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Characteristics of Na^+-dependent hexose transport in OK, an established renal epithelial cell line

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The characteristics of Na^+-dependent hexose uptake were determined for monolayers of OK, an established renal epithelial cell line derived from an opossum kidney. A comparison is made with other cultured cells, particularly LLC-PK_1. The capacity to accumulate α-methyl D-glucoside (AMG) in OK cells develops with time, reaching a maximum level of 18 nmol/mg protein per h, 3 days after confluency. In contrast to LLC-PK_1, this level is not influenced by the medium D-glucose concentration. AMG uptake in OK cells was characterized by an apparent K_m of 2.9 mM and a V_{max} of 17.1 nmol/mg protein per min. For Na^+-dependent phlorisin binding, a K_D of 0.025 μM and a B_{max} of 1.5 pmol/mg protein were found. A turnover frequency of 158/s was derived from our data. The hexose carrier of OK shares with the carrier of LLC-PK_1 a high level of expression, its substrate specificity and turnover frequency. It differs however with respect to the substrate binding site. The affinity for AMG and D-glucose is 3- and 10-fold lower, whereas the affinity for phlorizin is 3-times higher in OK than in LLC-PK_1. The Na^+ dependence of AMG uptake was also different for both cell lines and suggested for OK cells a 1:1, Na^+:substrate stoichiometry. In OK cells, the phlorizin-sensitive uptake rate of D-glucose is much lower than the one for AMG. Nevertheless, D-glucose interacts with the AMG binding site in a competitive way and with an affinity similar to AMG. This could indicate a malfunction of the carrier with D-glucose as a substrate at the level of the translocation step.

Introduction

The number of primary and established cell lines known to express a Na^+-coupled, phlorizin-sensitive hexose transporter is very limited [1]. This may be due to the fact that this carrier is not essential for survival of the cultured cells [2]. Most studies on sugar transport in cultured epithelial cells were done with LLC-PK_1, an established cell line derived from pig kidney. The characteristics of the Na^+-dependent hexose transport in this cell line are extensively reviewed [3–5]. Furthermore, Na^+-coupled sugar uptake was also described and characterized in primary cells from rabbit kidney [6–8] and in Caco-2, an adenocarcinoma cell line from human colon [9].Recently, two other renal established cell lines, JTC-12 [10] and OK [11,12] were shown to express Na^+-coupled sugar transport. JTC-12 was originated from a monkey kidney, while OK was derived from an American opossum kidney. For both cell lines, no kinetic characterization of the carrier was made. Malmström et al. [13] reported for the carrier of OK that it transports the non-metabolizable analog AMG, but not D-glucose.

The kinetics of Na^+-coupled sugar transport in renal and intestinal preparations have regained interest since it became evident that there is heterogeneity with respect to the type of carrier. For the kidney, evidence came from studies with isolated proximal tubule [14] and with cortical membrane vesicles [15,16]. The conclusion is that at least two systems with different biochemical characteristics are sequentially located along the proximal tubule. A high-capacity, low-affinity system with a Na^+: D-glucose stoichiometry of 1:1 is located in the early proximal tubule and a low-capacity, high-affinity system with a stoichiometry of 2:1 is found in the late proximal tubule. For intestine, no separation of brush-border membrane vesicles with different hexose transport systems could be achieved. Nevertheless, there is also evidence that two Na^+-dependent

Abbreviations: AMG, α-methyl-D-glucoside; 3OMG, 3-*O*-methyl-D-glucose; HBSS, Hanks' balanced salt solution; EMEM, Eagle's minimal essential medium; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; SDS, sodium dodecyl sulfate.

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transporters coexist [17,18]. Recent studies with vesicles from pig renal cortex [19] and from human fetal small intestine [20] suggest even more than two types of carrier with respect to their substrate affinity.

Cell cultures offer the opportunity to study the expression of a single type of hexose carrier. For LLC-PK_1, in particular, it was shown that only the high-affinity hexose carrier with a Na^+ : substrate stoichiometry of 2 : 1 is expressed [21–23]. In view of the observed heterogeneity, it was of interest to analyse in detail the properties of the hexose uptake in OK cells. We have performed this analysis in parallel for OK and for LLC-PK_1, which allows comparison in identical experimental conditions. This seemed to be important since literature data on LLC-PK_1 vary considerably.

Our data reveal that the carrier of OK shares properties with the carrier of LLC-PK_1, e.g., turnover frequency and carrier density. On the other hand, we find a significantly lower affinity for AMG uptake and no dramatic upregulation of the carrier level in response to a low D-glucose concentration in the medium. These two properties, together with a higher efflux rate of AMG, can account for the much lower steady-state accumulation of AMG for OK cells compared to LLC-PK_1. In contrast to the lower affinity for AMG, phlorizin is bound to the carrier of OK with a higher affinity. Although the interpretation of the Na^+ dependence is difficult in monolayer studies, our data are at least indicative of a 1 : 1, Na^+ : substrate stoichiometry in OK. Another point of interest was the poor transport of D-glucose reported for OK [13]. We found that, despite this, D-glucose interacts in a competitive manner with the AMG binding site of the Na^+-dependent hexose carrier. D-Glucose has an affinity for this site similar to that of AMG itself.

Material and Methods

LLC-PK_1 cells (American Type Culture collection, CRL-1392/CL 101) were obtained through Flow Laboratories and were used between passages 187 and 202. OK cells were a kind gift of Dr. J. Biber (Institute of Physiology, Zürich). They were used between passages 82 and 97. The cells were grown at 37 °C in a humidified 5% CO_2 atmosphere. The medium was EMEM supplemented with 3.5 mM L-glutamine, 0.9% (v/v) non-essential amino acids, 87 μg/ml streptomycin, 87 IU/ml penicillin and 10% fetal calf serum.

During the uptake procedure, the wells (12-well cluster, Costar, 3.8 cm^2) were fixed on a thermostated plate (37 °C) and placed on a mechanical shaker. The medium was aspirated and the monolayers were rinsed twice with 2.5 ml HBSS (Na^+ concentration: 140 mM) buffered to pH 7.4 with 10 mM Hepes. To each well 0.45 ml HBSS was added. The uptake was initiated by adding 0.05 ml of a stock solution containing ^{14}C-labelled AMG, ^{3}H-labelled 3OMG or ^{3}H-labelled D-glucose dissolved in HBSS. The final concentration of these sugars was 0.1 mM. The transport process was efficiently stopped by aspirating the medium and washing the monolayer twice with 2.5 ml ice-cold HBSS (total time, 20 s). The monolayers were solubilized in 2% SDS. Radioactivity was measured using Instagel II (Packard) as scintillation liquid. When the Na^+ concentration of the uptake medium was lowered, it was replaced by *N*-methyl-D-glucamine.

The Na^+-dependent phlorizin binding was determined at 37 °C in the presence or absence of 140 mM Na^+. The monolayers were first washed twice with 2.5 ml HBSS (+/− Na^+). Thereafter, 0.39 ml HBSS (+/− Na^+) was added. The binding study was started by adding 0.01 ml of a stock solution containing different concentrations of radioactive labelled phlorizin (0.6–6.6 μCi/ml). Before aspirating the medium after 12 min of incubation, a sample of this medium was taken. The monolayer was subsequently washed twice with 2.5 ml ice-cold HBSS (+/− Na^+) and solubilized in 2% SDS. Radioactivity was measured in a sample of the medium and in the solubilized cell material.

The D-glucose concentration in the growth medium was measured using a commercial test kit developed by Boehringer Pharma. The proteins were determined by the method of Lowry et al. [24] after precipitation of the proteins in 10% (w/v) ice-cold trichloroacetic acid.

[^{14}C]AMG, [^{3}H]3OMG and D-[^{3}H]glucose were from Amersham. [^{3}H]Phlorizin was from NEN. Nonradioactive AMG, 3OMG and phlorizin were from Sigma. D-Glucose was from Merck, phloretin was from Roth and cytochalasin B was from Aldrich. All other sugars were from Janssen Chimica.

Linear and non-linear fits were carried out using the Enzfitter (version 1.03) program (Elsevier Biosoft).

Results

Hexose transport in OK cells

The AMG uptake in OK monolayers is linear over the first 30 min and approaches a steady-state level after about 60 min (data not shown). When the initial velocity was required, an uptake period of 10 or 15 min was taken. In order to obtain a steady-state value, a 60 min uptake period was used. Qualitatively the same result is found for LLC-PK_1. We confirmed that the initial AMG uptake in OK cells is very low in the absence of Na^+ or in the presence of phlorizin [11,12]. In agreement with what was found for LLC-PK_1 [25], we conclude that uptake of AMG in OK cells occurs for more than 95% via the Na^+-dependent, phlorizin-sensitive pathway.

Compared to AMG, 3OMG is only a poor substrate for the Na^+-dependent hexose transporter [26]. Also for OK cells we find that 3OMG uptake is Na^+- and

phlorizin-insensitive (data not shown). 3OMG uptake is not a concentrative process and, as pointed out for LLC-PK_1 [27,28], the steady-state value can be used as an estimation for cell water. In the presence of 140 mM Na^+, the 3OMG space includes both cell water and the space under the monolayer (domes). We have found 8.4 and 14.4 μl/mg protein for OK and LLC-PK_1, respectively. In the absence of Na^+, the domes disappear. The values which then more closely reflect cell water were 7.3 and 8.6 μl/mg protein for OK and LLC-PK_1, respectively. This result also agrees with the observation that LLC-PK_1 develops more and larger domes than OK.

Development of the AMG accumulation and influence of medium D-glucose

We have evaluated the influence of the time in culture and of the D-glucose concentration in the medium on the steady-state accumulation in OK (Fig. 1A). The treatment was started after confluency was reached. This is done to avoid a possible effect of low or high D-glucose medium on the moment that confluency is reached. The D-glucose concentration was monitored throughout the experiment (Fig. 1B). The initial D-glucose concentration in the high D-glucose medium was 26.5 mM. The OK cells maximally lowered the medium D-glucose by 25%. This was accompanied by high acid production, as shown by the colour change of the medium. Medium with low D-glucose contained initially 1.4 mM D-glucose and the cells could lower the medium D-glucose to a level below the detection limit of our assay (less than 0.1 mM).

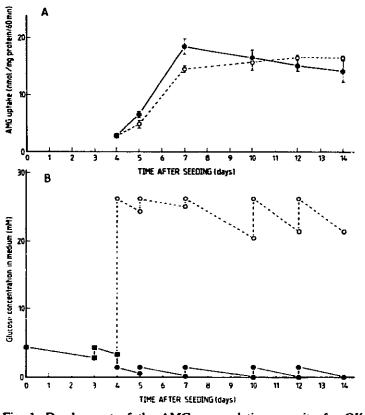

Fig. 1. Development of the AMG accumulating capacity for OK monolayers: Time dependence and influence of the medium D-glucose concentration. The same symbols are used to indicate the AMG uptake (A) and to give the respective D-glucose concentration in the medium (B). Cells were seeded on day 0 at a concentration of 20000 cells/cm^2. This was done in EMEM with a normal D-glucose concentration (approx. 5 mM, ■). On day 4, the medium was changed and two medium conditions were created: EMEM with a high D-glucose concentration (○) and EMEM with a low D-glucose concentration (●). A time period of 60 min was taken to attain the steady-state accumulation of AMG. Each point is an average of three determinations ± S.D.

Accumulation of AMG, in OK cells as well as in LLC-PK_1, only becomes detectable on day 4, at the time cultures reach confluency. From that moment on, the uptake capacity of OK increases to a maximal steady-state level of approx. 18 nmol/mg protein per h. This level is considerably lower than the highest uptakes found for LLC-PK_1. We found under the same conditions values for steady-state AMG accumulation in LLC-PK_1 between 100 and 150 nmol/mg protein per h (data not shown).

In LLC-PK_1, the steady-state accumulation level is affected to a large extent by D-glucose concentration in the medium [29]. The uptake level in OK is not changed by D-glucose concentration to the same extent as in LLC-PK_1. After 1 and 3 days of treatment, OK cells cultured in a low D-glucose medium showed a 36 and 28% higher uptake, respectively, compared to cells grown in a high D-glucose medium. After a longer period of treatment, no significant difference in AMG accumulation could be detected. Under the same conditions, we found for LLC-PK_1 after 1 and 3 days of treatment a 60 and 153% higher uptake, respectively (not shown). Reported effects for up- and downregulation in LLC-PK_1 amount to at least 5-fold higher uptake values in low compared to high D-glucose medium [30].

Na^+ : AMG stoichiometry

We have measured initial (15 min) AMG uptake in OK monolayers as a function of the Na^+ concentration in the uptake medium. The Na^+-dependent uptake follows a Michaelis-Menten-type dependence on the Na^+ concentration with a $V_{max} \pm$ S.D. of 4.0 ± 0.4 nmol/mg protein per min and an apparent $K_m \pm$ S.D. of 148 ± 24 mM (Fig. 2). If the data are analysed in a Hill plot (Fig. 2, inset), the slope ± S.D. of the least squares is 1.03 ± 0.03. An AMG concentration of 1 mM was used in order to approach more closely the saturation velocity. At 0.1 mM AMG, however, essentially the same behavior was found (V_{max} = 0.47 nmol/mg protein per min, K_m = 213 mM and n = 1.07). A similar experiment was done for LLC-PK_1 monolayers and this resulted in a Hill coefficient ± S.D. of 1.7 ± 0.3 (data not shown). This observation illustrates the difference between OK and LLC-PK_1 and is in favor of a different stoichiometry for both cell lines.

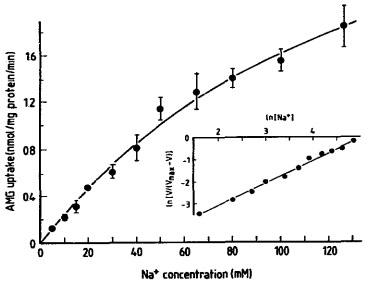

Fig. 2. Na^+-dependence of AMG uptake in OK monolayers. Cells were seeded at a concentration of 20000 cells/cm^2. Initial (15 min) uptakes were measured in the presence of 1 mM radioactive labeled AMG. The concentration of Na^+ was changed as indicated. Data points for Na^+-dependent AMG uptake were fitted by a Michaelis-Menten curve. The same data were analysed by a Hill plot (inset). V is the AMG uptake at a given Na^+ concentration and V_{max} was obtained from the Michaelis-Menten fit. Each point is an average of three determinations ± S.D.

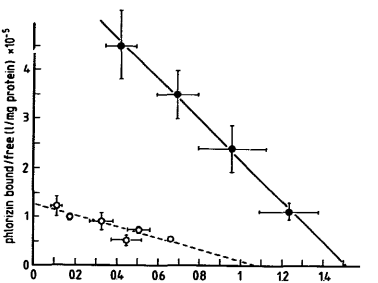

Fig. 3. Scatchard plot of the Na^+-dependent component of equilibrium [^{3}H]phlorizin binding to OK and LLC-PK_1 monolayers. Cells were seeded at a concentration of 20000 cells/cm^2. Binding was measured to 10-days-old OK (●) and 7-days-old LLC-PK_1 (○) cells for a phlorizin concentration range of 0.01–0.12 μM. For each point, phlorizin binding in the absence of Na^+ was subtracted from the corresponding value in Na^+-containing medium. Each value is an average of four determinations ± S.D. Straight lines were fitted to the data using least-squares determinations.

Kinetic analysis of AMG uptake

LLC-PK_1 cells, grown for 7 days in a low-D-glucose medium, show a 7-fold higher AMG accumulation than 10-days-old OK cells (Table I). This steady-state accumulation is dependent on four parameters: the affinity of the carrier for the substrate, the number of carriers, the turnover and the efflux rate.

The initial rate of AMG uptake (15 min) was measured as a function of the AMG concentration, ranging from 0.1 to 10 mM. Even at the highest AMG concentration, the AMG uptake was linear during the 15 min period. The kinetic parameters for AMG uptake in OK and in LLC-PK_1, measured under the same conditions, are summarized in Table I. The apparent K_m for AMG uptake is significantly higher for the carrier of OK as compared to the one of LLC-PK_1. It should be pointed out that AMG uptake in the presence of phlorizin (10 μM–1 mM; the ratio AMG/phlorizin was kept constant) contributes only a few % to the total uptake. This phlorizin-insensitive AMG permeability amounted to 0.1 μl/mg protein per min (data not shown).

The V_{max} is a function of the turnover frequency and of the number of carriers. This second parameter was determined by measuring the Na^+-dependent phlorizin binding after 12 min of incubation. It was verified that [^{3}H]phlorizin binding reached an equilibrium within 5 min (data not shown). The data for OK and for LLC-PK_1 are presented as a Scatchard plot (Fig. 3). The apparent dissociation constant (K_d) for Na^+-dependent phlorizin binding is significantly lower for OK (0.025 μM) than for LLC-PK_1 (0.086 μM). The $K_{0.5}$ for inhibition of AMG transport in OK cells by phlorizin is 0.035 μM. This value will be close to the K_i, since the substrate concentration (0.1 mM) is much below the apparent K_m for AMG uptake. Phlorizin thus binds with a higher affinity to the carrier of OK cells and

TABLE I

Summary of the kinetic data for the hexose carrier in OK and LLC-PK_1 cells

Cells were seeded at a concentration of 20000 cells/cm^2 in EMEM with an initial D-glucose concentration of 1 mM. The data were obtained after 7 days for LLC-PK_1 and after 10 days for OK.

	Accumulation at steady state (nmol/mg protein per h)	Apparent K_m ± S.D. (mM)	V_{max} ± S.D. (nmol/mg protein per min)	Efflux rate constant ± S.D. (1/h)
LLC-PK_1	118.4	1.1 ± 0.1	33.8 ± 2.3	0.35 ± 0.07
OK	16.6	2.9 ± 0.4	17.1 ± 0.9	0.69 ± 0.15

TABLE II

Influence of different sugars on the AMG uptake in OK and LLC-PK$_1$ cells

Initial uptakes (10 min) of [^{14}C]AMG (0.1 mM) were measured in the absence and presence of 10 mM of the different sugars. Averages ± S.D. of four measurements are given.

Sugar	AMG uptake relative to control ± S.D. (%)	
	LLC-PK$_1$	OK
Control	100 ± 10	100 ± 11
D-Fructose	99 ± 2	117 ± 24
D-Mannose	85 ± 13	93 ± 5
2-Deoxy-D-glucose	85 ± 13	93 ± 7
3OMG	94 ± 5	90 ± 13
D-Xylose	90 ± 6	86 ± 4
D-Galactose	31 ± 1	45 ± 9
AMG	10 ± 2	20 ± 2
D-Glucose	4 ± 1	21 ± 1

inhibits AMG uptake more efficiently than for LLC-PK$_1$ cells. From Fig. 3, it can also be observed that the number of phlorizin binding sites is slightly larger for OK (1.51 pmol/mg protein) than for LLC-PK$_1$ cells (1.09 pmol/mg protein). In a parallel experiment we have determined the V_{max} for AMG uptake in identical conditions as used for the phlorizin binding. From these data, turnover values were calculated. The values for OK (158/s) and for LLC-PK$_1$ (165/s) are nearly identical.

Steady-state accumulation is an equilibrium between influx and efflux. To determine the efflux rate, monolayers were incubated with radioactive labelled AMG for 1 h. Thereafter, the cells were transferred for different time periods to HBSS containing 10 μM phlorizin. An exponential curve was fitted to these data (not shown). In Table I the rate constants are given for both cell lines. The efflux rate constant for AMG is significantly larger for OK than for LLC-PK$_1$.

Effect of sugar analogs on AMG uptake

In order to determine the specificity of the Na$^+$-dependent hexose carrier of OK, initial uptakes (10 min) of 0.1 mM radioactive labelled AMG were measured in the presence of a number of sugar analogs at a concentration of 10 mM. The results are presented in Table II. D-Xylose had a slight inhibitory effect on AMG uptake. D-Galactose, D-glucose and AMG itself inhibited the uptake by 55, 80 and 79%, respectively. We found a similar result for LLC-PK$_1$, except that the inhibitory effect of D-galactose, D-glucose and AMG was larger: 69, 96 and 90%, respectively. The ratio of the inhibition by D-glucose and D-galactose is approximately the same for both cell lines.

We confirmed the effects of cytochalasin B and phlorizin on the initial uptake of D-glucose and AMG in OK cells [13]. Phlorizin inhibits AMG uptake almost completely, but has only a small inhibitory effect on D-glucose uptake (20%). On the other hand, D-glucose uptake is partly cytochalasin-B-sensitive (65%), while this inhibitor of the facilitated diffusion carrier has no effect on the AMG uptake in OK cells. Phloretin inhibited D-glucose uptake to the same extent as cytochalasin B (70%) and it was also found that inhibition by phlorizin was additive to the inhibition by either cytochalasin B or phloretin (cumulative inhibition: 85 and 87%, respectively).

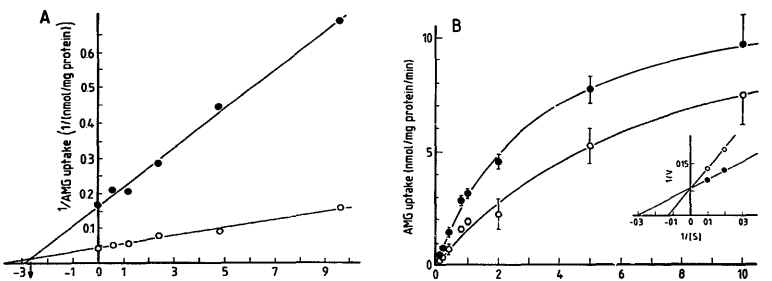

Fig. 4. Influence of D-glucose on AMG uptake in OK cells. (A) Initial (10 min) uptakes were measured in the presence of 0.1 mM (●) and 0.5 mM (○) radioactive labelled AMG. The concentration of D-glucose was varied as indicated. The results are presented as a Dixon plot. (B) The cells were seeded at a concentration of 15000 cells/cm^2. After 10 days, the apparent K_m and the V_{max} for AMG uptake were determined in the absence (●) and in the presence (○) of 2 mM D-glucose. Each point is an average of four determinations ± S.D. In both conditions, a Michaelis-Menten curve is fitted to the data. The inset shows a detail of the Lineweaver-Burk transformation of the Michaelis-Menten curves.

The phlorizin-sensitive part of the initial uptake of D-glucose in 12-days-old OK monolayers amounts to 0.03 nmol/mg protein per min. This is very low compared to the initial uptake of AMG, which is 0.70 nmol/mg protein per min under the same conditions. The interaction of D-glucose with the AMG binding site was therefore examined. D-Glucose (10 mM) is as effective in inhibiting [^{14}C]AMG uptake as 10 mM AMG (Table II). From a Dixon plot (Fig. 4A), an apparent K_i for D-glucose of 2.6 mM is derived. A nearly identical value (2.2 mM) was found when the same experiment was done in the presence of cytochalasin B (not shown).

To determine the nature of the inhibition, the influence of D-glucose on the apparent K_m and on the V_{max} for AMG uptake were determined. Initial uptakes of AMG (15 min) were measured in the presence of different initial AMG concentrations. This was done in the absence and presence of 2 mM D-glucose (Fig. 4B). A Michaelis-Menten curve was fitted to the data. In the absence of D-glucose, the calculated apparent $K_m \pm$ S.D. is 3.2 ± 0.2 mM and the $V_{max} \pm$ S.D. is 12.7 ± 0.4 nmol/mg protein per min. In the presence of 2 mM D-glucose, the $K_m \pm$ S.D. is 7.5 ± 1.5 mM and the $V_{max} \pm$ S.D. is 13.1 ± 1.5 nmol/mg protein per min. This indicates that D-glucose inhibits AMG transport in a competitive way.

Discussion

The steady-state AMG accumulation can reach a much higher value for LLC-PK$_1$ than for OK. For LLC-PK$_1$, it is reported that this accumulating capacity is dependent on the time in culture and on the D-glucose concentration in the growth medium [29]. For OK, the accumulating capacity also increases after confluency and reaches a maximal value 3 days after confluency. However, this level is not significantly altered by the D-glucose concentration in the growth medium (Fig. 1). The down- or upregulatory mechanism in a high or low D-glucose medium, respectively, seems to be absent in OK cells. This makes it easier to control the development of the carrier, as fluctuations of the D-glucose concentration throughout an experiment have no influence on the expression level of the carrier.

A lack of upregulation in response to a low D-glucose concentration can be responsible for the lower steady-state AMG accumulation in OK. However, this is partly also due to a difference in kinetic parameters. For OK,

TABLE III

Characteristics of the Na^+-dependent hexose transporter in cultured cells

M, monolayer; V, vesicle preparations; V_{max} and K_m, kinetic constants for substrate transport; K_d, phlorizin dissociation constant; K_i, kinetic constant for inhibition of hexose transport by phlorizin; B_{max}, number of phlorizin binding sites.

Cell type		Substrate	Na^+:S	V_{max} (nmol/mg protein per min)	K_m (mM)	Phlorizin binding		Ref.
						K_d (K_i) (μM)	B_{max} (pmol/mg protein)	
Primary cells								
rabbit proximal tubule	M	AMG	2:1	0.33	0.16	9		8
	M	AMG	2:1	0.14	0.80			7
	M	D-glucose			3.54			36
flounder renal tubule	M	D-glucose		5 [a]	0.16			38
Established cells								
LLC-PK$_1$	M	AMG		18.2 [a]	0.59			35
	M	AMG		3.5 [b]	0.75	1.2	0.3 [d]	25, 31
	M	AMG	2:1	10.8, 33.8 [c]	1.1	0.086	1.09	this study
	M	D-glucose	2:1	22 [a]	0.21			23, 35
	M					0.2	0.25, 0.8 [e]	30
	M					0.080	2.2	32
	V	AMG	2:1	3	2	0.2, 0.4, 2.5 [f]	11.6	21, 34
	V	AMG	2:1	5	10			22
	V	D-glucose		5.8	0.3			37
	V	D-glucose		5	3.8	0.033	0.67	33
OK	M	AMG	1:1	17.1	2.9	0.025	1.5	this study
CACO-2	M	AMG	2:1	0.41	3.1			9

[a] Data obtained by electrophysiological techniques, units are $\mu A/cm^2$.
[b] Units are μmol/h per mg DNA.
[c] Values at high and low D-glucose, respectively.
[d] Units are μmol/g DNA.
[e] Units are in pmol/10^6 cells for high and low D-glucose, respectively.
[f] Values at 500 mM, 266 mM and 100 mM Na^+, respectively.

a 2-times higher efflux rate and a considerably lower affinity for AMG were found (Table I). The latter is certainly important, taking into account that the AMG concentration in the uptake medium is much lower than the apparent K_m. As a consequence, when V_{max} values are compared, the differences between both cell lines are much smaller than these found for the steady-state AMG accumulation. The V_{max} values for OK and for LLC-PK_1 cells (Table I) are in any case much higher than the V_{max} values found for AMG transport in other cultured cells (Table III).

The comparatively high level of AMG transport is correlated to the number of hexose carriers as well as to the turnover frequency of these carriers. In this study, we have found a B_{max} for Na^+-dependent phlorizin binding of 1.5 pmol/mg protein for OK and of 1.09 pmol/mg protein for LLC-PK_1. The latter value is again very much dependent on the D-glucose concentration in the growth medium. Reported values for LLC-PK_1 monolayers (Table III), when expressed in the same units (pmol/mg protein), vary at least over a 10-fold range. The turnover values for OK (158/s) and for LLC-PK_1 (165/s) are very similar and are also in excellent agreement with reported values for LLC-PK_1 cells (170/s [32] and 125/s [33]).

Ample evidence has been obtained for the occurrence of both a low-affinity type of Na^+-dependent hexose transporter with 1:1 Na^+: substrate stoichiometry and a high-affinity type with 2:1 stoichiometry. Most cell lines examined thus far express the carrier with a Na^+ : substrate stoichiometry of 2:1 (Table III). This carrier is typical for the pars recta as found in preparations from rabbit outer medulla [16]. OK monolayers differ from LLC-PK_1 with respect to the Na^+ dependence of initial AMG uptake. It should be pointed out that the monolayer technique makes the interpretation of the Na^+ dependence more difficult due to possible effects on other cellular parameters such as the membrane potential. A more reliable determination of the stoichiometry requires a study with membrane vesicles in which the membrane potential is short-circuited [13,34]. Nevertheless, our observations are at least indicative for different kinetics in both cell lines, and suggest a 1:1 stoichiometry in OK cells.

A comparison of the apparent K_m values for the substrates (AMG or D-glucose) reveals further heterogeneity. The LLC-PK_1 cell line is very well documented in this respect, but there is considerable variability among the data, particularly for the vesicle preparations (Table III). When only the studies in monolayers are taken into account, the experimental parameters are more constant ($Na^+ \approx 140$ mM, temperature 37°C) and the data agree much better. It then becomes evident that the apparent K_m for AMG uptake in the primary cells and in LLC-PK_1 is below or around 1 mM. By direct comparison in identical experimental conditions we have found that the affinity for AMG is 3-times lower in OK cells than in LLC-PK_1 cells. Moreover, with respect to D-glucose as a substrate, there is an even larger ($\approx$ 10-fold) difference between both cell lines. This is concluded from our observation that the affinity of the OK carrier for D-glucose is approximately the same as for AMG itself (Fig. 4A and Table II), whereas the LLC-PK_1 carrier has a 3-fold higher affinity for the former substrate [35]. The difference in the substrate binding site of the hexose carrier in both cell lines is also evident from a comparison of the phlorizin binding. In this case, however, it is the carrier of OK which has the higher affinity. The inverse relationship between the interaction with the substrates on one hand and with phlorizin on the other suggests that the binding site of the OK carrier favors hydrophobic interactions.

A peculiar characteristic of the OK cell line, already observed by Malmström et al. [13], is that D-glucose seems to be a poor substrate for the phlorizin-sensitive hexose carrier. The low activity of phlorizin-sensitive D-glucose transport in OK is not due to a dramatic difference in the affinity of the carrier for D-glucose compared to AMG. D-Glucose was as effective in inhibiting the phlorizin-sensitive [^{14}C]AMG uptake as AMG itself (Table II). Furthermore, the inhibition of AMG transport by D-glucose is purely competitive and the inhibition constant (K_i) is of the same order of magnitude as the K_m for AMG transport (Fig. 4A, B).

It is evident from the effect of inhibitors on the initial uptake of D-glucose that this uptake is primarily via a cytochalasin B-sensitive pathway. The phlorizin-sensitive pathway accounts for only 20% of the total D-glucose uptake. That a similar behavior is not observed for LLC-PK_1 could be due merely to a higher permeability of the intercellular junctions in OK, favoring the basolateral pathway of cytochalasin B-dependent uptake. However this does not explain why the initial rate of phlorizin-sensitive uptake in OK is about 20-times lower than the initial uptake of AMG measured under the same conditions (0.1 mM substrate). Moreover, a very poor phlorizin-sensitive uptake rate for D-glucose, as compared to AMG, was also found in vesicle preparations from OK [13]. These data, taken together with the observation that there is no difference in the affinity for both substrates indicate a much lower V_{max} for D-glucose than for AMG uptake. This also implies a much lower turnover frequency for D-glucose. This is in contrast to LLC-PK_1 cells, where very similar V_{max} values for both substrates were found [35]. The result for OK cells could be due to a malfunction in the translocation step with D-glucose as a substrate or an effect of D-glucose on the intracellular binding site. The latter, however, seems unlikely, since cytochalasin B had no significant influence on the inhibitory effect of D-glucose on AMG transport.

In conclusion, our data show that the hexose carrier

of OK shares some characteristics with the carrier of LLC-PK_1, but differs with respect to the substrate binding site and with respect to the Na^+ dependence of AMG uptake. This suggests that OK cells express a carrier which is similar in some respect to the low-affinity carrier with 1:1 stoichiometry found in the early part of the proximal tubule. It also supports the view that multiple types of carrier may coexist in the proximal tubule covering a broad range of substrate affinities. Furthermore, our data indicate that OK cells are a valuable alternative to LLC-PK_1 for the study of the development and expression of the Na^+-dependent hexose carrier. The absence of up- and downregulation of the carrier by the D-glucose concentration in the medium makes it easier to examine and interpret the influence of other parameters. Finally, the malfunction with respect to the turnover frequency for D-glucose could be important in unravelling the molecular mechanism of Na^+-dependent hexose transport.

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