it activates the transport of sugar. The dependence of the final level of accumulation of α-methyl-D-glucose upon sodium levels, also explored in this study, is consistent with our interpretation of the uptake data. In inhibition studies of this system, phlorizin was found to inhibit uptake with a K_i of about $9 \cdot 10^{-6}$ M; phloretin also inhibits α-methyl glucose uptake, but to a lesser degree.

Materials and Methods

Rabbit proximal tubule preparation and culture. The technique for isolation of proximal tubule was adapted from the method described by Brandel and Meezan [5]. Adult white New Zealand rabbits (2–3 kg) were killed by stunning, and the kidneys excised and placed in sterile medium (F-12 Ham and Dulbecco 1 : 1). An 18 gauge plastic catheter was placed in the renal artery and kidney perfused via the catheter with phosphate-buffered saline (PBS, 10 mM phosphate/130 mM NaCl (pH 7.4)) until the venous return was free of blood. 5 ml of 5% iron hydroxide solution was then injected into the renal artery. Iron particles were trapped in the glomeruli and later were removed by a magnetic stirring bar.

The cortex was removed, minced and homogenized with a Dounce glass homogenizer. The homogenate was passed through a 250 and an 85 μm nylon sieves in series. Tissue retained on the 85 μm sieve was resuspended in the medium and exposed to type IV collagenase and soybean trypsin inhibitor in final concentrations 1% and 0.02%, respectively for 3 min. The tissue was washed and plated on 35 mm culture dish.

Medium. The culture medium consists of F-12 Ham and Dulbecco's modified Eagle's medium (DMEM), containing 25 mM Hepes supplemented by 5 μg/ml insulin, 5 μg/ml human transferrin and $5 \cdot 10^{-8}$ M hydrocortisone; the medium also includes 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were incubated in 95% air, 5% CO_2 at 37 °C. Outgrowth started within 24 h, with formation of a monolayer in 10–15 days.

Uptake measurement. α-[^{14}C]Methyl glucose uptake was measured in the 2-week-old confluent monolayer at room temperature. The medium was poured off, and the cell monolayer was washed with phosphate-buffered saline three times. The uptake assay was initiated by addition of 1 ml Hank's solution (142 mM NaCl/6.0 mM KCl/1.2 mM $CaCl_2$/1 mM $MgCl_2$/0.33 mM Na_2HPO_4/0.44 mM KH_2PO_4 (pH 7.4)) containing 0.1 μCi α-[^{14}C]methyl glucose and a specified concentration of unlabeled α-methyl glucose. When inhibitors were used, they were added to the uptake-assay medium. The uptake was terminated by decanting the incubation medium and washing the cells with ice-cold phosphate-buffered saline. The cells were solubilized by 0.1 M NaOH, and a portion of cell lysate was neutralized by 0.1 M HCl and counted for radioactivity using a Packard scintillation counter (model Tri-Carb 4640). The other portion of cell lysate was used for protein determination by the modified Lowry method [6].

All data are expressed as moles of α-methyl glucose per mg cell protein. In Na^+-free Hank's solution, NaCl was replaced by equimolar choline chloride. 3-*O*-methyl-D-glucose uptake was measured in the same manner using 3-*O*-[^{14}C]methyl-D-glucose as a tracer. The uptake at 'zero time' was measured by removing incubation medium immediately (within 5 s) after it was added. Washing the cells with ice cold PBS, with or without phlorizin before addition of incubation medium, did not affect the zero-time uptake. The zero time uptake was measured in all experiments and substracted from the uptake at subsequent points in

time. Each point is a mean ± S.E. of 4 to 18 determinations.

Materials. α-[^{14}C]Methyl glucose, and 3-*O*-[^{14}C]Methyl-D-glucose were purchased from New England Nuclear (Boston, MA). All hormones, human transferrin and other chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Culture medium, penicillin, streptomycin, and trypsin inhibitor were purchased from GIBCO (Grand Island, NY), Type IV collagenase was purchased from Worthington Biochemicals (Freehold, NJ).

Results

Time-course of α-methyl glucose uptake

Time-courses of α-methyl glucose uptake in the presence and in the absence of several concentrations of Na^+ with a fixed concentration (1 mM) of α-methyl glucose, are shown in Fig. 1. The uptake at each Na^+ concentration is almost linear for the first ten minutes, and then it slowly levels off reaching a steady-state level in 90 min. With 142 mM Na^+, 50% of the stady-state uptake was reached in approximately 25 min, and within 60 min it had reached about 90% of the steady state. In the absence of Na^+, 50% of the uptake occurred in approximately 10 min and 90% of the stady state was achieved in 30 min.

The initial rates of α-methyl glucose uptake were assessed from the linear portion of the time-course. The initial rates measured for various concentrations of α-methyl glucose and Na^+ data were analyzed using the double-reciprocal plot of Lineweaver and Burk [7]. The results are shown in Fig. 2. For each Na^+ concentration, the α-methyl glucose uptake rate shows a simple form of saturation kinetics as indicated by the linearity of the plot. V_{max} is not affected by the presence of Na^+ at different concentrations, remaining at 0.33 nmol/mg protein per min throughout the range of Na^+ concentrations tested. The K_m of the sugar flux, however varies with Na^+ concentration (Table I), ranging from 0.16 mM at 142 mM Na^+ to 2.3 mM in the absence of Na^+.

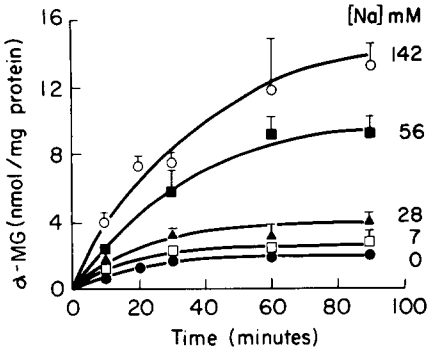

Fig. 1. Time-course of α-methyl glucose (α-MG) uptake in the presence of 1 mM α-methyl glucose, for various concentrations of Na^+, from 0 to 142 mM. Each point represents an average of 6 to 18 determinations with bar indication of the standard error.

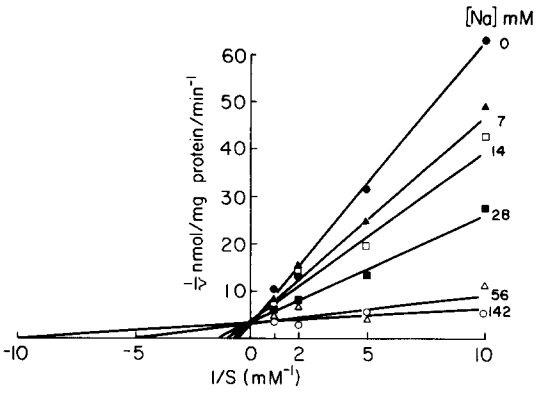

Fig. 2. Initial rate (10 min) uptake of α-methyl glucose (v) plotted as a function of sugar concentration (S) using a double-reciprocal plot. Na^+ concentration was varied from 0 to 142 mM. Each point is an average of 6 to 18 determinations. The curves are the least-squares best fit for the data.

Kinetic analysis of the Na^+ effect

Our data suggested that glucose uptake, as measured by α-methyl glucose uptake in these cultured cells is similar to that of proximal tubular cells in vivo. We have undertaken the analysis of the initial α-methyl glucose uptake as a function of Na^+ concentration within the context of the kinetic model shown in Fig. 3. This model is a restricted case of the generalized cotransport scheme considered by Turner [8]. In general, the model represents the translocation of sugar by means of a carrier molecule, C, which can bind both sugar and sodium ion. Rate constants for the processes of membrane permeation, and equilibrium constants for the carrier-sugar-sodium association reactions are defined in the footnote of Fig. 3.

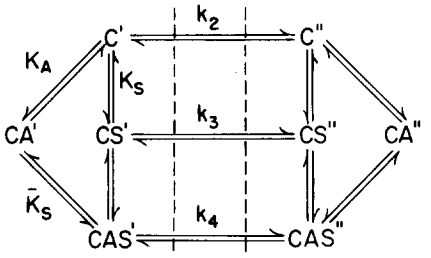

Fig. 3. Schematic representation of the Na^+-α-methyl glucose cotransport model used in this paper.

Reaction sequence assumed as the basis for the kinetic model, in which C represents free carrier; A, the activator; (Na^+ in this case); and S the permeating sugar substrate. Single and double primes indicate external and intracellular concentrations, respectively. k_2, k_3 and k_4 are the first-order permeation coefficients for the carrier and its complexed forms, while K_A, K_S and $\bar{K}_S$ represent the equilibrium association constants for the reaction processes indicated. It is assumed that the association reactions are sufficiently rapid to maintain equilibrium, and that the carrier activator complex (CA) cannot penetrate the membrane. With these assumptions, the α-methyl glucose flux, V, can be expressed:

$$V = \frac{k_2 C_t k_3 K_s (S' - S'') + k_4 K_A K_S (A'S' - A''S'')}{\Delta} \qquad (1)$$

in which the denominator, Δ, has the form

$$\Delta = (1 + K_s S' + K_A A' + K_A K_S A'S') \times (k_2 + k_3 K_S S'' + k_4 K_A \bar{K}_S A''S'') + (1 + K_S S'' + K_A A'' + K_A \bar{K}_S A''S'') \times (k_2 + k_3 K_A S' + k_4 K_A \bar{K}_S A'S')$$

Further simplifying assumptions lead to the more compact equations shown in the text.

In order to simplify the analysis and reduce the number of parameters to be fitted, we assume that the permeation processes are rate limiting, and correspondingly, that the distribution of the carrier among its free and associated forms is at equilibrium. It is also assumed that the corresponding association constants are equal on the two sides of the the membrane. Under these conditions, one can readily find an expression for the net permeation velocity as a function of the Na^+ and sugar concentrations on the two sides of the membrane.

Further simplication is achieved by setting the internal α-methyl glucose concentration (S'') to zero, and letting the intracellular sodium (A'') be an undetermined constant. It is reasonable to expect these conditions to be approximated during the initial phase of the uptake measurements. The expression for permeation velocity, under these circumstances, can be reexpressed in a hyperbolic form analogous to the conventional enzyme rate equation

$$V = \frac{V_{max} S'}{K_m + S'} \qquad (2)$$

In this form, the Michaelis-Menten parameters are related to the model parameters as follows

$$V_{max} = \frac{k_2 C_T}{1 + K_A A'' + k_2/k_4} \qquad (3)$$

$$K_m = \frac{2 + K_A A'' + K_A A'}{[1 + k_4/k_2 (1 + K_A A'')][K_S + \bar{K}_S K_A A']} \qquad (4)$$

In arriving at these equations, we have also set $k_3 = k_4$, a relation necessary to ensure that V_{max} is independent of external sodium concentration as our observations suggest.

From Eqn. 4, we note that the dependence of

TABLE I

ANALYSES OF THE Na^+ DEPENDENCE OF α-METHYL GLUCOSE FLUX

$[Na^+]$ (mM)	$[Na^+]^2$ $(mM)^2$	K_m Expt.	K_m Case I	K_m Case II
0	0	2.3	2.39	2.49
7	49	2.2	1.13	2.01
14	196	1.1	0.74	1.30
28	784	1.03	0.44	0.57
56	3136	0.2	0.24	0.22
142	20164	0.10	0.10	0.10
			$R = 0.47$	$R = 0.14$

Case I: $K_m = K_m^0 \frac{1}{1 + \beta[Na^+]}$ $\alpha = 0$, $K_m^0 = 2.39$, $\beta = 0.16$

Case II: $K_m = K_m^0 \frac{1 + \alpha[Na^+]^2}{1 + \beta[Na^+]^2}$ $\alpha = 1.54 \cdot 10^{-4}\ (mM)^{-2}$, $K_m^0 = 2.49$, $\beta = 5 \cdot 10^{-3}\ (mM)^{-2}$

the apparent K_m upon Na^+ can be put in the form

$$K_m = \frac{K_m^0(1+\alpha A')}{1+\beta A'} \quad (5)$$

with

$$\alpha = \frac{K_A}{2+K_A A''} \text{ and } \beta = \frac{\bar{K}_S K_A}{K_S} \quad (6)$$

The results of our efforts to fit these relations to the experimental observations are tabulated in Table I. If sodium is considered to bind with a stoichiometry of 1 (Case I), the best fit is obtained with $\alpha = 0$, which, from Eqn. 6, would also require K_A to vanish. This case then, would correspond to an ordered carrier association reaction in which the sugar binds first.

A much closer fit was obtained by assuming a stoichiometry of 2 for sodium association ($A' = [Na^+]^2$), as shown in Fig. 4 and by the relative residual values, R, in Table I. The values obtained for β and α in the fitting procedure have a ratio β/α of about 25. From the relations (Eqn. 6), we also have

$$\beta/\alpha = (2+K_A A'')\bar{K}_S/K_S$$

suggesting that this case (Case II) corresponds to a system in which the sugar has considerably greater affinity for the sodium-loaded carrier than for the free carrier.

In the extreme, one could consider the system in which this ratio becomes infinitely large, with $K_s \to 0$. This case would correspond to the ordered association reaction, with sodium binding first. These circumstances, however, are incompatible with the data at hand, since they would require the sugar permeation to vanish in the absence of external sodium. In contrast, the data reported here indicate invariance of V_{max} with Na^+ concentration, while K_m remains finite for zero external sodium ion concentration.

Relationship of Na^+ concentration to final accumulation of α-methyl glucose

The levels of α-methyl glucose accumulated in the cells were measured following 90 min of incubation. These incubations were conducted in medium containing various concentrations of Na^+, while concentrations of α-methyl glucose were raised from 0.1 to 1 mM. The data show that the final accumulation of α-methyl glucose is dependent not only on glucose concentration, but also on the external Na^+ concentration (Fig. 5). The steady state of α-methyl glucose accumulation, plotted as a function of external sodium, is generally sigmoid in shape. Na^+ concentrations below 15 mM have a minimal effect on the final sugar uptake. At the other extreme, Na^+ concentrations above 60–120 mM (depending upon the concentration of α-methyl glucose did not substantially increase the accumulation of α-methyl glu-

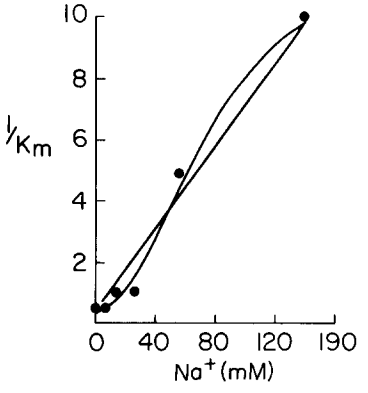

Fig. 4. $1/K_m$ values obtained from the theoretical model (Table I) plotted versus Na^+ concentration. The theoretical relation is linear for 1:1 stoichiometry (Case I), becoming sigmoid for 2:1 stoichiometry (Case II). The experimental data are most closely fit by Case II.

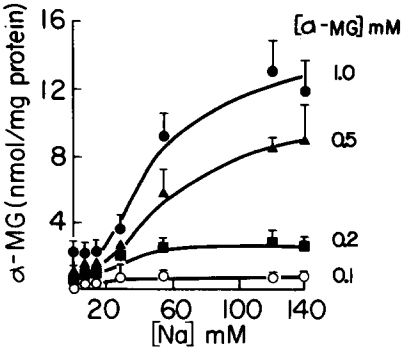

Fig. 5. The final level of α-methyl glucose (α-MG) uptake accumulated in the cells after 90 min incubation, plotted as a function of Na^+ concentration. α-Methyl glucose concentration was varied from 0.1 to 1 mM. Each point is the mean of 6 to 18 determinations, with the standard error indicated.

cose. For 1 mM α-methyl glucose in the presence of 142 mM Na^+, the final accumulation is about 7-fold of that found in the absence of sodium ion. In contrast, the steady-state uptake of 3-*O*-methyl-D-glucose did not exhibit sodium dependence (data not shown). A steady-state value of 1.97 ± 0.13 nmol/mg protein was achieved within 2 to 3 minutes in the presence of 1 mM 3-*O*-methyl-D-glucose and 142 mM Na^+ in the medium (Fig. 6). This accumulation is quite similar to that of α-methyl glucose, (also at 1 mM α-methyl glucose in the medium) in the absence of Na^+ (Fig. 1).

Effect of sugar analogs on α-methyl glucose uptake

Initial rates of 0.5 and 0.2 mM α-methyl glucose uptake in the presence of 10 and 25 mM of different sugar analogs were measured in order to quantitate the possible inhibitory effect of these analogs on α-methyl glucose uptake (Table II). α-Methyl glucose was inhibited by 35% when D-galactose was present at a concentration 50-times higher than that of α-methyl glucose. D-Glucose inhibits α-methyl glucose uptake by 70%. Relative potencies in this inhibition are D-glucose > α-methyl glucose > D-galactose = 3-*O*-methyl-D-glucose. D-Fructose showed a slight effect, while L-glucose and D-mannose did not show any significant effect under these conditions. These observations suggest that D-glucose, α-methyl glucose and D-galactose compete for the same carrier. Insulin at concentrations of $1.6 \cdot 10^{-8}$ and $1.6 \cdot 10^{-7}$ M did not affect the initial uptake of α-methyl glucose (4.02 ± 0.2 vs. control 3.3 ± 0.4 nmol/mg protein per 10 min; *P*, not significant).

Effect of inhibitors on α-methyl glucose uptake

Phlorizin, a known inhibitor of glucose uptake at the luminal membrane, also inhibits α-methyl glucose uptake in these cultured cells. Analysis of the data of the initial 10 min uptake of α-methyl glucose measured in the presence of different concentrations of phlorizin (Fig. 7) revealed that the inhibition was a simple, saturable function of phlorizin concentration with $K_i = 9 \cdot 10^{-6}$ M.

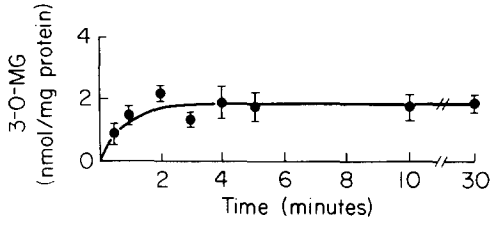

Fig. 6. The time-course of 3-*O*-methyl-D-glucose (3-*O*-MG) uptake in the presence of 142 mM Na^+. The sugar concentration is 1 mM. Each point is the mean of 3 to 9 determinations.

TABLE II

EFFECT OF SUGAR ANALOGS ON 10 MINUTE UPTAKE OF α-METHYL GLUCOSE

Figures presented are means ± S.D. ($n = 4$–7). α-MG, α-methyl lucose; 3-*O*-MG, 3-*O*-methyl-D-glucose.

Sugar (mM)	[α-MG] (mM)	α-MG uptake (nmol/mg protein)
Control	0.2	0.65 ± 0.04
	0.5	3.27 ± 0.1
D-Fructose, 10	0.2	0.49 ± 0.06
25	0.5	2.7 ± 0.1
L-Glucose, 10	0.2	0.66 ± 0.1
25	0.5	2.7 ± 0.1
D-Mannose, 10	0.2	0.69 ± 0.06
25	0.5	2.6 ± 0.2
3-*O*-MG 10	0.2	0.4 ± 0.08 *
25	0.5	2.5 ± 0.1 *
D-Galactose, 10	0.2	0.42 ± 0.04 *
25	0.5	2.2 ± 0.1 *
α-MG, 10	0.2	0.3 ± 0.04 *
25	0.5	0.89 ± 0.05 **
D-Glucose, 10	0.2	0.24 ± 0.04 *
25	0.5	0.74 ± 0.05 **

* $P < 0.01$.
** $P < 0.001$.

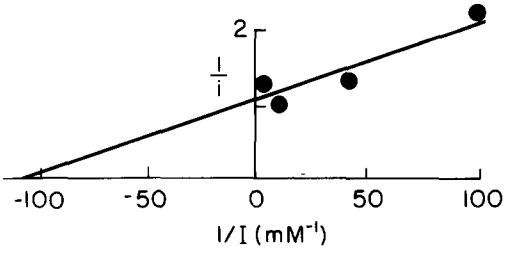

Fig. 7. Effect of phlorizin on initial rates of α-methyl glucose uptake. The initial rates correspond to the first 10-min α-methyl glucose uptakes measured in the presence of varying concentrations (0.01–2.5 mM) of phlorizin (*I*). The concentration of α-methyl glucose is 1 mM. The inhibition coefficient (*i*) is defined as $(V_o - V_i)/V_o$, where V_o and V_i are the initial rates measured in the absence and presence of the inhibitor, respectively [23]. The least-squares regression line is shown.

TABLE III

EFFECT OF INHIBITORS ON ACCUMULATION OF 1 mM α-METHYL GLUCOSE

The α-methyl glucose level was masured at a steady state after 90 min incubation, in the absence (control) and presence of inhibitors. Figures presented are means ± S.D. α-MG, α-methyl glucose.

Inhibitor	Concn. (mM)	α-MG level (nmol/mg protein)
Control	-	11.6 ±1.7
Phlorizin	0.01	2.9 ±0.08
	0.1	0.75 ± 0.01
Phloretin	0.01	10.05 ± 1
	0.1	4.35 ± 0.5
Ouabain	1.0	7.2 ±2

The final accumulation of α-methyl glucose at the steady state, was also inhibited by phlorizin, phloretin and ouabain (Table III). Ouabain at 1 mM concentration decreases α-methyl glucose accumulation by approximately 40%. Phloretin, an inhibitor of glucose transport at basolateral membrane, inhibits the α-methyl glucose accumulation by 13% and 60% at respective concentrations of 0.01 and 0.1 mM. Phlorizin at 0.1 mM concentration eliminated 90% of the α-methyl glucose accumulation. Phlorizin was 5-times more potent in inhibiting of α-methyl glucose than phloretin (Table III).

Discussion

This report provides evidence that cultured rabbit proximal tubule cells transport α-methyl glucose, a non metabolized D-glucose analog, by a saturable mechanism (Fig. 1). The uptake of this sugar is almost linear during the first 10 min and reaches steady state in approximately 90 min at 24°C. Our results are in agreement with Amsler and Cook's finding in the LLC-PK_1 cell lines [9].

The most important findings of this study concern the effect of Na^+ concentration on the α-methyl glucose flux. Na^+ concentration influences K_m but does not affect the V_{max} of α-methyl glucose upake (Fig. 2). In the presence of 142 mM Na^+, K_m is about 0.1 mM and V_{max} is 0.33 nmol/mg protein per min. This value of K_m is similar to that of the high-affinity, low-capacity carrier found in vesicles prepared from late or straight portion of rabbit proximal tubules, as reported by Moran ($K_m = 0.34 \pm 0.05$ mM). The value for V_{max} in our monolayer cell culture system is 10-fold less than that found by Moran et al. [10] in the brush-border vesicle preparation ($V_{max} = 4.1 \pm 0.03$ nmol/mg protein per min). These differences may reflect a considerable enrichment of the carrier in the vesicle preparations.

In the absence of Na^+, K_m is 2.3 mM, and it decreases with an increasing concentration of Na^+. Our finding that Na^+ affects K_m but not V_{max} is consistent with the findings reported by Rabito et al. in LLC-PK_1 cell line [11], Aronson and Sacktor in rabbit brush-border membrane vesicle [12], and micropuncture in the rat nephron by Ullrich [13]. However, our results contradict the findings by Kleinzeller in rabbit kidney slices [14], and Hopfer in brush-border vesicles prepared from rabbit kidney [15]. Both of these studies showed a dependence of V_{max}, but not of K_m on Na^+. More recently, in LLC-PK_1 cell monolayers placed in a Ussing chamber, Misfeldt and Sanders reported electrophysiological findings suggesting that Na^+ increases the affinity of the carrier for glucose, a finding consistent with ours.

Our data on the effects of Na^+ on K_m (Fig. 4 and Table I) suggest that binding of two Na^+ to each carrier is required for this effect. This 2:1 stoichiometry is consistent with the finding of Turner et al. [17] who reported 2:1 ratio for Na^+-sugar cotransport system in brush-border vesicle prepared from the LLC-PK_1 cell line. The 2:1 stoichiometry, however, contrasts with the 1:1 stoichiometry reported by Rabito and Ausiello [11] in the same cell line, and the findings of Hopfer [18] in bursh-border vesicles prepared from rabbit kidney . The nature of these discrepancies is not clear.

In the absence of external Na^+, the apparent α-methyl glucose space is essentially the same as that of 3-*O*-methyl-D-glucose, being 1.80 ± 0.34 and 1.97 ± 0.13 nmol/mg protein, respectively at substrate concentrations of 1 mM (Figs. 1 and 6). This would indicate no significant uphill movement of α-methyl glucose in the absence of Na^+. In the presence of Na^+, however, the steady-state level of α-methyl glucose accumulated in the cell is much higher, reaching 13.3 ± 1.4 nmol/mg pro-

tein, in the presence of 142 mM Na^+. This level represents a 7-fold accumulation of the sugar (Fig. 1). The rate of uptake, as well as the final level of accumulation of α-methyl glucose, are dependent upon the sugar concentration in the medium (Fig. 5).

Phlorizin is an inhibitor of glucose transport in the intestine and proximal tubular cell. It inhibits mainly the Na^+-dependent portion of glucose transport. Our data demonstrate that phlorizin inhibits α-methyl glucose uptake by these primary cultured cell with $K_i = 9 \cdot 10^{-6}$ M. Phlorizin inhibits glucose uptake ($K_i = 2 \cdot 10^{-4}$ M) in the brush-border vesicle prepared from rabbit kidney [19], and in rat intestine brush-border vesicle in the presence of 100 mM Na^+ gradient ($K_i = 2.7 \cdot 10^{-6}$ M) [18]. Phlorizin binds to rabbit proxiaml tubule preparation with a K_d (dissociation constant) of $8 \cdot 10^{-6}$ M [20]. The low sensitivity of α-methyl glucose uptake to inhibition by phlorizin observed in this cell culture preparation is similar to the low sensitivity of the outer medullary preparation reported by Moran et al. [10].

Stereospecificity of the carrier-substrate interaction was evaluated by studying the inhibition of α-methyl glucose uptake by other hexose. The order of inhibition observed is D-glucose > α-methyl glucose > D-galactose = 3-*O*-methyl-D-glucose. D-Glucose and D-galactose are the hexose known to share the high affinity (G site) carrier in the proximal tubule cell [21]. L-Glucose, mannose and fructose, on the other hand, did not have a significant effect on α-methyl glucose uptake. This sequence of selectivity is similar to that reported for the rat kidney [22], rabbit kidney cortex slices [14], LLC-PK_1 cell line [10,11], and vesicles prepared from the outer medullary portion of the rabbit kidney [17].

Our method of isolating proximal tubules does not allow the separation of the convoluted from the straight portion of the proximal tubule. Unfortunately, based only upon the data available, one cannot determine of which segment of the proximal tubule these cells are most characteristic.

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