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Identification of proximal tubular transport functions in the established kidney cell line, OK

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OK cells, derived from an American opossum kidney, were analyzed for proximal tubular transport functions. In monolayers, L-glutamate, L-proline, L-alanine, and α-methyl-glucopyranoside (α-methyl D-glucoside) were accumulated through Na^+-dependent and Na^+-independent transport pathways. D-Glucose and inorganic sulfate were accumulated equally well in the presence or absence of Na^+. Influx of inorganic phosphate was only observed in the presence of Na^+. Na^+/α-methyl D-glucoside uptake was preferentially inhibited by phlorizin and D-glucose uptake by cytochalasin B. An amiloride-sensitive Na^+-transport was also identified. In isolated apical vesicles (enriched 8-fold in γ-glutamyltransferase), L-glutamate, L-proline, L-alanine, α-methyl D-glucoside and inorganic phosphate transport were stimulated by an inwardly directed Na^+-gradient as compared to an inwardly directed K^+-gradient. L-Glutamate transport required additionally intravesicular K^+. D-Glucose transport was similar in the presence of a Na^+- and a K^+-gradient. Na^+/α-methyl D-glucoside uptake was inhibited by phlorizin whereas cytochalasin B had no effect on Na^+/D-glucose transport. An amiloride-sensitive Na^+/H^+ exchange mechanism was also found in the apical vesicle preparation. It is concluded that the apical membrane of OK cells contains Na^+-coupled transport systems for amino acids, hexoses, protons and inorganic phosphate. D-Glucose appears a poor substrate for the Na^+/hexose transport system.

Introduction

Established cell lines of renal origin are frequently used for analyzing renal transport functions and their regulation. LLC-PK_1 [1] and MDCK [2] are examples of well characterized renal cell lines, which often are employed as model systems for the proximal [3–6] and the distal [7,8] tubule, respectively. LLC-PK_1 cells, in contrast to proximal tubular epithelial cells, contain no or only a few parathyroid hormone (PTH)-receptors and Na^+-coupled phosphate transport is not under control of PTH or cyclic AMP in these cells [9]. Recently, an established cell line derived from an American opossum kidney (OK) was shown to have PTH receptors and to hormonally regulate the Na^+/phosphate cotransport [9–11].

In previous studies on monolayers of OK cells, Na^+-dependent transport for phosphate, L-alanine and α-methyl-D-glucopyranoside (α-methyl D-glucoside) have been described [9,10]. In the present study, we have identified Na^+-coupled transport systems for acidic and neutral amino acids, for

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; Mes, 4-morpholineethanesulfonic acid; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid.

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hexoses, for inorganic phosphate and a Na^+/H^+ antiporter. These transport systems are present in the apical membrane, as shown by experiments with isolated membrane vesicles. Thus, the OK cell line may turn out to be an alternative model system to LLC-PK_1, since hormonal receptors and transport functions [9,11] are more typical for the proximal tubule than the ones present in LLC-PK_1 cells.

Methods

Cell cultures

OK cells were maintained in culture by serial passage between passage number 80 and 100 as described [9]. The growth medium was Dulbecco's minimum essential medium HAMS F 12 (1 : 1) supplemented with 5 mM glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin and 10% fetal calf serum, with 20 mM Hepes and 22 mM $NaHCO_3$ (pH 7.4) as buffer system.

For transport experiments the cells were plated ($(1–2) \cdot 10^5$ cells/2 ml) in 35 mm diameter plastic petri dishes (NUNC; Denmark) and grown for 5 days in a humidified 5% CO_2/95% air atmosphere. The cell monolayers were confluent when used.

For membrane isolation the cells were grown in glass roller bottles (840 cm^2) (Flow Laboratories, Baar, Switzerland) to a dense confluent stage (8–10 days). The medium was changed on the fourth day and thereafter every second day.

Membrane isolation

Cells from two roller bottles were used for the preparation. The monolayer was rinsed three times with a medium containing (in mM): 300 mannitol, 5 EGTA, 12 Tris-HCl (pH 7.1). The cells were harvested after scraping them into 30 ml of above medium, and exposing them to a short centrifugation step ($400 \times g = 1800$ rpm for 5 min in a Sorvall RT 6000). The cells were resuspended in 7 ml of medium, diluted to 20 ml with distilled water and pressurized for 30 min at 750 lb/$inch^2$ nitrogen in a Parr pressure homogenizer. The cells were fragmented by nitrogen cavitation and the homogenate was collected dropwise. $MgCl_2$ was added to a final concentration of 10 mM and the homogenate was left for 20 min on ice. The aggregated organelles, nuclei etc. were removed by centrifugation ($2400 \times g = 4500$ rpm for 10 min in a Sorvall SS-34 rotor). The supernatant was subjected to a second centrifugation step ($30\,000 \times g = 16\,000$ rpm for 30 min in a Sorvall SS-34 rotor). The pellet containing the apical membrane vesicles was washed once in 20 ml of a medium containing (in mM): either (a) 300 mannitol, 20 Hepes-Tris (pH 7.4); or (b) 300 mannitol, 50 KCl, 20 Hepes-Tris (pH 7.4) (for L-glutamate); or (c) 300 mannitol, 20 potassium gluconate, 50 Mes-Tris (pH 5.5) (for Na^+/H^+ exchange), centrifuged at $30\,000 \times g$ and resuspended in the appropriate medium to a final concentration of 1.5–2 mg/ml.

Assay of marker enzymes activities

The specific activities of marker enzymes were measured on the day of the preparation. The enzyme activities were determined semi-automatically using either a LKB reaction rate analyzer (model 8600) or the kinetic analysis program of a Bausch and Lomb spectrophotometer (model 2000). The activity of $(Na^+ + K^+)$-ATPase (EC 3.6.1.37) was assayed as described by Berner and Kinne [12]; leucine aminopeptidase (EC 3.4.11.2) as described by Haase et al. [13]; the γ-glutamyltransferase (EC 2.3.2.2) as described by Wahlefeld and Bergmeyer [14]; the KCN-resistant NADH-oxidoreductase (EC 1.6.99.2) was assayed according to Sottocasa et al. [15] and *N*-acetyl-β-glucosaminidase (EC 3.2.1.30) according to Scalera et al. [16]. All enzyme activities were measured at 37 °C.

Transport studies

Cell monolayers. The monolayer was rinsed once with a medium containing (in mM): 137 NaCl (or for Na^+-independent transport, 137 *N*-methyl-D-glucamine (HCl), 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$, 10 Hepes-Tris (pH 7.4). The transport was carried out at 37 °C and initiated by adding 1 ml of the above medium supplemented with a radioactively labelled substrate (0.1 mM); $KH_2{}^{32}PO_4$ (0.5 μCi/ml), L-[^{3}H]alanine (2.0 μCi/ml), L-[^{3}H]proline (1.5 μCi/ml), L-[^{3}H]glutamate, (1.5 μCi/ml), D-[^{14}C]glucose (0.5 μCi/ml) or α-[^{14}C]methyl-D-glucopyranoside (0.4 μCi/ml). For Na^+ transport a slightly different protocol was used: Prior to the transport measurement, the cells were depleted of Na^+ by rinsing the monolayer once and then

leaving the cells for 2 h in a medium containing (in mM): 137 *N*-methyl-D-glucamine/HCl, 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$ and 10 mM Hepes (pH 7.4). Five minutes before starting the uptake, the cells were exposed to a medium where 100 mM *N*-methyl-D-glucamine had been replaced with 100 mM KCl and 1 mM ouabain. The Na^+ uptake was measured in this medium (37 °C) supplemented with 10 mM ^{22}Na (1 μCi/ml). The Na^+ fluxes related to Na^+/H^+ exchange was determined as the amiloride-sensitive (1 mM) Na^+ flux. The transport of the various substrates was terminated after the appropriate incubation time, by aspirating off the medium and washing the monolayer four times with an ice-cold stop-solution containing (in mM): 137 NaCl, 14 Tris-HCl (pH 7.4). Subsequently, the monolayer was solubilized in 1 ml 0.5% Triton X-100. Aliquots of 150 μl or 300 μl were used for scintillation-counting to determine the amount of accumulated substrate.

Vesicles. 20 μl of membrane vesicles (15–20 μg) were added to 100 μl prewarmed (37 °C) medium, final concentration (in mM): (a) 300 mannitol, 100 NaCl (or KCl), 20 Hepes-Tris (pH 7.4) and 0.1 substrate (such as L-[^{3}H]alanine (2 μCi/20 μl), D-[^{14}C]glucose (1 μCi/20 μl), α-[^{14}C]methyl D-glucoside (0.5 μCi/20 μl) and $H_2{}^{32}PO_4$ (1 μCi/20 μl)); (b) 300 mannitol, 50 KCl, 100 NaCl (or 100 KCl), 20 Hepes-Tris (pH 7.4) and 0.1 L-[^{3}H]glutamate (1.0 μCi/20 μl); or (c) 300 mannitol, 20 potassium gluconate, 50 Mes-Tris at either pH 5.5 or 7.4, 0.1 μg/ml valinomycin, and 0.1 $^{22}Na_2SO_4$ (1.0 μCi/20 μl). At predetermined times 20-μl aliquots were removed and the uptake was terminated by injecting 2 ml of ice-cold stop-solution containing in mM: 100 mannitol, 150 NaCl, 5 Tris-HCl, and for phosphate transport 5 potassium phosphate (pH 7.4). The samples were filtered through a Sartorious filter (0.65 μm pore size) and washed three times with 4 ml of ice-cold stop-solution. The filters were dissolved in 2 ml of Packard scintillator 299 and counted in a liquid scintillation counter.

Protein determination

The protein content of cell monolayers were determined according to Lowry as modified by Dulley and Grieve [17], using bovine serum albumin as standard. The protein content of 10 random monolayers was determined and an average was calculated per experiment. The protein content of apical membrane vesicles was determined according to Bradford [18] with γ-globulin as standard.

Materials

The cell culture reagents were obtained from Amimed, Basle, Switzerland. The radioisotopes (transport substrates) were from New England Nuclear, Boston, MA; cytochalasin B and valinomycin from Sigma, St. Louis, MO; phlorizin from Roth, Karlsruhe, F.R.G. Amiloride was a kind gift from Merck, Sharp & Dome Research Lab., Rahway, NJ. All other reagents were of highest purity available.

The results are presented as representative experiments performed in triplicate. Similar results were obtained in at least three separate experiments.

Results

Na^+-coupled amino acid transport

In the proximal tubule, most amino acids are reabsorbed across the brush-border membrane coupled to the Na^+ flux [19]. We have chosen three amino acids, L-alanine, L-proline and L-glutamate to evaluate some of the properties of amino acid transport in OK cells. As shown in Fig. 1, L-glutamate and L-proline were transported preferentially in a Na^+-dependent way, whereas L-alanine transport largely was independent of Na^+. The L-glutamate uptake was linear for at least 6 min, the L-proline maintained its linearity up to 10 min and L-alanine only for about 2 min. These findings suggest that some neutral amino acids (e.g. L-proline) and some acidic amino acids (e.g. L-glutamate) require Na^+ for optimal cellular accumulation, whereas some neutral amino acids (e.g. L-alanine) can be accumulated also without a Na^+ gradient.

Na^+-coupled inorganic anion transport

In the proximal tubule, inorganic phosphate and inorganic sulfate are transported via separate Na-dependent systems [20]. Fig. 2a shows that in

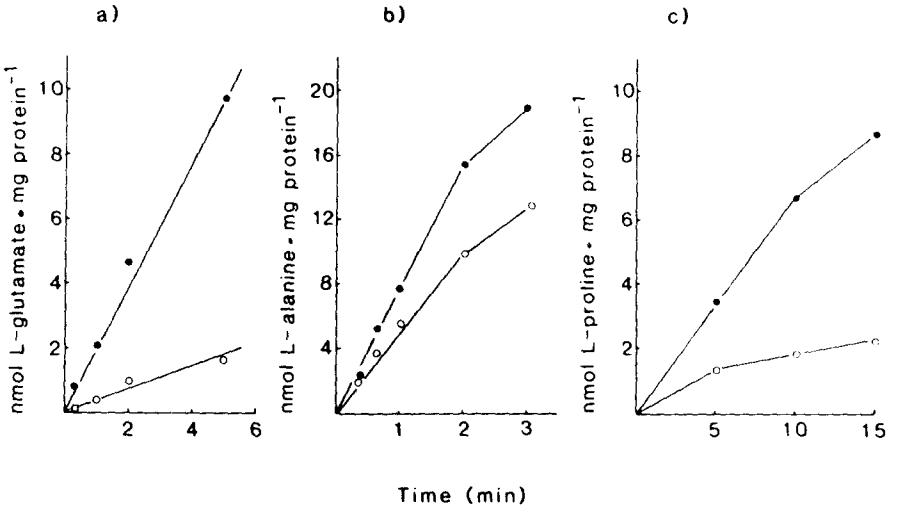

Fig. 1. Time-course of L-amino acid transport in cell monolayers. The transport was measured in the presence of 137 mM NaCl (●) or 137 mM *N*-methyl-D-glucamine (○) using 0.1 mM substrate. (a) L-Glutamate, (b) L-alanine, (c) L-proline.

OK cells inorganic phosphate was transported very rapidly in the presence of Na^+ and that a linear uptake proceeded for about 6 min. The Na^+-independent portion which was measured by replacing Na^+ with *N*-methyl-D-glucamine was extremely small (about 1% of the total uptake). Therefore the transport of phosphate in the presence of Na^+ represents almost exclusively the Na^+-coupled transport and the Na^+-independent influx can be neglected. We found that sulfate is transported differently from phosphate with no or an extremely small Na^+ requirement. Almost the same amount of sulfate (approx. 200 pmol per mg after 1 min) was accumulated by the OK cells irrespectively of the presence or absence of Na^+ (data not shown).

Na^+-coupled D-glucose transport

D-Glucose and the non-metabolizable analogue α-methyl-D-glucopyranoside (α-methyl D-glucoside) were used to evaluate the hexose transport in OK cells. As is documented in Fig. 3 the linear

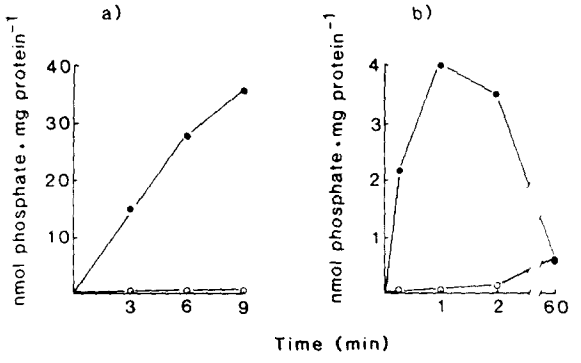

Fig. 2. Time-course of phosphate transport in OK cells. The phosphate transport (0.1 mM) was measured: in (a) monolayers in the presence of 137 mM NaCl (●) or 137 mM *N*-methyl-D-glutamine (○) and in (b) apical membrane vesicles with a Na^+ gradient (100 mM) (●) or a K^+ gradient (100 mM) (○).

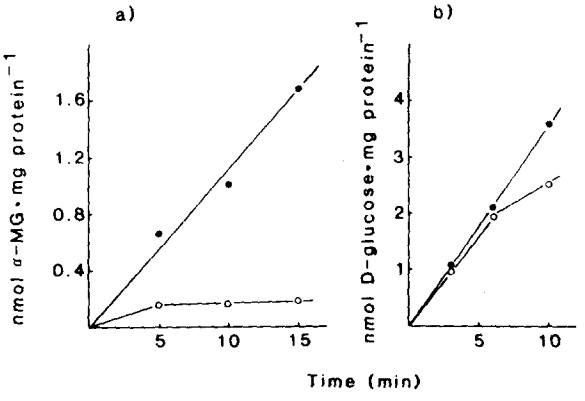

Fig. 3. Time-course of hexose transport in cell monolayers. The transport was measured in the presence of 137 mM NaCl (●) or 137 mM *N*-methyl-D-glucamine (○) using 0.1 mM substrate. (a) α-Methyl D-glucoside (α-MG), (b) D-glucose.

phase, which lasted for about 15 min, is similar for the two transport substrates. α-Methyl D-glucoside transport was stimulated by the presence of Na^+, whereas D-glucose transport was accumulated to the same extent in the presence or absence of Na^+. This was an indication that the accumulation of D-glucose and α-methyl D-glucoside by OK cells do not occur via the same transport pathways. To clarify further the mechanisms of hexose transport in OK cells we studied the effect of phlorizin and cytochalasin B on D-glucose and α-methyl D-glucoside transport (Table I). Phlorizin, which inhibits Na^+-coupled glucose transport [29], reduced α-methyl D-glucoside transport by approx. 90% and the D-glucose by 35% in the presence of Na^+, but had no effect on the Na^+-independent transport of the two sugars. Cytochalasin B, which inhibits the Na^+-independent glucose transport [29,30], reduced in the presence of Na^+, D-glucose by 72% and α-methyl D-glucoside transport by 27%. Cytochalasin B did not affect the Na^+-independent α-methyl D-glucoside transport but decreased the Na^+-independent D-glucose transport by about 87%. From these results we have concluded that α-methyl D-glucoside is transported mainly by the phlorizin sensitive Na^+-dependent glucose carrier whereas D-glucose is transported preferentially by the cytochalasin B sensitive, Na^+-independent glucose carrier.

TABLE I

INFLUENCE OF PHORIZIN AND CYTOCHALASIN B ON D-GLUCOSE AND α-METHYL D-GLUCOSE TRANSPORT IN OK CELL MONOLAYERS

The uptake of 0.1 mM D-glucose and 0.1 mM α-methyl D-glucoside were followed for 10 min at 37 °C in the absence and in the presence of 100 μM phlorizin or 10 μM cytochalasin B. The values are means ± S.D. *n* indicates the number of experiments.

	Uptake ($nmol \cdot mg^{-1} \cdot (10\ min)^{-1}$)	
	Na^+ medium	*N*-methyl-D-glucamine medium
D-Glucose		
Control ($n = 6$)	4.8 ± 0.7	3.0 ± 0.4
+ phlorizin ($n = 6$)	2.8 ± 0.2	2.4 ± 0.5
+ cytochalasin B ($n = 6$)	1.2 ± 0.1	0.4 ± 0.03
α-Methyl D-glucoside		
Control ($n = 6$)	2.2 ± 0.2	0.25 ± 0.06
+ phlorizin ($n = 3$)	0.2 ± 0.01	0.17 ± 0.09
+ cytochalasin B ($n = 3$)	1.6 ± 0.1	0.25 ± 0.03

Na^+ transport

A Na^+/H^+ exchange system is present in the apical membrane of the proximal tubular epithelial cell [32,33]. The Na^+ fluxes via the Na^+/H^+ antiporter are amiloride-sensitive [33]. We have determined the Na^+ fluxes in cells which had been depleted of Na^+ for 2 h (see Methods) using 10 mM external Na^+. The initial linear rate lasted for 2 min and thereafter a plateau was reached (data not shown). These Na^+ fluxes (0.5 min uptake value = 30 nmol Na^+/mg protein at 10 mM ^{22}Na) were inhibited to about 60–70% by 1 mM amiloride, indicating that a major portion of the Na^+ flux occurs via the Na^+/H^+ antiporter (see also Fig. 4).

Apical membrane vesicles; enzymatic characterization

The apical membrane vesicles were isolated as described in Methods. The purity was estimated by measuring the enzyme activity for the luminal membrane (γ-glutamyltransferase), the basolateral membrane ($(Na^+ + K^+)$-ATPase), the endoplasmic reticulum (NADH-oxidoreductase) and for the lysosomes (*N*-acetyl-β-glucosaminidase). The specific activities, the enrichment factors and the yields are presented in Table II. Two of the

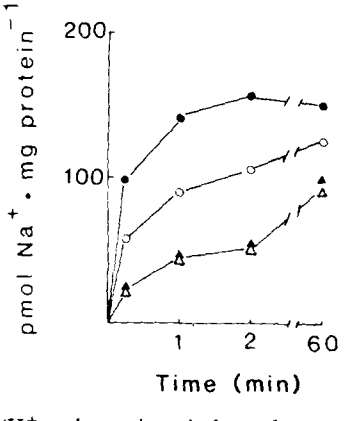

Fig. 4. Na^+/H^+ exchange in apical membrane vesicles: Na^+ uptake in the presence of a H^+ gradient ($pH_i = 5.5$ and $pH_o = 7.4$) (●, ○); and without H^+ gradient $pH_i = pH_o = 5.5$ (▲, △). Control (●, ▲); 1.0 mM amiloride (○, △).

TABLE II

ENZYMATIC CHARACTERIZATION OF APICAL MEMBRANES ISOLATED FROM OK CELLS

Specific activities are given as $\mu mol \cdot min^{-1} \cdot (mg\ protein)^{-1}$. The enrichment factor is derived by dividing specific activities of the isolated membrane fraction by that of the homogenate. Yield (%) is the enzyme activity recovered in the final membrane as compared to the total activity in the initial homogenate. The date are means ± S.D. n.d., not detectable; *n* indicates number of experiments.

Enzyme	Apical membrane fraction		
	spec. act.	Enrichment	Yield (%)
Alkaline phosphatase	n.d.		
Leucine aminopeptidase	151 ± 41	5.4 ± 1.2	5.6 ± 5.6
γ-Glutamyltransferase	175 ± 59	7.7 ± 1.1	19 ± 6
$(Na^+ + K^+)$-ATPase (5)	125 ± 58	1.9 ± 0.6	4.3 ± 1.9
NADH-oxidoreductase (2)	426 ± 124	0.9 ± 0.35	2.6 ± 1.9
Glucosaminidase (2)	173 ± 94	1.5 ± 1.1	4.3 ± 4.4

common marker enzymes for brush-border membranes, alkaline phosphatase and leucine aminopeptidase could not be used since alkaline phosphatase was not detectable and the leucine aminopeptidase was found mainly in the soluble fraction of the homogenate (85%; data not shown). Only 6 ± 6% of the leucine aminopeptidase activity was retained in the apical membrane fraction. The purified apical membrane fraction retained about 20% of the original activity of the γ-glutamyltransferase and the enrichment was about 8-times. The membrane fraction was also enriched slightly in basolateral membranes and lysosomes but was depleted of endoplasmic reticulum. The yield of each contaminating organelle was less than 5%.

Na^+-coupled transport systems in the apical membrane

For transport experiments, we examined first the resistance of the vesicle-preparation towards freezing (liquid nitrogen) and thawing. The freezing-thawing step seems, however, to change the membrane permeability, since Na^+-coupled transport is decreased and is similar to the transport in the absence of Na^+. The vesicles were, therefore, always prepared and used on the day of the experiment.

By analyzing which of the Na^+-dependent transport systems, identified in cell monolayer, were retained in the partially purified apical membrane vesicles fraction, an indication of their location can be acquired. The transport was measured in parallel experiments with an inwardly directed Na^+ gradient ($Na_o^+ > Na_i^+$) or inwardly directed K^+ gradient ($K_o^+ > K_i^+$). The results in Fig. 5 show that the uptake of amino acids were stimulated in the presence of a Na^+ gradient. A transient 'overshoot' over the equilibrium was found for all three amino acids. Noteworthy is that the Na^+ dependency of glutamate is apparent only after preloading the vesicles with potassium. The glutamate uptake in the absence of intravesicular potassium is not stimulated by a Na^+ gradient (data not shown). Such a requirement for internal K^+ (most likely K^+ efflux) for Na^+-dependent L-glutamate uptake was found in the proximal

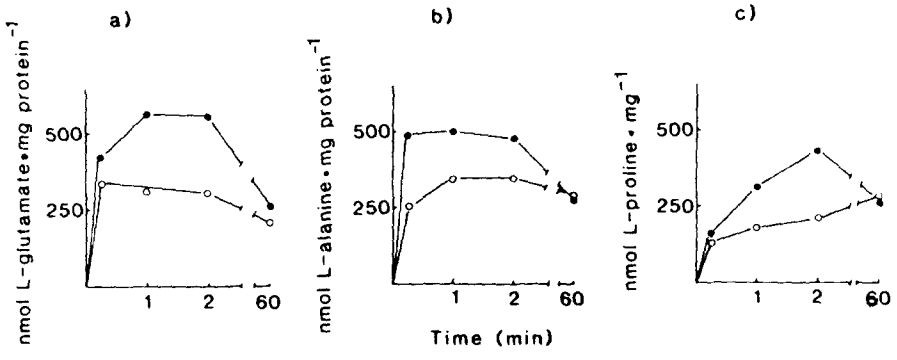

Fig. 5. Time-course of L-amino acid uptake in apical membrane vesicles. The uptake of amino acids (0.1 mM) was measured in the presence of a Na^+ gradient (100 mM) (●) or K^+ gradient (100 mM) (○). (a) L-Glutamate, (b) L-alanine, (c) L-proline.

tubular brush-border membrane vesicles [22,23]. We concluded that the apical membrane of OK cells contains Na^+-dependent transport systems for the amino acids L-proline, L-glutamate and L-alanine.

Similar to the observations on hexose transport in monolayers we found that α-methyl D-glucoside required Na^+ for optimal transport rates (Fig. 6a) and that D-glucose, exhibited identical transport in the presence of a Na^+ gradient or a K^+ gradient (Fig. 6b). The sensitivity of the hexose transporters to phlorizin and to cytochalasin B was analyzed at an early time point (1 min) and at the equilibrium value (60 min) (Table III). In analogy to monolayers, the α-methyl D-glucoside transport in the presence of Na^+ was inhibited by phlorizin to 50% and reached a value identical to the uptake in the presence of a K^+ gradient. Cytochalasin B did not inhibit transport of α-methyl D-glucoside neither in the presence of Na^+ nor in the presence of K^+. The transport of D-glucose was not diminished by phlorizin or cytochalasin B under the two different ionic conditions. These observations indicate that the Na^+-dependent hexose carrier is localized in the apical membrane but that it cannot transport D-glucose. Furthermore, the Na^+-independent and cytochalasin B-sensitive hexose carrier–seen in monolayers– is most likely not localized in the apical membrane. The stimulation of D-glucose transport by cytochalasin B

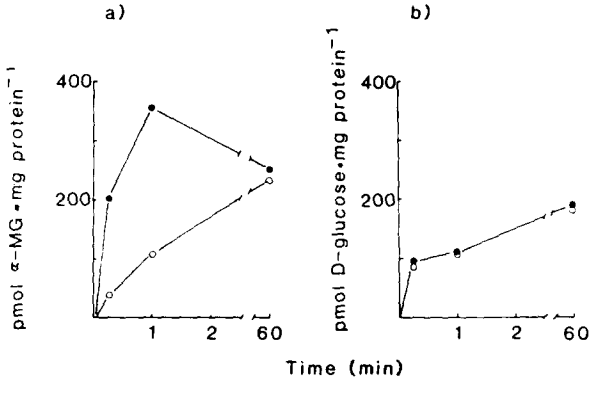

Fig. 6. Time-course of hexose uptake in apical membrane vesicles. The uptake of hexoses (0.1 mM) was measured in the presence of a Na^+ gradient (100 mM) (●) or a K^+ gradient (100 mM) (○). (a) α-Methyl D-glucoside (α-MG), (b) D-glucose.

TABLE III

EFFECT OF PHLORIZIN AND CYTOCHALASIN B ON α-METHYL D-GLUCOSIDE AND D-GLUCOSE UPTAKE IN APICAL MEMBRANE VESICLES FROM OK CELLS

The uptake of 0.1 mM α-methyl D-glucoside (α-MG) or D-glucose was measured with a Na^+ gradient ($Na_o^+ > Na_i^+$) and a K^+ gradient ($K_o^+ > K_i^+$). The concentration of phorizin was 100 μM and cytochalasin B 10 μM. The data are mean ± S.D. of one or two experiments performed in triplicate.

Condition	Uptake (pmol · mg^{-1})			
	1 min		60 min	
	Na^+ medium	K^+	Na^+ medium	K^+
α-Methyl D-glucoside				
Control (2)	76 ± 6	29 ± 1	80 ± 13	64 ± 6
+ phlorizin (2)	37 ± 7	32 ± 3	78 ± 22	81 ± 14
+ cytochal. B (2)	86 ± 13	46 ± 11	101 ± 33	97 ± 32
D-Glucose				
Control (1)	48 ± 2	49 ± 1	188 ± 6	201 ± 2
+ phlorizin (1)	54 ± 4	51 ± 2	250 ± 9	211 ± 6
+ cytochal. B (1)	85 ± 1	63 ± 1	263 ± 9	266 ± 12

(Table III) is an intriguing observation and as yet it cannot be explained.

Phosphate was the only inorganic anion to be investigated in the vesicle preparation since sulfate transport did not show any Na^+ dependency in monolayers (data not shown). We measured the phosphate accumulated under identical conditions as described for the amino acids and hexose transport. In Fig. 2b, the time-course is illustrated. A transient overshoot over the equilibrium value (about 6-fold) was observed. The phosphate uptake in the presence of a K^+ gradient was very slow although the same equilibrium was reached eventually. Thus, the Na^+/phosphate cotransport system is present in the apical membrane.

Finally we wanted to verify the presence of the Na^+/H^+ antiporter in the apical membrane. We measured ^{22}Na-uptake in the presence of an outwardly directed H^+ gradient ($pH_i = 5.5$ and $pH_o = 7.4$) and no H^+ gradient ($pH_o = pH_i = 5.5$). The uptake was stimulated by a H^+-gradient (Fig. 4). Furthermore, the H^+ gradient stimulated Na^+-uptake was inhibited by 1 mM amiloride (to approx. 40%), whereas the Na^+ uptake in the absence of a proton gradient was not changed. The data sug-

gest that also the Na^+/H^+ antiporter is present in the apical membrane.

Discussion

The present study describes some transport characteristics of the established opossum kidney cell line OK. By using cell monolayers and isolated vesicles, we have shown that the OK cells possess Na^+-coupled transport systems for amino acids, hexoses, phosphate and protons and that they are present in the apical membrane.

In monolayers, the transport of L-glutamate and L-proline are strongly Na^+-dependent, whereas transport of L-alanine does not require Na^+. In isolated apical vesicles, all studied amino acids were transported in a Na^+-dependent manner. This observation suggests, that a large component of Na^+-independent L-alanine flux in monolayers is via a sodium independent pathway located preferentially in the basolateral membrane, e.g. via the L system [24,25]. The present study does not allow to attribute Na^+-dependent uptake to one of the specific Na^+-coupled pathways for amino acids (e.g. the A or ASC system, Refs. 24, 25) because detailed studies on specificity have not been performed. However, the existence of (separate) Na^+-coupled transport of acidic amino acids and of neutral amino acids is clearly documented by the data obtained with isolated vesicles. In isolated apical vesicles Na^+-coupled transport of acidic amino acids but not of neutral amino acids requires internal K^+.

The Na^+-coupled glucose transport in the luminal membrane of the proximal tubule is inhibited, competitively, by phlorizin [29] and the Na^+-independent glucose transport across the basolateral membrane is inhibited by phloretin and cytochalasin B [29,30]. Thereby, transport across the basolateral membrane seems to be similar to D-glucose transport in nonepithelial plasma membranes, e.g. erythrocytes [26], adipocytes [27] and fibroblasts [28]. The Na^+-dependent D-glucose carrier accepts both D-glucose and α-methyl D-glucoside as substrate, whereas the Na^+-independent glucose carrier accepts D-glucose but not α-methyl D-glucoside [31]. Since, in OK cells, phlorizin inhibited mainly the Na^+-dependent transport of α-methyl D-glucoside and cytochalasin B mainly D-glucose transport in the presence or absence of Na^+, it could be concluded that α-methyl D-glucoside is transported by the Na^+-dependent hexose carrier and the larger portion of the D-glucose by the Na^+-independent hexose carrier. In apical membrane vesicles isolated from OK cells phlorizin inhibited Na^+-dependent α-methyl D-glucoside transport completely but not D-glucose transport in the presence or absence of Na^+, and cytochalasin B rather increased the transport rate for both α-methyl D-glucoside and D-glucose. This may suggest that the distribution of the Na^+-dependent and the Na^+-independent hexose transporters in OK cells is similar to that in the intact proximal tubule. The large Na^+-independent and cytochalasin B-sensitive flux in the monolayer experiments must be explained by accumulation of D-glucose across the basolateral membrane. Surprisingly, D-glucose seems to be in OK cells a rather poor substrate for the Na^+-dependent transport system for hexose.

Transport of inorganic phosphate in OK cells is in analogy to renal proximal tubule: Na^+/phosphate cotransport is present in the apical membrane. The rate of this transport mechanism is under hormonal control and PTH, intracellularly mediated by cAMP, reduces the Na^+ phosphate cotransport activity [9,10]. Unlike proximal tubular epithelial cells [20], transport of inorganic sulfate in OK cells seems to proceed via a Na^+-independent mechanism.

We have also shown that the Na^+/H^+-antiporter in OK cells is present in the apical membrane. This was done by studying the Na^+ uptake into the OK cells and into isolated apical vesicles. A large part of the Na^+ flux turned out to be amiloride-sensitive which is an indication that the Na^+ flux goes through the Na^+/H^+ antiporter. Pollock et al. [21] have studied this Na^+/H^+ exchange function extensively in OK cells (monolayers) and our limited goal was to determine its apical localization. The experimental conditions used to analyze ^{22}Na uptake in monolayers are rather unphysiological and the high extracellular K^+ concentration will lead to massive cell swelling.

In summary we have carried out a study on the identification of some transport functions in the OK cell line, to better understand its character

with respect to proximal tubular transport phenomena. The existence in the apical membrane of Na^+-coupled transport systems for glutamate (dependent on internal K^+), L-proline and L-alanine, for inorganic phosphate and of Na^+/H^+ exchange is consistent with data on proximal tubule transport. The lack of Na^+-coupled sulfate transport and the observation on hexose transport is intriguing. The Na^+-dependent transport system for hexoses is present in the apical membrane but it is conceivable that its affinity for D-glucose is very low. The D-glucose influx in OK cells occurs most likely via a Na^+-independent transporter in the basolateral membrane.

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References

1 Hull, R.N., Cherry, W.R. and Weaver, G.W. (1976) In Vitro 12, 670–677
2 Madin, S.H. and Darby, N.B. (1958) Proc. Natl. Soc. Exp. Biol. Med. 98, 574–576
3 Rabito, C.A. and Ausiello, D.A. (1980) J. Membrane Biol. 54, 31–38
4 Rabito, C.A. and Karish, M.W. (1982) J. Biol. Chem. 257, 6802–6808
5 Rabito, C.A. and Karish, M.W. (1983) J. Biol. Chem. 258, 2543–2547
6 Biber, J., Brown, C.D.A. and Murer, H. (1983) Biochim. Biophys. Acta 735, 325–330
7 Herzlinger, D.A., Easton, T.G. and Ojakian, G.K. (1982) J. Cell. Biol. 93, 269–277
8 Rindler, M.J., Chuman, L.M., Shaffer, L. and Saier, M. (1979) J. Cell. Biol. 81, 635–648
9 Malmström, K. and Murer, H. (1986) Am. J. Physiol. 251, C23–C31
10 Caverzasio, J., Rizzoli, R. and Bonjour, J.-P. (1986) J. Biol. Chem. 261, 3233–3237
11 Teitelbaum, A.P. and Strewler, G.J. (1984) Endocrinology 114, 980–985
12 Berner, W. and Kinne, R. (1976) Pflügers Arch. 361, 269–277
13 Haase, W., Schäfer, A., Murer, H. and Kinne, R. (1978) Biochem. J. 172, 57–62
14 Wahlefeld, A.W. and Bergmeyer, H.U. (1983) in Methods of Enzymatic Analysis (3rd Edn.) (Bergmeyer, H.U., ed.), Vol. 3, pp. 352–356, Verlag Chemie, Weinheim
15 Sottocasa, G.L., Kuylenstierna, L., Ernster, L. and Bergstrand, A. (1967) J. Cell. Biol. 32, 415–438
16 Scalera, V., Storelli, C., Storelli-Joss, C., Haase, W. and Murer, H. (1980) Biochem. J. 186, 177–181
17 Dulley, J.T. and Grieve, P.A. (1975) Anal. Biochem. 64, 136–141
18 Bradford, M. (1976) Anal. Biochem. 72, 248–252
19 Ullrich, K.J. (1979) Annu. Rev. Physiol. 41, 185–195
20 Ullrich, K.J. and Murer, H. (1982) Phil. Trans. R. Soc. London, B. 299, 549–558
21 Pollock, A.S., Warnock, D.G. and Strewler, G.J. (1986) Am. J. Physiol. 250, F217–F225
22 Schneider, E.G. and Sacktor, B. (1980) J. Biol. Chem. 255, 7645–7649
23 Burkhardt, G., Kinne, R., Stange, G. and Murer, H. (1980) Biochim. Biophys. Acta 559, 191–201
24 Christensen, H.N. (1979) Adv. Enzymol. 49, 41–101
25 Mircheff, A.K., Van Os, C.H. and Wright, E. (1980) J. Membr. Biol. 52, 85–92
26 LeFevre, P.G. (1961) Pharmacol. Res. 13, 39–57
27 Ludvigsen, C. and Jarret, J. (1979) J. Biol. Chem. 254, 1444–1446
28 Christopher, C.W., Colby, W.W. and Ullrey, D. (1976) J. Cell. Physiol. 89, 683–692
29 Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M. and Sachs, G. (1975) J. Membrane Biol. 21, 375–395
30 Kashara, M., Inui, K., Takano, M. and Hori, R. (1985) Biochem. Biophys. Res. Commun. 132, 490–496
31 Kleinzeller, A. and McAvoy, E.M. (1973) J. Gen. Physiol. 62, 169–184
32 Murer, H., Hopfer, U. and Kinne, R. (1976) Biochem. J. 154, 597–604
33 Aronson, P.S. (1985) Annu. Rev. Physiol. 47, 545–560