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Polarity of transport of 2-deoxy-D-glucose and D-glucose by cultured renal epithelia (LLC-PK$_1$)

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At least two types of glucose transporter exist in cultured renal epithelial cells, a Na^+-glucose cotransporter (SGLT), capable of interacting with D-glucose but not 2-deoxy-D-glucose (2dglc) and a facilitated transporter (GLUT) capable of interacting with both D-glucose and 2dglc. In order to examine the polarity of transport in cultured renal epithelia, 2dglc and D-glucose uptakes were measured in confluent cultures of LLC-PK$_1$ cells grown on collagen-coated filters that permitted access of medium to both sides of the monolayer. The rates of basolateral uptake of both 1 mM glucose (K_m 3.6 mM) and 1 mM 2dglc (K_m 1.5 mM) were greater than apical uptake rates and the (apical-to-basolateral)/(basolateral-to-apical) flux ratio was high for glucose (9.4) and low for 2dglc (0.8), thus, confirming the lack of interaction of 2dglc with the apical SGLT. Specific glucose transport inhibitor studies using phlorizin, phloretin and cytochalasin B confirmed the polarised distribution of SGLT and GLUT in LLC-PK$_1$ cells. Basolateral sugar uptake could be altered by addition of insulin (1 mU/ml) which increased 2dglc uptake by 72% and glucose uptake by 50% and by addition of 20 mM glucose to the medium during cell culture which decreased 2dglc uptake capacity at confluence by 30%. During growth to confluence, 2dglc uptake increased to a maximum, then decreased at the time of confluence, coincident with a rise in uptake capacity for α-methyl-D-glucoside, a hexose that interacts only with the apical SGLT. It was concluded that the non-metabolisable sugar 2dglc was a useful, specific probe for GLUT in LLC-PK$_1$ cells and that GLUT was localised at the basolateral membrane after confluence.

Introduction

Sugar uptake by mammalian cells occurs by three different routes: (1) simple diffusion through the lipid bilayer; (2) facilitated diffusion, or (3) Na^+-glucose cotransport [1]. Renal proximal tubule cells have been shown to have both an apical Na^+-glucose cotransport system [2,3] involving an SGLT transporter [4,5] and a basolateral Na^+-independent facilitated diffusion system [1,6] involving a GLUT-1 or GLUT-2 transporter isoform [4,7–10]. In order to simplify the study of transport mechanisms, cell culture systems have been established as models for renal epithelial transport [11]. The pig kidney cell line LLC-PK$_1$ has proven useful for studying the renal Na^+-glucose cotransport system [4,12–14] that is localised to the apical membrane of the cells [15]. The nucleotide sequence of SGLT has been determined in this cell line [4] and found to be homologous to the Na^+-coupled transporter SGLT1 of intestinal cells [5]. The Na^+-independent facilitated diffusion system of LLC-PK$_1$ cells involving the GLUT family of isoforms [7,8] has been found to be of the erythroid/brain type GLUT-1 [4,16]. None of the other GLUT isoforms were detectable in LLC-PK$_1$ cells [4]. GLUT-1 was originally cloned from human hepatoblastoma HepG2 cells by Mueckler et al. [9].

In uptake studies in LLC-PK$_1$ cells, dissociation of sugar transport from intracellular metabolism of the transported sugar has been possible through the use of non-metabolisable substrates such as α-methyl-D-glucoside (AMG) for SGLT1 and 3-O-methyl-D-glucose (OMG) or 2-deoxy-D-glucose (2dglc) for GLUT-1. GLUT-1 in LLC-PK$_1$ has been characterised by Mullin et al. [17] using 2dglc, a hexose that is phosphorylated in the cell but not metabolised further. Mullin et al. [18] have also succeeded in producing LLC-PK$_1$ mutants with reduced uptake capacities for 2dglc and these cell lines may prove useful in determining the role played by GLUT-1 in normal glucose reabsorption by the kidney. Various factors can alter the rate of facilitated glucose transport by mammalian cells, in-

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cluding glucose availability [1,4,16,17] and the presence or absence of insulin [1,7,19,20]. The aim of the present investigation was to first determine if GLUT-1 has a polarised distribution in confluent LLC-PK_1 cells by assessing unidirectional uptakes of 2dglc and their specific inhibitor sensitivities. Secondly, the aim was to compare differences in uptake between a non-metabolisable sugar (2dglc) that interacts only with GLUT-1 and a metabolisable sugar (glucose) that interacts with both GLUT-1 and SGLT1. A third aim was to determine the effects of insulin, extracellular glucose and cell density on the expression of GLUT-1 in culture.

Materials and Methods

Cell culture. LLC-PK_1 cells between passages 186 and 203 were maintained as described previously [12]. The culture medium consisted of Minimum Essential Medium (MEM, Flow Laboratories) without antibiotics and supplemented with 10% fetal bovine serum (FBS, Flow Laboratories) and vitamins as formulated for Eagle's Basal Medium. Cells were routinely passaged at weekly intervals using 0.05% trypsin, 0.02% EDTA.

Sugar transport. For sugar uptake measurements from the apical or basolateral medium, cells were grown in chambers consisting of a lucite ring with a collagen-coated (Ethicon) polycarbonate filter bottom (4.2 cm inside diameter) as previously described [21]. The ring was placed into a plastic culture dish, thus, separating the apical medium inside the ring from the basolateral medium outside the ring in the dish. This permitted unidirectional uptake measurements, as well as transcellular flux measurements. The ring was held in position slightly above the bottom of the culture dish by plastic tabs that extended over the outside edge of the dish, thus, allowing the medium in the dish to bathe the underside of the cell monolayer. For experiments with confluent cultures, rings were seeded with $5 \cdot 10^6$ cells per ring; cultures were refed every day, and uptake measurements were performed 3 days after seeding. Only confluent monolayers with a transepithelial potential difference of $\geqq 0.3$ mV (apical negative) were used in the study.

The standard saline for the sugar uptake measurements (Mopsal) consisted of (mM) 119 NaCl, 5.3 KCl, 6.6 sodium acetate, 2.8 $CaCl_2$, 1.3 $MgSO_4$, 2.0 KH_2PO_4, 6.6 $NaHCO_3$ and 9.3 sodium morpholinopropanesulfonate (Mops) at pH 7.22. Various additions were made to the apical or basolateral solutions according to the experimental protocol. D-[^{14}]glucose, 2-deoxy-D-[^{3}H]glucose (2dglc) and α-methyl-D-[^{14}C]glucose (AMG) were added to only one side of the ring for measurement of apical or basolateral uptake. The rings containing the cell monolayers were washed in Mopsal at room temperature and suspended in a 60-mm plastic culture dish containing 5 ml Mopsal (basolateral medium). An additional 5 ml of Mopsal was pipetted into the ring (apical medium) and the ring was placed on a Clinical Rotator (Thomas, Philadelphia, PA) at a slow speed and incubated at room temperature. The transcellular flux of isotope during an incubation was monitored as previously described [21].

At the finish of an incubation, the ring was taken out of the dish and adherent uptake solution was removed by aspiration. The rings were then washed 3 times in successive changes of ice-cold Mopsal containing 200 μM each of phloretin (Pht) and phlorizin (Phz), followed by a brief aspiration of adhering wash solution. The ring was then immediately placed cell side up onto filter paper (Whatman No. 1) soaked in ice-cold Mopsal sitting on top of a cold, solid metal cylinder. The filter with its attached cells was cut free, divided in half and each half transferred to a separate centrifuge tube containing 2 ml of 0.4 M perchloric acid (PCA). The complete washing procedure required about 40 s.

Analysis of PCA extracts. Radioactivity and DNA content of the cells were measured as previously described [12]. The values obtained for both half filters from a single ring were averaged and presented as a single datum point in the results. For distinguishing between free sugar and sugar phosphate, 1.5 ml of the 0.4 M PCA extract following freeze-thawing and DNA precipitation [12] was neutralized with 2 M KOH using 0.2% Bromthymol blue as a pH indicator. After centrifugation for 5 min in a Dynac tabletop centrifuge (Clay Adams) at speed setting No. 50, 0.5 ml of supernatant was removed and the radioactivity in the sample counted for estimation of total sugar in the extract. Sugar phosphates were then precipitated by addition of 0.5 ml of 5% $ZnSO_4$ followed by addition of 0.5 ml of 0.3 M $Ba(OH)_2$. After mixing, the samples were left at room temperature for 20 min, then centrifuged as before. A 1 ml sample of supernatant was counted for radioactivity to estimate free sugar in the sample. Sugar phosphate was taken as the difference between total sugar and free sugar. For a metabolisable sugar like glucose, the non-precipitable radioactivity will not be equal to the free glucose concentration in the cell because the supernatant will also contain the metabolites lactate, alanine, or glutamate [18,22]. Chromatographic analysis on Whatman 3MM paper using 4:1 isopropanol/water as solvent was, thus, used to determine the amount of intracellular free glucose.

The concentration of sugar in cell water (H_2O) was calculated from cell volumes determined by OMG uptake or from DNA content using a conversion factor of $0.98 \cdot 10^{-4}$ ml cell water per μg DNA [12]. DNA was measured colorimetrically by the diphenylamine reaction of Burton [23].

Statistical analysis. All data are presented as the mean ± S.E. Statistical significance of the data at the

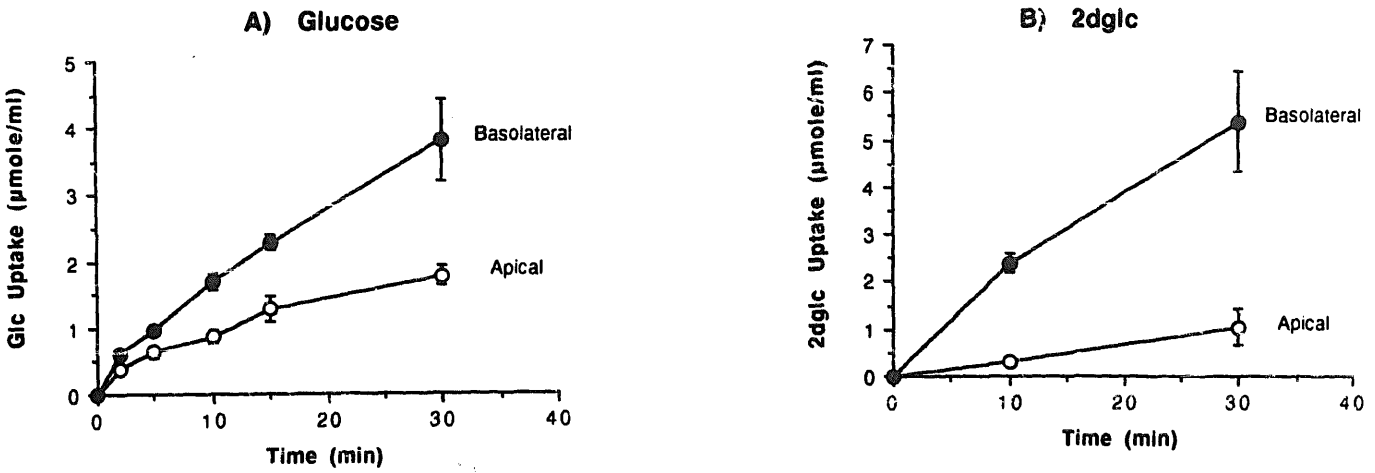

Fig. 1. Polarised sugar uptake by LLC-PK$_1$. The unidirectional uptakes of 1 mM D-glucose (A) and 2-deoxy-D-glucose (2dglc) (B) by confluent monolayers of LLC-PK$_1$ cells grown on collagen-coated rings are presented as the mean uptakes of total sugar (µmol/ml cell water) ± S.E. The sample sizes are 4 for glucose and 4–18 for 2dglc. Uptake was at room temperature.

95% confidence level was determined by the unpaired Student's *t*-test or by analysis of variance (ANOVA).

Materials. All chemicals used were analytical grade. Unlabeled sugars and inhibitors were from Sigma. Phloretin-2'-(2-deoxy-D-glucoside) was synthesized by Dr. R.F. Sullivan (Smith, Kline & French Laboratories) and its structure was confirmed by C and H analysis, mass spectrometry and determination of 2-deoxy-D-glucose in an acid hydrolysate. The purified material used here was a gift from Mr. L. Petka, Research and Development Division, Smith Kline & French Laboratories, Phildelphia, PA). 2-deoxy-D-[1-^{3}H]glucose was purchased from Amersham and D-[^{14}C]glucose ([^{14}C]glucose)(U), α-methyl-D-[^{14}C]glucopyranoside ([^{14}C]glucose)(U) and 3-*O*-methyl-D-[^{14}C]glucose ([^{14}C]glucose)(U) were purchased from New England Nuclear. LLC-PK$_1$ cells were originally obtained from Dr. R.N. Hull [24].

Results

Polarity of sugar uptake by LLC-PK$_1$

D-Glucose or 2-deoxy-D-glucose (2dglc) were added to the apical or basolateral side of confluent monolayers of LLC-PK$_1$ cells (Fig. 1). In saline containing a normal Na^+ concentration of 141.5 mM, the basolateral uptake rate was greater than the apical rate for both sugars. The rate of uptake of 2dglc from the apical side was very low and may in part be explained by 2dglc movement between cells followed by transport into the cells across the basolateral membrane. This explanation is supported by the fact that most apical 2dglc uptake was phloretin sensitive (Fig. 2). Sugar concentrations were varied and basolateral uptake rates were measured after incubations of 30 min (2dglc) or incubations of 3 min (glucose) at room temperature. An Eadie-Hofstee plot for 2dglc uptake gave a high affinity, low capacity uptake component with K_m and V_{max} values of 1.5 mM and 290 nmol/ml per min, respectively (one experiment, $n = 5$–11 rings at each of seven 2dglc concentrations). A low-affinity, high-capacity component of uptake was also present and presumably represented passive uptake. K_m and V_{max} values were also estimated for D-glucose uptake and found to be 3.6 mM and 5320 nmol/ml per min, respectively (mean of 2 experiments, $n = 4$ at each of five glucose concentrations).

Various sugar transport inhibitors were tested for their ability to inhibit apical or basolateral uptake. The effects of phlorizin (Phz), phloretin (Pht) and cytochalasin B (CB) on 10 min glucose and 2dglc uptake

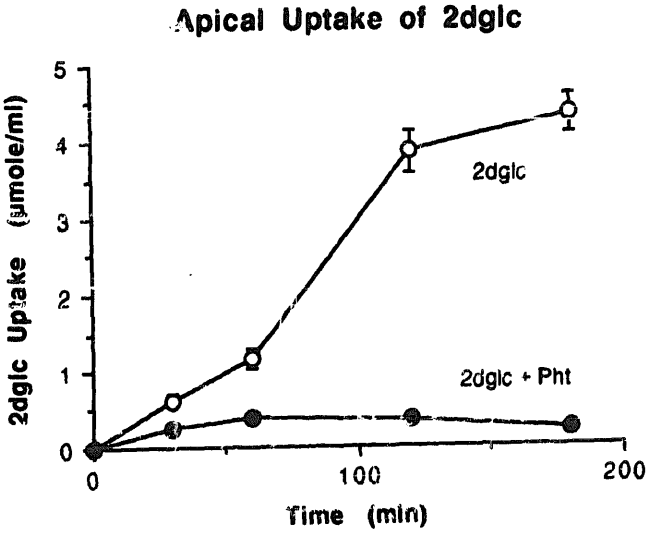

Fig. 2. Effect of basolateral phloretin on apical uptake of 2dglc. Uptake conditions were similar to Fig. 1. Uptake is expressed as total sugar in µmol/ml cell water (± S.E.). 1 mM 2dglc was presented to the apical side of the ring in the presence ($n = 4$–6) or absence ($n = 7$–11) of 200 µM phloretin added to the basolateral side only. Data were collected from two or three separate culture experiments.

rates are shown in Fig. 3. Uptakes were only measured for 10 min to reduce sugar crossing the monolayer and entering the cells from the opposite membrane. Apical uptake of glucose was greatly inhibited by Phz and Pht; apical 2dglc uptake was slightly blocked by Pht but not affected by CB (Fig. 3a). The slight inhibition by Pht may be due to basolateral uptake after paracellular flux across the monolayer as discussed for Fig. 2. In Fig. 3b it can be seen that basolateral uptake of both glucose and 2dglc is highly sensitive to Pht and CB. Phz had no effect on the basolateral uptake of glucose in a short 10 min incubation. A K_i for CB was calculated to be 3.9 μM, based on an experiment in which the medium concentration of CB was varied from 0.05 μM to 50 μM (data not shown). These CB results are similar to those reported previously [17].

Replacement of the glucose moiety in phlorizin by 2dglc to produce 2dglc-phloretin reduced the effect of this inhibitor on apical uptake of AMG, but the potency of 2dglc-phloretin on basolateral uptake of glucose (total sugar) was similar to that of Pht on its own (Table I). 2dglc-phloretin had a much reduced potency compared with Pht on basolateral uptake of 2dglc. The data indicate that for an interaction of Phz with SGLT1, the presence of the entire glucose moiety, including a free hydroxyl group on C-2, is required. On the other hand, the glucose moiety plays no role in the interaction of the phloretin moiety with GLUT-1 at the basolateral border.

OMG is known to be transported by GLUT but not by SGLT in kidney. Its ability to compete with basolateral glucose for uptake into LLC-PK$_1$ cells is shown in Table II. OMG inhibited glucose uptake in a dose-dependent manner.

TABLE I

Phloretin, 2-deoxyglucose-phloretin and phlorizin effect on uptake of sugars by LLC-PK$_1$

Rings were incubated at room temperature for 60 min. 2dglc-phloretin refers to phloretin-2'-(2-deoxy-D-glucoside). Sugars are present at 1 mM and inhibitors are present at 100 μM. Mean values ± S.E. of total sugars (free sugar + sugar phosphate + other metabolites) (n = 4–6) are given in μmol/ml cell H_2O. Significance vs. control; * $P < 0.02$; ** $P < 0.01$.

	Inhibitor	Total sugar uptake (μmol/ml H_2O_i)
Apical		
α-Methyl-D-glucoside	none (control)	3.01 ± 0.10
	phlorizin	0.10 ± 0.01 **
	phloretin	1.66 ± 0.06 **
	2dglc-phloretin	2.47 ± 0.11 *
Basolateral		
D-Glucose	none (control)	7.5 ± 0.13
	phloretin	3.9 ± 0.10 **
	2dglc-phloretin	4.6 ± 0.21 *
2-Deoxy-D-glucose	none (control)	21.0 ± 0.5
	phloretin	1.1 ± 0.05 **
	2dglc-phloretin	13.0 ± 0.8 **

Transcellular fluxes

The fluxes of 2dglc and glucose across LLC-PK$_1$ monolayers were measured as previously described for AMG [21]. The flux ratio for glucose, apical to basolateral (J_{ab}) vs. basolateral to apical (J_{ba}) was 9.4 (Fig. 4a), whereas the ratio of respective fluxes for 2dglc (0.8) was much lower (Fig. 4b). The low J_{ab} flux for 2dglc may represent movement through the paracellular pathway.

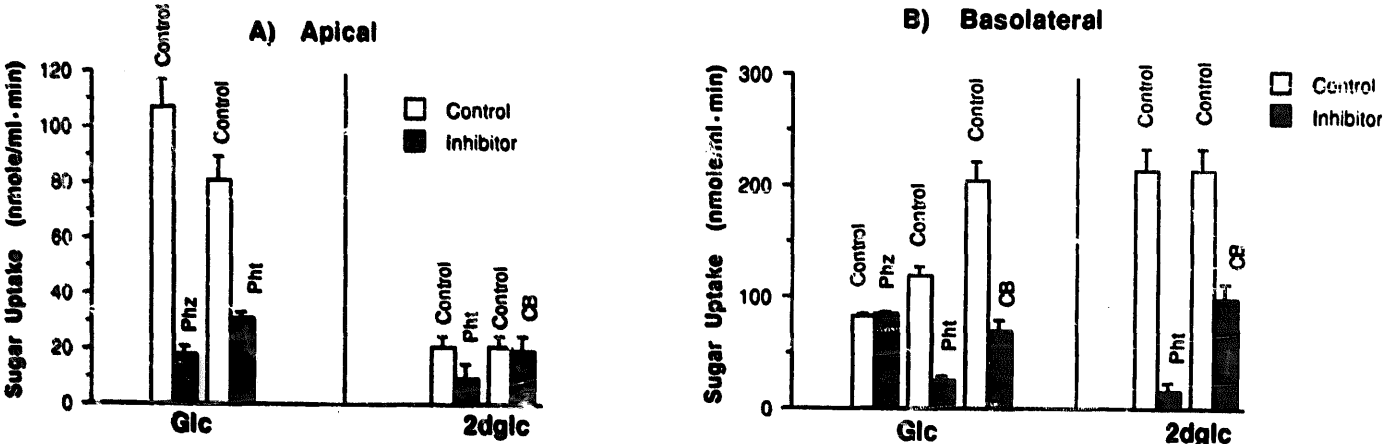

Fig. 3. Inhibitor sensitivity of polarised sugar uptake by LLC-PK$_1$. Apical (A) and basolateral (B) unidirectional D-glucose (Glc) and 2-deoxy-D-glucose (2dglc) uptakes were measured in confluent cell cultures grown on collagen-coated rings in the presence and absence of inhibitors. Incubation was for 10 min at room temperature. Inhibitors for 1 mM glucose uptake were 100 μM phlorizin (Phz), 100 μM phloretin (Pht), or 40 μM cytochalasin B (CB). Inhibitors for 1 mM 2dglc uptake were 200 μM Pht and 5 μM CB. Sample size was 4 – 6 for glucose and 5 – 18 for 2dglc. Values are given as the mean uptake of total sugar (nmol/ml cell water per min) ± S.E.

TABLE II

Effect of 3-O-methyl-D-glucose on basolateral uptake of glucose by LLC-PK$_1$

OMG concentration (mM)	Glucose uptake (μmol/ml H_2O_i)	(n)
0	1.88 ± 0.24	5
1	1.60 ± 0.09	4
5	1.18 ± 0.06 *	4
10	0.93 ± 0.04 **	4

Rings were incubated at room temperature for 15 min. Glucose was present at 1 mM and 3-*O*-methyl-D-glucose (OMG) as indicated. Mean values ± S.E. of total sugar are given in μmol/ml cell H_2O. Significance vs. control (no OMG); * $P < 0.05$; ** $P < 0.02$.

Intracellular free sugar values

After uptake, most intracellular 2dglc and glucose is present as phosphorylated sugar. In the control experiments of Table I, 2dglc-phosphate represented 89% of the total intracellular 2dglc in the cells after 1 h of uptake and glucose 6-phosphate represented 75% of the total intracellular glucose after 1 h of uptake. Apical or basolateral 1 mM sugar uptakes of 1–2 h incubation were carried out to ensure that uptake had reached steady-state conditions and 'free sugar' and phosphorylated sugar were compared in the cells. In the case of a non-metabolised sugar like AMG, SGLT1 is capable of setting up a 3-fold concentration gradient in LLC-PK$_1$ (Table I). In normal Na^+-containing medium, intracellular 'free glucose' under steady-state conditions, however, was not different from extracellular (1.11 ± 0.04 mM, $n = 6$) when glucose was presented to the apical side of the cell but it exceeded extracellular when it was presented to the basolateral side (2.5 ± 0.19). Similar results were found for 2dglc. Intracellular 2dglc was 0.81 ± 0.19 mM (apical) and 2.29 ± 0.20 mM (basolateral). In order to test whether the 'free glucose' concentration in the cell during apical uptake could be increased by blocking efflux, total and free sugar values were measured after 60 min uptakes in the presence or absence of 40 μM CB. Total glucose was unaffected by CB treatment (3.37 ± 0.17 vs. 3.61 ± 0.16 in the presence of CB); whereas a slight increase in the steady-state 'free glucose' concentration was observed after CB treatment (1.11 ± 0.04 vs. 1.37 ± 0.04 in the presence of CB) ($n = 6$, $P < 0.001$). Calculations of actual free glucose levels in cell extracts after a 60 min uptake by chromatography analysis gave true intracellular sugar to extracellular sugar ratios of only 0.10 ± 0.03 ($n = 4$) for apical uptake and 0.55 ± 0.13 ($n = 4$) for basolateral uptake in the absence of CB. These results may be artificially elevated due to extracellular 'unrinsed' free glucose.

Insulin effect on sugar uptake

Insulin stimulates the transport of sugars across the basolateral membrane of LLC-PK$_1$ cells, as shown in Fig. 5. Both glucose and 2dglc uptake from the basolateral side of the monolayer were significantly increased after treatment of the cells with 1 mU/ml insulin ($P < 0.02$ for glucose and $P < 0.0001$ for 2dglc). Insulin, however, had no effect on the apical uptake of AMG although it slightly, but non-significantly, stimulated the apical uptake of glucose, possibly by an effect on glucose metabolism. Postulating a role for protein kinase A in the above insulin effects, results not reported here in detail showed a relatively small stimulatory effect of 1 mM dibutyryl cAMP on both the apical and basolateral uptakes of glucose and a significant

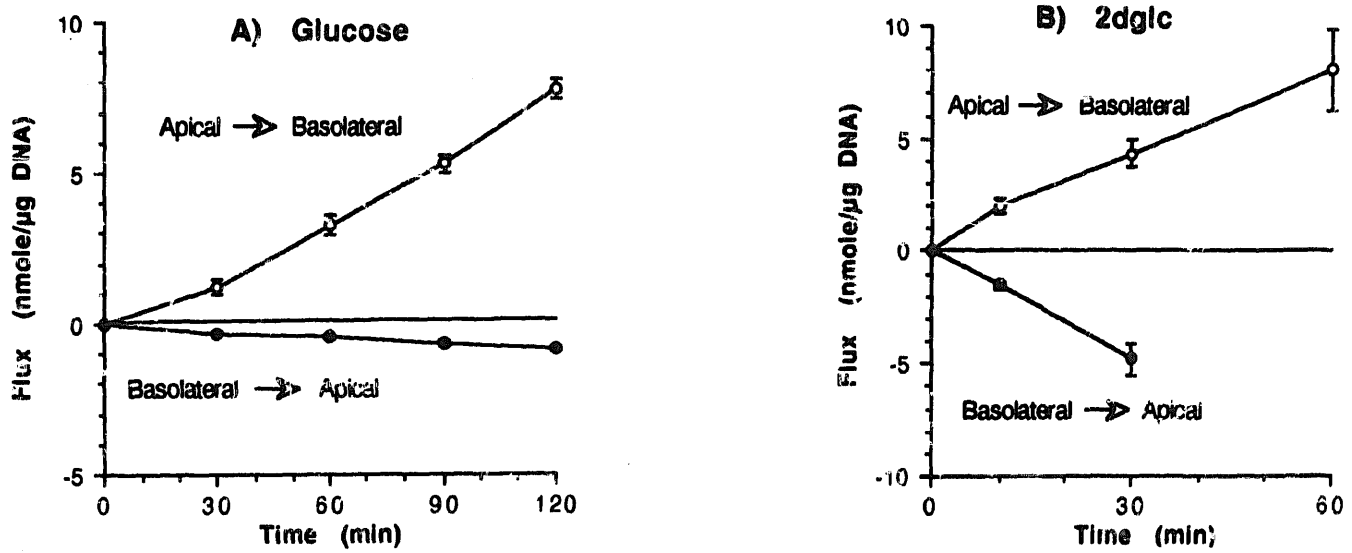

Fig. 4. Transcellular fluxes of glucose and 2-deoxy-D-glucose across LLC-PK$_1$ cell sheets. Transcellular fluxes across confluent LLC-PK$_1$ monolayers grown on collagen-coated rings of D-glucose (A) and 2-deoxy-D-glucose (2dglc) (B) were measured at room temperature. Sugars were present at 1 mM on one side of the monolayer only. Fluxes are given as the mean value (nmol/μg DNA) ± S.E. Sample size equals 6–29 rings.

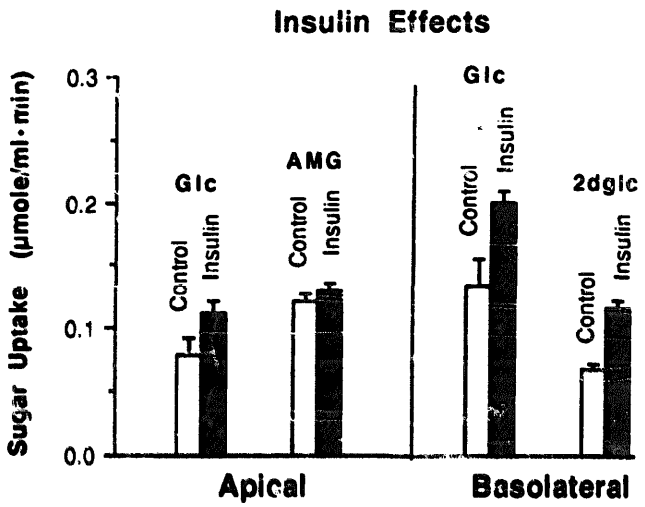

Fig. 5. Insulin effects on glucose, 2-deoxy-D-glucose and α-methyl-D-glucoside uptake by LLC-PK$_1$ cells. Insulin (1 mU/ml) was added to the same side of confluent LLC-PK$_1$ monolayers grown on collagen-coated rings as the sugar and unidirectional uptakes of D-glucose (Glc), 2-deoxy-D-glucose (2dglc), and α-methyl-D-glucoside (AMG) were measured. Sugar was present at 1 mM and uptakes were measured at room temperature for 10 min (Glc), 30 min (2dglc), or 60 min (AMG). Insulin was added at the same time as isotope. Values are expressed as total sugar taken up (μmol/ml cell water per min) ± S.E. Sample size equals 6 rings.

inhibitory effect of 10 mM theophylline on the basolateral uptake of glucose.

Development of sugar transport polarity in culture

Before LLC-PK$_1$ cultures attain confluence, Phz-sensitive AMG uptake is virtually absent whereas Pht-sensitive 2dglc uptake is high (Fig. 6). At confluence (day 7), AMG uptake begins to increase as SGLT1 is inserted in the apical membrane [15] and 2dglc uptake begins to decrease. The decrease of the latter may be due to segregation of GLUT-1 to the basolateral side of the cells. Extracellular glucose can also affect the development or activity of the sugar transport systems in culture. LLC-PK$_1$ cells maintained in culture in 25 mM glucose compared with 5 mM glucose have a significantly reduced capacity (30% decrease) for taking up 2dglc (Table III). AMG uptake was also decreased but not significantly. Since glucose was not present during the uptake measurements, this reduced uptake was not a competitive effect but presumably represented down-regulation of transporters and/or a decrease in their activity or their affinity for hexoses.

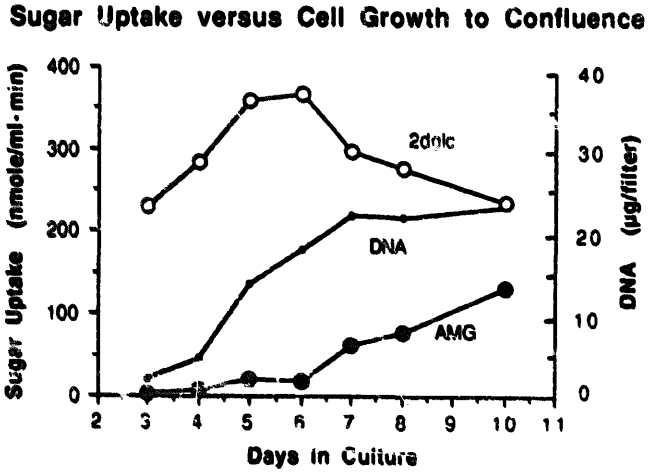

Fig. 6. Sugar uptake by LLC-PK$_1$ cells vs. days in culture. At day 0, collagen-coated rings were seeded with $2 \cdot 10^5$ cells/cm^2 and cultures were refed daily. Beginning at day 3, sugar uptakes were measured for 1 mM 2-deoxy-D-[^{3}H]glucose (2dglc) in the presence and absence of 0.1 mM Pht and for 1 mM α-methyl-D-[^{14}C]glucoside (AMG) in the presence and absence of 0.2 mM Phz. Uptakes were at room temperature for 30 min (2dglc) or 60 min (AMG), respectively. Cultures were confluent at day 7. The mean DNA content per ring (μg DNA/filter) is given ($n = 5$). Pht and Phz-sensitive sugar uptakes from two separate culture experiments are presented as the mean value (nmol/ml H_2O_i per min) from 4 rings for 2dglc and 2 rings for AMG.

TABLE III

Effect of growth in high glucose medium on sugar uptake by LLC-PK$_1$

	2dglc uptake (n) (μmol/ml cell H_2O per min)	AMG uptake (n) (μmol/ml cell H_2O per min)
Control	0.27 ± 0.02 (4)	0.15 ± 0.05 (2)
High glucose	0.19 ± 0.01 (4) *	0.12 ± 0.03 (2)

Cells were grown on rings in standard medium (control) containing 5 mM D-glucose or in standard medium supplemented with 20 mM D-glucose (25 mM final concentration). Cultures were refed daily. For uptake of sugars, rings were incubated at room temperature for 10 min in the case of 2dglc uptake or 60 min in the case of AMG uptake. Mean values ± S.E. of 200 μM phloretin-sensitive uptake (2dglc) or 200 μM phlorizin-sensitive uptake are presented as μmol/ml cell H_2O per min. Significance versus control: * $P < 0.05$.

Discussion

A family of five or more mammalian glucose transporters of the facilitated diffusion type have now been identified and the kidney appears to have at least four of these types, GLUT-1, GLUT-2, GLUT-3, and GLUT-5 [1,4,7–10]. Recent work has identified the facilitated system in LLC-PK$_1$ as the GLUT-1 isotype [4] which is also known to be present in renal proximal tubule cells [10]. The present study is consistent with this conclusion, since the low K_m for 2dglc uptake (1.5 mM) indicates a high affinity transport system consistent with the high affinity GLUT-1 isoform [10]. The LLC-PK$_1$ cell line originally isolated by Hull et al. [24] has proven to be an effective model for investigating sugar uptake by the renal proximal tubule. The cell line possesses an apical SGLT1 for Na^+-glucose entry [2,4,12–14] and a Na^+-independent GLUT-1 system for sugar efflux [4,16–18]. Although the cell line has the sugar transport features characteristic of the proximal tubule cell, it may be considerably modified in other aspects, including the ability to carry out gluco-

neogenesis [13] or transport organic acids [25]. Mullin et al. [17] characterised Na^+-independent sugar transport in LLC-PK_1 cells using the non-metabolisable hexose 2dglc. In the present study, GLUT-1 was investigated further for both 2dglc and D-glucose transport and its basolateral location was firmly established. Basolateral uptake of 2dglc and glucose was much faster than apical uptake (Fig. 1) and the inhibitor sensitivities (Fig. 3) and insulin effects (Fig. 5) of the unidirectional uptakes indicated unique differences between the apical and basolateral borders of the cells, thus, confirming the polarised distribution of the transporters. Similar inhibitor sensitivities of Na^+-independent hexose uptake have been reported for LLC-PK_1 cells [17] and for basolateral membrane vesicles isolated from rabbit kidney cortex [6].

Phosphorylation of both glucose and 2dglc is extensive and rapid; therefore, the rate-limiting step in uptake appears to be the membrane-transport step [17]. This conclusion is supported by the fact that the K_m for hexokinase-induced 2dglc phosphorylation (27 μM) [26] is much lower than that for 2dglc uptake (1.5 mM). In addition, a hexose that is not phosphorylated by intracellular hexokinase, OMG, reduces the rate of basolateral glucose uptake, presumably by competitive inhibition (Table II). 2dglc (K_m 1.5 mM) was a better competitor of its own uptake than glucose (K_m 3.6 mM). The glucose K_m is somewhat less than the 9.9 mM reported for Na^+-independent D-glucose uptake into basolateral membrane vesicles of the rabbit [6]. Apical uptake of 2dglc, although minimal, could be explained either by leak across the monolayer followed by basolateral uptake, hence its Pht sensitivity (Fig. 2), or by passive diffusion. A lack of apical interaction of 2dglc in LLC-PK_1 differs from the studies by Silverman [3] in the dog kidney, indicating that 2dglc was capable of interacting only with the brushborder transporter (GLUT) and not the antiluminal system (SGLT). In LLC-PK_1 cells, 2dglc appears to be an effective and specific probe of GLUT-1.

Unlike 2dglc, D-glucose is presumably capable of interacting with both the apical SGLT1 and basolateral GLUT-1. Previous studies have confirmed that glucose interacts with the apical Na^+-glucose cotransporter of LLC-PK_1 cells [12,14]. Since glucose can interact with both transporters, it was necessary to confirm that the basolateral glucose uptake seen in the present study was not occurring by passage through the cell sheet and uptake by SGLT1. This appeared not to be the case, since the non-metabolisable sugar OMG, which does not interact with SGLT1 [12], was able to compete for glucose uptake at the basolateral side of the cell sheet (Table II). In addition, the lack of effect of Phz on basolateral glucose uptake and its high sensitivity to Pht (Fig. 3b) confirmed that glucose entry into the cell from the basolateral side was via GLUT-1. As further evidence, a non-metabolisable sugar transported exclusively by the apical SGLT1, AMG (10 mM), had no effect on the basolateral uptake of 1 mM glucose (data not presented), thus, providing additional support for the conclusion that basolateral D-glucose uptake is via GLUT-1. The physiological significance of this uptake in intact renal tubules is not known; however, it may be nutritional, especially in those portions of the nephron where luminal glucose has already been absorbed. Although proximal tubule cells in the kidney have a high capacity for gluconeogenesis [20,27], most LLC-PK_1 cells lack fructose 1,6-biphosphatase activity required for this process, although some gluconeogenic strains have been isolated from LLC-PK_1 cultures [28]. In the intact kidney, renal proximal tubule cells show little glycolytic activity relative to thick ascending limb or distal tubule cells [20,27]. The capacity of the proximal tubule to produce ATP from glucose (132 nmol/mg protein per min) is only about one third that of medullary thick ascending limb (390 nmol/mg protein per min) [27]. Mullin et al. [18,22] showed significant glycolytic conversion of glucose to lactate and subsequent conversion of lactate to alanine in LLC-PK_1. Various studies have demonstrated a close linkage between glucose metabolism, energy state and solute transport in renal cells [20,27]. It is not known to what extent glucose uptake by proximal cells is involved in cell nutrition during the process of glucose reabsorption; however, in renal cortex approximately 18% of metabolism is supported by glucose [29]. Glucose that enters the metabolic pool may enter the cell from either border, and therefore, at least part of the transported glucose enters the metabolic sink. Thus, uptakes and transcellular fluxes will be affected by both the actual rate constants for transport as well as the rate of glucose metabolism, the latter affecting intracellular concentrations of glucose.

Under steady-state conditions, the intracellular free sugar concentration for 2dglc exceeds the extracellular. Uphill (2–3 fold) transport of 2dglc at the basolateral side in LLC-PK_1 is consistent with studies on basolateral uptake of 2dglc in flounder kidney [30]. The mechanism of apparent uphill transport by a facilitated diffusion system remains to be elucidated. In the case of glucose, correction of the 'free' glucose value for its metabolised products indicates that the actual free glucose concentration in the cell is well below the extracellular concentration, as pointed out previously by Mullin et al. [18]. This argues against a standard interpretation of the Crane-Schultz model of glucose transport. It is difficult to propose a model for transcellular glucose transport (J_{ab}) if the intracellular free glucose concentration is below 1.0, since the basolateral exit of glucose would appear to proceed via a facilitated diffusion system against its own concentration gradient. Perhaps compartmentation of trans-

ported glucose could explain this paradox. Compared to glucose uptake, AMG is concentrated inside LLC-PK_1 cells (Table I). This difference may be explained on the basis of complete lack of AMG efflux via the basolateral transporter [21]. The intracellular 'free' glucose concentration resulting from apical uptake of glucose was not greatly enhanced, however, by blocking glucose efflux across the basolateral border with 40 μM CB.

The J_{ab}/J_{ba} transcellular flux ratios for glucose and 2dglc were 9.4 and 0.8, respectively (Fig. 4). The glucose flux ratio is only about 2/3 of the AMG ratio of 15 [21], possibly because less glucose is offered to the basolateral side for efflux from the cell than in the case of AMG due to a portion of cell glucose being diverted to metabolism. In addition, glucose, but not AMG, is able to enter the cell via the basolateral GLUT-1, thus, increasing the J_{ba} flux component. The very low flux ratio for 2dglc is as expected, since 2dglc does not interact with SGLT1 in LLC-PK_1 cells and, therefore, can only enter the cell rapidly from the basolateral side. Exit into the apical medium is presumably by passive diffusion of free sugar.

Up-regulation and down-regulation of GLUT isoforms have been studied in various cell lines [1] and serum glucose and various growth factors can lead to changes in gene transcription and mRNA accumulation. In LLC-PK_1 glucose availability during the growth and maintenance of the cells can affect the rate of glucose transport by both SGLT1 [4,16,31,32] and GLUT-1 [4,17]. Withdrawal of glucose from the medium surrounding LLC-PK_1 cells [4,16,17], as in other cell lines [1], during culture causes an increase in transport rate [17] and an increase in GLUT-1 mRNA [4]. In the present study, high glucose (25 mM) during culture had the expected opposite effect on the uptake of 2dglc and glucose, causing decreases in the uptake capacities at both the apical and basolateral cell borders (Table III).

Insulin is known to affect glucose metabolism and glucose uptake in a number of cell types, especially skeletal muscle and adipocytes where it is believed to increase a unique population of transporters of the GLUT-4 isoform that are not present in the plasma membrane in the non-hormone-stimulated state [1,7]. In adipocytes GLUT-1 is also increased by insulin although to a much lesser extent [1]. Insulin, however, seems to have little effect on renal cells. Some studies have shown that insulin may stimulate Na^+ transport and phosphate transport and inhibit gluconeogenesis [19,20]. There are no known direct effects of insulin on glucose metabolism although there is some evidence that the Na^+ transport effect referred to above is glucose-dependent [20] and involves an alteration of the apical Na^+/H^+-exchanger [19]. There is also no clear evidence in the literature for a direct insulin effect on glucose uptake by renal cells, although such an effect has been postulated [19,33]. In the present study, insulin directly stimulated 2dglc and glucose uptake by LLC-PK_1 cells (Fig. 5), but it was only effective when applied to the basolateral side of the cell. This finding supports more indirect evidence on the localisation of the insulin-sensitive transport step in renal cortical cells in flounder kidney [33]. The extent of stimulation (72%) was much less than in insulin-regulated cells like adipocytes where 8-fold or greater enhancements are observed [1]. It seems unlikely, therefore, that insulin is stimulating insertion of GLUT-1 in the basolateral membrane in a manner similar to the insertion of GLUT-1 and GLUT-4 in adipocytes. Therefore, the effect of insulin on 2dglc uptake may be relatively non-specific, involving effects on metabolism or on Na^+ or phosphate transport [19].

Growing cells and terminally differentiated cells have very different sugar transport profiles. It was previously shown that confluent LLC-PK_1 cultures have low uptake capacities for 2dglc compared to subconfluent cultures [17] whereas AMG uptakes are greater in confluent cultures compared to growing cells [12]. In the present study, Pht-sensitive 2dglc uptake increased to a peak activity just before confluence and then fell after confluence when the cells terminally differentiated (Fig. 6). This pattern is similar to the profile for basolateral amino-acid uptake in this cell line [34].

In conclusion, the GLUT-1 Na^+-independent 2dglc transport system of LLC-PK_1 cells, originally described by Mullin et al. [17,18], was shown to be localised at the basolateral border of confluent cell monolayers. Even in normal Na^+-containing medium, there was no interaction of 2dglc with the apical Na^+-glucose co-transporter SGLT1, thus, confirming the usefulness of 2dglc as a specific probe for GLUT-1 in LLC-PK_1 cells. The transport system was responsive to insulin and capable of transporting D-glucose in a similar manner to 2dglc. The development of 2dglc and AMG transport activity during culture was dependent on cell growth and differentiation and could also be affected by the glucose concentration in the culture medium.

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