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Na^+-Dependent Sugar Transport in a Cultured Epithelial Cell Line from Pig Kidney

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Summary. A Na^+-dependent hexose transport system with similar characteristics to that observed in the kidney is retained in a cultured epithelial cell line from pig kidney (LLC-PK_1). The active transport of α methyl-D-glucoside (α MGP), a nonmetabolizable sugar, which shares the glucose-galactose transport system in kidney cells is mediated through a Na^+-dependent, substrate-saturable process. The kinetic analysis of the effect of Na^+ on the uptake of αMGP indicated that the Na^+-sugar cotransport system is an affinity type system in which the binding of either sugar or Na^+ to carrier increases the affinity for the other ligand without affecting the V_{max}. The sequence of selectivity for different sugars studied by the inhibition produced in the uptake of αMGP is very similar to that reported in rat kidney, rabbit kidney cortex slices, and rabbit renal brush border membrane vesicles. Phlorizin, even at very low concentration, almost completely inhibits αMGP uptake. Conversely, phloretin at the same low concentration stimulated the sugar accumulation by inhibition of efflux, probably at the level of the basolateral membrane. Sulfhydryl group inhibitors also blocked the αMGP uptake, suggesting that these groups were required for normal functioning of the sugar carrier system. This sugar transport system is an important functional marker to study the molecular events associated with the development of polarization in epithelial cells.

Key words: Epithelial cell asymmetry – α Methyl-D-glucopyranoside – phlorizin – phloretin – sulfhydryl group inhibitors.

Physiologic studies with intact animal, renal cortical slices, and isolated proximal tubules on the reabsorption of D-glucose by the kidney have indicated that D-glucose transport is carried out by an uphill, saturable, stereospecific, Na^+ and energy-dependent transport system [31]. Micropuncture studies [30] and the development of techniques for isolated renal brush border membranes [2, 14] have localized the luminal membrane as the site of the uphill transport step. The sugar carrier system localized in these renal brush borders is also specifically susceptible to the competitive inhibitory effect of the phenolic glucoside, phlorizin [11, 14]. According to these data the Na^+ gradient hypothesis postulates that glucose, which accumulates within the cell by the luminal Na^+-cotransport mechanism, leaves the cell passively at the contraluminal cell side. Results from studies on basolateral membrane vesicles [14] indicate that the sugar transfer at this cell side, is probably Na^+-insensitive, inhibited by phloretin rather than by phlorizin, and apparently with a different specificity, which, however, has not yet been totally clarified. Thus, the basolateral carrier system exhibits properties similar to those known to exist in nonepithelial cells such as the glucose carrier in erythrocytes [18] adipocytes [19] and fibroblasts [7]. The specific location of both sugar transport systems in each section of the plasma membrane is another expression of the functional polarization of epithelial cells that plays such an important role in the transepithelial transport of solutes and water. Several observations indicate that the morphological and functional polarity of epithelial cells *in vivo* are retained in cultured epithelial cells [6, 20, 21, 22][1]. These model epithelia membranes can be readily dissociated into individual cells and then the epithelial sheet can be reconstituted through the normal processes of plating out and growing in culture medium[2]. This provides a

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[2] Rabito, C.A., Tchao, R. 1979. (^3H) ouabain binding during the monolayer organization and cell cycle in a cell line (MDCK) derived from canine kidney. *Am. J. Physiol.* (*in press*).

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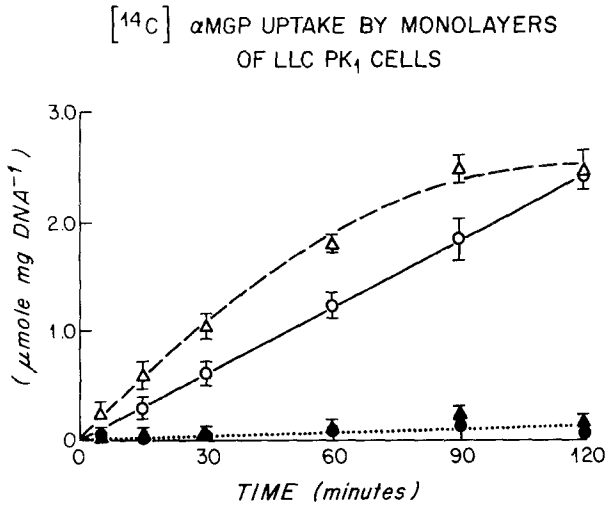

Fig. 1. Uptake of ∝ MGP by LLC-PK_1 monolayer. The monolayers were incubated in 10 ml EBSS containing 1 mM ∝MGP with (○·····○) or without Na^+ (●·····●) and 10 mM ∝ MGP with (△——△) or without Na^+ (▲·····▲) plus 0.25 μCi/ml (^{14}C) ∝MGP 1 μCi/ml (^3H) inulin as extra cellular maker. The monolayers were allowed to take up the (^{14}C) ∝ MGP for various times at 37°. Each point is an average of 9–12 monolayers ± SEM

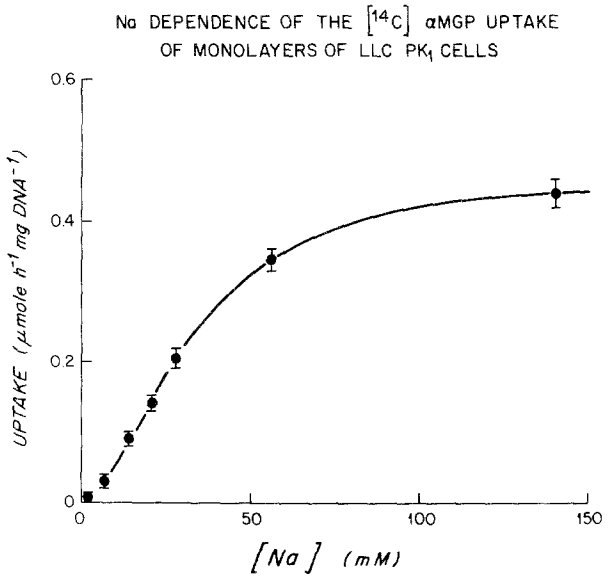

Fig. 3. Relation of∝MGP uptake to the medium Na^+ concentration. Monolayers were incubated for 60 min in 0.1 mM∝MGP. Na^+ was replaced by choline in EBSS to produce the desired Na^+ concentrations. Each point is an average of 6 determinations ± SEM

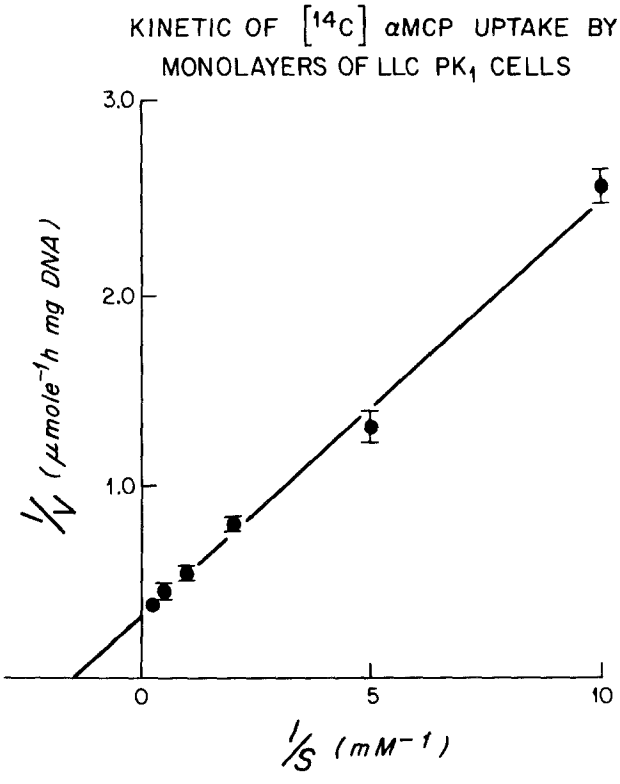

Fig. 2. Lineweaver-Burk plot of∝MGP uptake. V is velocity expressed as μmol hr^{-1} mg DNA^{-1}. S is∝MGP concentration in mM. The line was fitted to the data using least squares determinations. From these data the apparent K_m and V_{max} calculated for ∝MGP uptake were 0.75 mM and 3.03 μmol hr^{-1} mg DNA^{-1}, respectively. Each point is an average of 10 determinations ± SEM

powerful tool for studying the development or loss of polarization in epithelia. In the present study, experiments are described showing that a Na^+-dependent hexose transport with similar characteristics to that observed in kidney is also retained in cultured epithelial cells from pig kidney, providing another functional marker to study the molecular events associated with the polarization of epithelial cells.

Materials and Methods

Cell Cultures and Monolayer Preparation

LLC-PK_1 cells obtained from the American Type Culture Collection were maintained by serial passages in plastic tissue culture dishes. The cells were fed with Dulbecco's modified Eagle's medium with L-glutamine, supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and Fungizone (0.25 μg/ml). All cultures were maintained in an atmosphere of 5% CO_2-95% air at 37°. When cell growth reached saturation density, subcultures were prepared using 0.02% EDTA and 0.05% trypsin solution.

Monolayers on a permeable support were prepared using a polycarbonate filter membrane with 5 μm pore size and 25 mm diameter (Nucleopore Corp.). The filters were covered with a very thin film of 0.5% collagen dispersion (Ethicon) and applied to a standard microscope slide. The methods for collagen aggregation and sterilization of the collagen-coated membrane are described elsewhere [22].

Reagents

∝ -Methyl (^{14}C)-D-glucopyranoside (∝MGP) and methoxy (^3H) inulin were purchased from New England Nuclear Corp. D-xylose

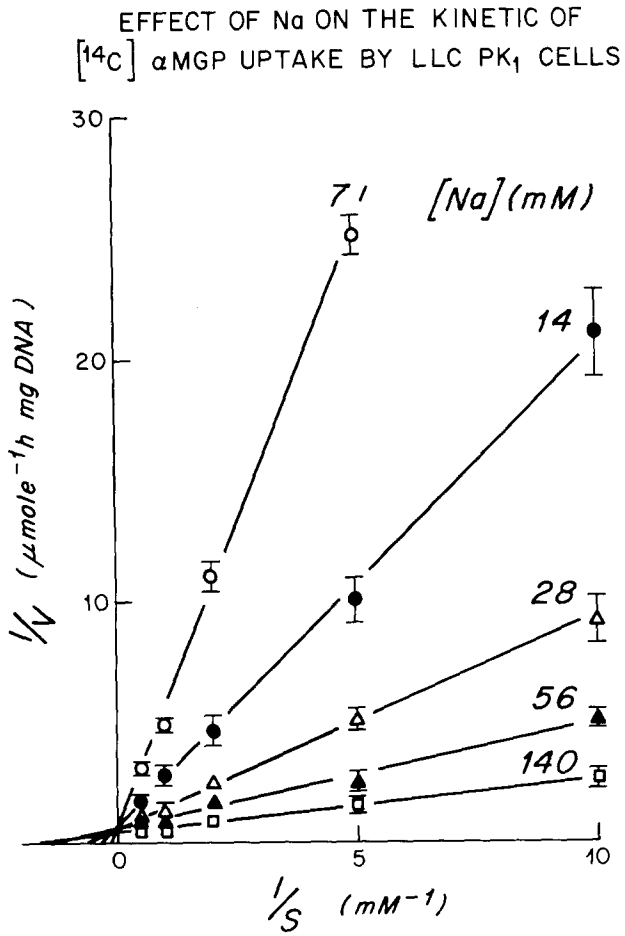

Fig. 4. Effect of Na^+ on the kinetics of αMGP uptake. The rates of uptake of αMGP were measured in the presence of 7, 14, 28, 56 or 140 mM Na^+. V is velocity expressed as μmol hr^{-1} mg DNA^{-1}. S is αMGP concentration (0.1 to 2 mM). The calculated apparent K_m value decreased from 21.5 mM at 7 mM Na to 0.75 mM at 140 mM Na^+. The V_{max} was unaffected and had an average value of 3.5 μmol hr^{-1} mg DNA^{-1}. Each point is an average of 6 determinations ± SEM

Table 1. Uptake of (^{14}C) αMGP by LLC PK_1 cells: Effect of pre-loading the cells with αMGP

Condition	Concentration αMGP (mM)	Uptake ± SEM (μmol hr^{-1} mg DNA^{-1})	n	P
Control	0.1	0.440 ± 0.018	25	
Pre-loaded[a]	0.1	0.960 ± 0.047	11	< 0.001

[a] The monolayers were preincubated for 15 min with 0 mM (control) and 10 mM (experimental) αMGP and then transferred to flask with 0.1 mM αMGP plus 1 μCi/ml ^{14}CαMGP for 15 min uptake.

was obtained from Fisher Scientific Company and 3-O-methyl-D-glucose from Pfanstiehl Laboratories, Inc. All other monosaccharides used in this study were obtained from the Sigma Chemical Company. Culture media, fetal bovine serum, trypsin-EDTA solution and antibiotic-antimycotic solution were obtained from Grand Island Biological Co.

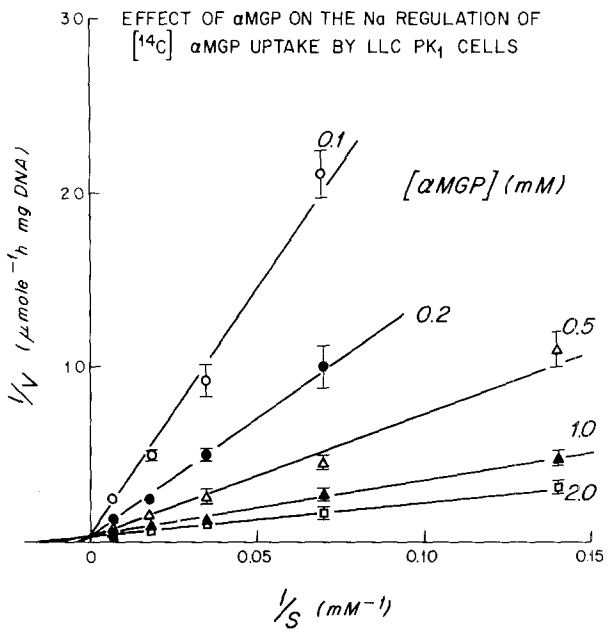

Fig. 5. Sugar uptake rates (V) in the presence of 0.1, 0.2, 0.5, 1.0, and 2.0 mM αMGP were measured using Na^+ concentration (S) of 7 to 140 mM. The calculated apparent K_{Na} value decreased from 130 to 45 mM when the αMGP concentration was increased from 0.1 to 2.0 mM. The data are the same as shown in Fig. 4. Each point is an average of 6 determinations ± SEM

Table 2. Effect of its analogs on the αMGP uptake by LLC-PK_1 monolayer

Sugar	Concentration (mM)	Uptake ± SEM (μmol hr^{-1} mg DNA^{-1})	Inhibition (%)	n	P
Control		0.440 ± 0.018	0	25	
D-fructose	10	0.524 ± 0.013	+19	9	< 0.001
L-glucose	10	0.517 ± 0.015	+18	9	< 0.01
D-mannose	10	0.508 ± 0.029	+15	9	N.S.
2-deoxy-D-glucose	10	0.473 ± 0.011	+ 8	9	N.S.
D-xylose	10	0.366 ± 0.010	−17	9	< 0.01
3-O-methyl-D-glucose	10	0.278 ± 0.004	−37	9	< 0.001
D-galactose	10	0.210 ± 0.009	−52	9	< 0.001
p-nitrophenyl-D-glucoside	10	0.061 ± 0.003	−86	9	< 0.001
αMGP	10	0.031 ± 0.001	−93	9	< 0.001
D-glucose	10	0.016 ± 0.001	−96	9	< 0.001

Uptake Assays

For uptake assays, Earle's balanced salt solution was used. Under an atmosphere of approximately 5% CO_2/95% air, the pH of the solution was 7.4. For sodium-free assays the Na_2HPO_4 was replaced with K_2HPO_4; NaCl with choline chloride, and $NaHCO_3$

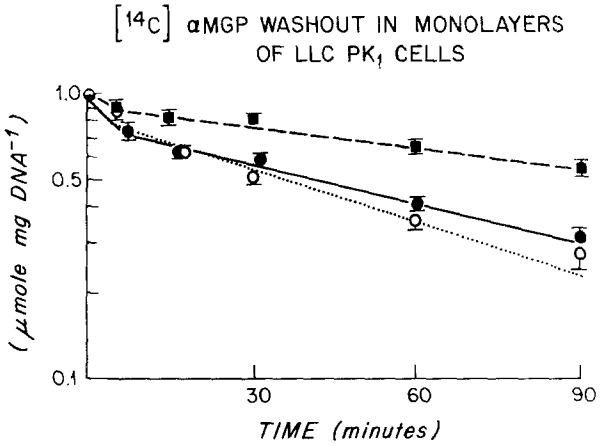

Fig. 6. Effect of Na^+ and phloretin on the efflux of αMGP from LLC-PK_1 monolayers. Monolayers accumulated radioactive glycoside for 60 min at 37 °C in complete EBSS. Efflux of the glycoside was then followed in EBSS with (o·····o) or without Na^+ (●——●) and in the presence of 0.010 mM phloretin in Na^+-free medium (■----■). Each point is an average of 6 determinations ± SEM

Table 3. Effect of phlorizin and phloretin in the uptake of αMGP by LLC-PK_1 cells

Condition	Concentration inhibitor (mM)	Concentration αMGP (mM)	Uptake ± SEM (μmol hr^{-1} mg DNA^{-1})	*n*	*P*
Control		0.1	0.440 ± 0.018	25	
Phlorizin	0.1	10	0.190 ± 0.010	5	< 0.001
	0.1	1.0	0.070 ± 0.020	10	< 0.001
	0.010	0.1	0.012 ± 0.001	16	< 0.001
	0.005	0.1	0.019 ± 0.003	16	< 0.001
Phloretin	0.1	0.1	0.265 ± 0.027	11	< 0.001
	0.010	0.1	0.597 ± 0.022	8	< 0.01

with choline bicarbonate. The cells grown as monolayers on a collagen-coated Nucleopore filter membrane, were removed from the microscope slide to give access to the αMGP to both sides of the filters. The monolayers were washed with Earle's solution and then allowed to take up the (^{14}C) αMGP for various times at 37 °C. The uptake medium was Earle's solution with or without sodium, containing (^{14}C) αMGP (0.5 μCi/ml) and methoxy (^3H) inulin (1 μCi/ml). At the end of each uptake period the filters were washed for 15 sec with ice-cold Earle's solution. After dissolving the samples with tissue solubilizer (NCS tissue solubilizer, Amersham Co.), the radioactivity was measured by liquid scintillation using Dimilume-30 (Packard Instrument Co.) scintillation fluid. Corrections for interstitial trapping were made, measuring the amount of methoxy (^3H) inulin associated with each sample. The results were normalized for the DNA content of each filter.

DNA Assay

DNA was measured by a modification of the fluorometric micromethod of Switzer and Summer [29]. Essentially, the filters were placed in a 5% (W/V). Ice cold trichloroacetic acid solution for 30 min. They were then washed for 5 min in a 0.01 N potassium acetate solution in absolute ethanol and then air-dried overnight at room temperature. DNA standards were prepared, dissolving calf thymus deoxyribonucleic acid (Sigma Chemical Co.) in a 1.0 N ammonium hydroxide solution. The DNA content of the samples and standards was measured using a 2-M 3,5-diaminobenzoic acid hydrochloride (J.T. Baker Chemical Co.) solution. The fluorometric determinations were performed in a Model 111 Turner Fluorometer (G.K. Turner Associates) used with a 406 nM interference primary filter and a Wratten No. 58 secondary filter. No interferences from the collagen-coated Nucleopore filter membrane were observed.

Table 4. Effect of different inhibitor on the αMGP uptake by LLC-PK_1 monolayers

Condition	Concentration inhibitor (mM)	Uptake ± SEM (μmol hr^{-1} mg DNA^{-1})	*n*	*P*
Control		0.440 ± 0.018	25	
Ouabain	0.005	0.067 ± 0.004	9	< 0.001
p-hydroxy mercury benzoate	0.1	0.005 ± 0.002	6	< 0.001
N-ethyl maleimide	0.5	0.014 ± 0.001	8	< 0.001

Results

Time Course of αMGP Uptake

The uptake of αMGP by LLC-PK_1 monolayer cells either in complete Earle's balanced saline solution or in a medium in which sodium was replaced by choline, is illustrated in Fig. 1. In the presence of Na^+ there is a continual accumulation of the sugar. At a concentration of 1 mM the rate of uptake remained constant up to 120 min. Increasing the sugar concentration to 10 mM increased the uptake velocity, which, however, started to decline after 60 min, suggesting that αMGP accumulation by this preparation was a saturable process. The similar uptakes observed at 120 min in both concentrations are rather surprising. The value at 120 min in 10 mM seems to be an unusually low value. Further studies will be required to clarify this possibility. In the absence of Na^+ the uptake of sugar was greatly reduced. The differences in the uptake in medium with or without Na^+ after 1 hr accumulation was 11.5 and 18.8 times that for 1 and 10 mM sugar concentration, respectively.

The apparent intracellular concentration of the tested sugar was calculated after correction for the extracellular space. The intracellular water, determined as previously described [20], was 3.49 ± 0.13 kg/kg dry wt ($n=8$) or 89 ± 5.3 μl/mg DNA^{-1} ($n=8$). The value normalized to DNA content allows the calculation of an apparent αMGP intracellular concentration of 4.9, 15.5, and 21.2 mM after 1 hr accumulation in 0.1,

1, and 10 mM sugar medium concentrations, respectively.

Sugar Concentration and Initial Uptake Velocity

Initial uptake velocity was determined during 15 min over a 100-fold range of initial αMGP concentration in the incubation medium. The velocities were plotted in Fig. 2 by the double reciprocal method of Lineweaver and Burk. The straight line was fitted to the data using least squares determinations ($r^2 = 0.9983$). From these data the apparent K_m and the V_{max} calculated for αMGP uptake were 0.75 mM and 3.03 μmol hr^{-1} mg DNA^{-1}, respectively.

Medium Sodium Concentration and αMGP Uptake

The results presented in Fig. 1 indicate that the αMGP uptake is a Na^+-dependent process. In Fig. 3 is described the effect of different concentrations of NaCl on the uptake velocity of 0.1 mM αMGP by LLC-PK_1 monolayers. In these experiments, there was an increased stimulation of uptake with increased concentration of Na^+ over the entire range of concentrations tested. However, at 140 mM NaCl, the highest concentration used, almost a complete saturability with respect to Na^+ was found. At low concentrations the curve is nonhyperbolic.

Effect of Na^+ on Kinetics of αMGP Uptake

The rates of uptake of αMGP were measured using sugar concentrations of 0.1 to 10 mM in the presence of 7, 14, 28, 56 or 140 mM Na^+. As shown in Fig. 4, the effect of increasing Na^+ concentration in the external medium was to lower the apparent K_m for uptake. The calculated apparent K_m values were 21.5 and 0.75 mM at 7 and 140 mM Na^+, respectively. The V_{max} was apparently unaffected and had an average value of 3.5 μmol hr^{-1} mg DNA^{-1}.

Relationship Between αMGP Concentration, Na^+ concentration, and Sugar Uptake

The effect of Na^+ concentration on the rate of sugar uptake in the presence of different αMGP concentrations is illustrated in Fig. 5. The uptake rates of αMGP were measured using Na^+ concentrations of 7 to 140 mM in the presence of 0.1, 0.2, 0.5, 1.0, and 2.0 mM αMGP. The calculated apparent K_{Na} value decreased from 130 to 45 mM when the concentration of αMGP was increased from 0.1 to 2.0 mM.

Effect of Preloading with αMGP on its Uptake by LLC-PK_1 Monolayers

In the experiments reported in Table 1 the monolayers were preincubated with 0 and 10 mM αMGP at 37° for 15 min prior to initiating sugar uptake measurements. The final concentration of αMGP in the uptake medium was 0.1 mM. The monolayers which were preincubated in the absence of αMGP were used as controls. A 118% increase in rate of αMGP uptake was found with preincubation with αMGP.

Effect of Analogs on the Uptake of αMGP by LLC-PK_1 Monolayers

Selected analogs at a concentration of 10 mM were evaluated as inhibitors of the rate of uptake of 0.1 mM αMGP. The results reported in Table 2 show that the uptake of αMGP was highly sensitive to D-glucose, αMGP, *p*-nitrophenyl-β-D-glucoside, and D-galactose. A slight inhibition was found with 3-O-methyl-D-glucose and D-xylose (37 and 17%, respectively). None of the other sugars, including 2-deoxy-D-glucose, D-mannose, D-fructose, and L-glucose inhibited the αMGP uptake. On the contrary D-fructose and L-glucose produce a slight but significant stimulation (19 and 18%, respectively) of the αMGP uptake.

Effect of Phlorizin and Phloretin in the Uptake of αMGP by LLC-PK_1 Monolayers

The effect of 0.1 and 0.005 mM phlorizin and phloretin on the rate of uptake of 0.1 mM αMGP by LLC-PK_1 was estimated, and the results are illustrated in Table 3. The uptake of αMGP was almost totally blocked by phlorizin even at a concentration as low as 0.005 mM. In contrast to these findings, phloretin at a concentration of 0.1 mM was markedly less effective as inhibitor of αMGP uptake than phlorizin at the same concentration. However, at a concentration of 0.010 mM there was not only no inhibition but a stimulation of αMGP uptake by phloretin in LLC-PK_1 monolayers. This result is clearly in contrast with the almost complete inhibition produced by 0.005 mM phlorizin. As an additional observation, Table 3 shows that the inhibitory effect of phlorizin decreased when the concentration of αMGP increased.

Effect of Different Inhibitors of the αMGP Uptake by LLC-PK$_1$ Monolayers

The effect of different inhibitors on the αMGP uptake by LLC-PK$_1$ monolayers are shown in Table 4. The monolayers were incubated for 30 min in the presence of the inhibitor at concentrations shown in the table. The uptake of 0.1 mM αMGP was then determined for another 30-min period in the presence of the same concentration of inhibitor. The results show that 0.005 mM ouabain produced an 85% inhibition from 0.440 ± 0.018 μmol hr^{-1} mg DNA^{-1} to 0.067 μmol hr^{-1} mg DNA^{-1} ($P < 0.001$) in the sugar uptake by the monolayers. Similarly, two modifiers of −SH groups *p*-hydroxy mercurybenzoate at a concentration of 0.1 mM and N-Ethylmaleimide at a concentration of 0.5 mM almost completely blocked the α MGP uptake ($P < 0.001$ for both inhibitors compared with the control condition).

Efflux of αMGP from Monolayers of LLC-PK$_1$ Cells

In these studies the monolayers were incubated for 60 min to accumulate the ^{14}C labeled glycoside and then transferred to flasks containing EBSS with or without Na^+ or EBSS without Na^+ plus phloretin at a concentration of 0.010 mM for measurement of loss of radioactive sugar. Results showing the effect of Na^+ and phloretin on the efflux are presented in Fig. 6. Contrary to the almost complete inhibition of αMGP uptake produced by the total substitution of Na^+ by choline in the uptake medium a small but nonsignificant ($P < 0.1$) inhibition was observed in the efflux of sugar in the absence of Na^+ in the wash-out medium. The efflux constant calculated from the slopes after the first 5 min in Na^+-free medium was 0.0105 ± 0.0003 min^{-1} ($n = 6$) compared with 0.0148 ± 0.0016 min^{-1} ($n = 6$) obtained in EBSS solution with a normal Na^+ concentration. Phloretin, on the contrary, at a concentration as low as 0.010 mM produced a clear inhibition on the sugar efflux as shown by the reduction in the efflux constant to 0.0057 ± 0.0005 min^{-1} ($n = 6$) ($P < 0.01$) for the difference with the efflux constant in control conditions.

Discussion

The present results indicate that α MGP, a nonmetabolizable sugar, which shares the glucose-galactose transport system in kidney cells [17, 25] is actively transported by monolayers of LLC-PK$_1$ cells derived from a pig kidney. This sugar transport is mediated through a Na^+-dependent system which conforms to the kinetics of a substrate-saturable process. From the kinetic data the apparent K_m calculated for α MGP uptake was 0.75 mM which was very close to 1.2 mM obtained in rabbit kidney cortical slices [15] although different from 6 mM reported in rat kidney [26]. This discrepancy between K_ms from different species and the lack of comparable data from pig kidney prevent using, only kinetic data, the establishment of any conclusive similarity between this sugar transport system and the one reported in mammalian kidney. The results presented in Figs. 1 and 2, indicating that the α MGP uptake is a Na^+-dependent process, agree with previous reports on the influence of Na^+ on the reabsorption of sugar in the kidney, as studied *in vitro* by renal cortical slices [16] and renal brush border membrane vesicles [2] and in intact animals by microperfusion of proximal tubules [24] and in clearance measurements [23]. The kinetic analysis of the effects of Na^+ on the uptake of α MGP by LLC-PK$_1$ monolayers show that the increase of Na^+ in the uptake medium from 7 to 140 mM decreases the K_m for αMGP by a factor of 29 without a change in V_{max} which remains practically constant throughout the entire range of Na^+ concentrations tested. These findings agree with those reported from micropuncture studies of αMGP transport in rat nephron [33] and with studies on D-glucose transport in isolated brush border membrane vesicles from rabbit kidney [3] but contrast with those found for α MGP transport in rabbit renal cortex slices [17]. In the more complex kidney slice it was reported that Na^+ affected V_{max} and not K_m. Our data indicate that the Na^+-sugar cotransport system is an affinity type system in which the binding of either glucose or Na^+ to the carrier increases the affinity for the other ligand, without affecting the mobility of the carrier-sodium-glucose complex within the membrane. Further studies will be needed to analyze the nonhyperbolic relationship between sodium concentration and αMGP uptake.

The mechanisms by which Na^+ enhances the sugar transport in the kidney remain controversial. However, the results from renal brush border membrane vesicles indicate a sugar-coupled Na^+ influx into the cell through the apical membrane that occurs along an electrochemical gradient for Na^+ ions [3]. According to the mechanism proposed by Crane for intestinal transport [8], it is the ouabain-sensitive active extrusion of Na^+ across the basolateral plasma membrane of the cell that maintains this Na^+ gradient across the apical membrane. The almost complete inhibition produced by ouabain on the αMGP uptake by LLC-PK$_1$ monolayers support this idea and confirm the ouabain inhibition of D-glucose reabsorption in the kidney [9] and the accumulation of αMGP

in renal cortical slices [1]. The results in Table 1 show that the intracellular unlabeled αMGP accelerates the uptake of the labeled compound from the extracellular medium. The results of these experiments indicate the occurrence of counterflow, a strong indication that the αMGP transport is a carrier mediate process in which the rate-limiting step is the mobility of the free carrier within the membrane. Similar results for αMGP were obtained in rat renal cortical slices [12].

The active transport of αMGP was inhibited by other sugars listed in descending order: D-glucose > *p* nitrophenyl β D-glucose > D-galactose > 3-O-methyl-glucose > D-xylose. D-mannose and 2-deoxy glucose had no effect, while D-fructose and L-glucose produced a light stimulation. This sequence of selectivity is very similar to that reported in rat kidney by micropuncture experiments [32], rabbit kidney cortex slices [17], and renal brush border membrane vesicles [3]. From this sequence of selectivity we can conclude that the molecular requirements of the sugar to be transported by the system are; the presence of an hydroxyl group in the D-glucose configuration in C-2, and an hydroxyl group on C-3 in the same configuration as in D-glucose (partial requirement). Deviation in C-4 reduced the active transport to 52%; deviation in C-1 did not modify this transport rate. Another molecular requirement is that the ring must be in the pyranose form. A definitive explanation cannot yet be provided for the stimulation of αMGP uptake by D-fructose and L-glucose.

Glucose reabsorption in the renal proximal tubule [4] and its accumulation by renal cortical slices [16] can be inhibited effectively by a relatively small concentration of phorizin. Studies in renal brush border membrane vesicles indicated that the sugar-carrier system localized in this membrane is specifically susceptible to the competitive inhibitory effect of the phenolic glycoside without being translocated itself across the membrane by the glucose transporter [27]. On the contrary, the phlorizin's aglycone phloretin inhibits the Na^+-independent facilitated diffusion transport system for sugars associated with the basolateral membrane of epithelial cells [13]. Our results on the effect of phlorizin and phloretin in the uptake of αMGP by LLC-PK_1 cells are in total agreement with these observations. Phlorizin, even at a concentration as low as 0.005 mM, almost completely inhibited the αMGP uptake. In contrast, however, phloretin at the same concentration stimulated sugar accumulation, probably by inhibiting the efflux of sugar through the basolateral membrane. The efflux inhibition of αMGP from LLC-PK_1 monolayer produced by phoretin support this idea. Although these observations are consistent with a simple two compartment system – cells and bath since the filter is equilibrated with the bath – where influx and efflux can be measured, the exact localization of sugar entry and exit in the apical and basolateral cell borders still remains to be elucidated.

Since sulfhydryl group inhibitors *p*-chloromercuribenzoate and N-ethylmaleimide blocked the glucose-sensitive phlorizin binding in renal brush border membranes [5], one may conclude that the same group is involved in the active translocation of αMGP in LLC-PK_1 monolayers. In conclusion, the data in the present study suggest, but so far does not prove, that –SH groups are involved in the sugar carrier system since the effect of both inhibitors could be mediated by dissipation of the Na^+ electrochemical gradient via an unspecific effect on the cell viability. Recent results, however, have shown that *p*-chloromercuribenzoate stimulated the active Na^+ transport in frog skin [10] and toad urinary bladder [28] indicating a complete preservation of the cell viability in these preparations.

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Note Added in Proof

After this manuscript was submitted, it was brought to our attention that Mullins *et al.* (1979, Fed. Proc. **38**:*Abstr.* 4394) reported, in abstract form, similar results to some experimental data reported here.

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