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The bovine renal epithelial cell line NBL-1 expresses a broad specificity Na^+-dependent neutral amino acid transport system (System B^0) similar to that in bovine renal brush border membrane vesicles

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(1) In the bovine renal epithelial cell line NBL-1, transport of alanine and glutamine is highly Na^+-dependent while the transport of leucine and phenylalanine is also stimulated by Na^+ although to a much lesser extent. (2) Na^+-dependent alanine transport is insensitive to inhibition by methyl AIB, lysine and glutamate but is inhibited by a range of other neutral amino acids. (3) Inhibition of Na^+-dependent alanine transport by glutamine, phenylalanine and leucine is competitive indicating that these amino acids are transported on a common carrier. (4) Amino acid transport in these cells appears to be localised preferentially on the basolateral membrane. (5) The results are consistent with the presence in confluent cells of a broad-specificity Na^+-dependent neutral amino acid transport system (System B^0) similar to that in bovine BBMV. An Na^+-independent system with high activity similar to System L is also present. (6) It is argued that previous results in the literature are consistent with the occurrence of this broad-specificity system in other renal cell lines.

Introduction

Na^+-dependent transport of neutral amino acids in bovine renal BBMV is mediated by a single transport system of broad specificity which catalyses the uptake of alanine, glutamine, branched-chain and aromatic amino acids [1]. This system differs in specificity from the classical A and ASC systems found in non-epithelial cells; following discussion with Professor H.N. Christensen we propose to refer to this transport system as System B^0. This differentiates the system from the somewhat similar system described in blastocysts by Van Winkle et al. [2] and designated $B^{0,+}$ which transports basic in addition to neutral amino acids. The term B is retained to imply broad substrate specificity. A transport system of similar broad specificity has been implicated in amino acid transport in rabbit intestinal brush border membrane vesicles [3].

Nothing is known at present about the adaptive or hormonal regulation of System B^0. In order to investigate such regulation, it was decided to determine whether a transport system of similar specificity is expressed in intact cells of renal origin. Amino acid transport has been studied in three renal epithelial cell lines. It has been reported that Na^+-dependent transport in the cell line MDCK from canine kidney is mediated by the classical A and ASC systems [4]. In the LLC-PK_1 cell line from pig kidney, very little transport via System A was detected, and Na^+-dependent amino acid transport was ascribed to a single system resembling System ASC [5]. In OK cells from Opossum kidney, the uptake of a number of amino acids including phenylalanine and valine is partially Na^+-dependent and electrogenic [6]. The possibility of a Na^+-dependent system of broad specificity different from Systems A and ASC was not specifically considered in these investigations.

Since the previous work on System B^0 was performed on bovine renal BBMV, it was decided to use

Abbreviations: methyl AIB, 2-(methylamino)isobutyric acid; BCH, 2-aminonorbornane-2-carboxylic acid; AOA, aminooxyacetate; Hepes, 4-(2-hydroxyethyl)piperazineethanesulphonic acid; BBMV, brush border membrane vesicles.

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the epithelial cell line NBL-1 (MDBK) derived from bovine kidney. Although this cell line has been extensively used for studies in other areas, no reports of amino acid transport in these cells are available.

In this paper, evidence is presented that the NBL-1 cell line expresses a transport activity of similar specificity to that characterised in renal brush border membrane vesicles. It is argued that previous work on other cell lines is also consistent with the widespread occurrence of System B^0 in epithelial cells.

Methods

Cell culture

NBL-1 cells were obtained from Flow Laboratories (McLean, VA, USA) at passage number 121 and cultured in Ham's F-12 medium supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin G, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B, 50 μg/ml gentamycin, at 37°C in a gas phase of 5% CO_2/95% air. Cells were grown in 75 cm^2 flasks for continuous culture and 55-mm diameter petri dishes for experiments. Cells were trypsinized once a week, seeded into petri dishes and fed every other day until confluent (5–7 days) when they were used for experiments.

Transport in cell monolayers

Transport was measured using a modification of the method of Quamme et al. [7]. Briefly, cells were washed in the following transport medium without substrate: 137 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM Hepes adjusted to pH 7.4 with Tris. Amino acid uptake was initiated by adding 1ml of the above medium containing appropriate concentrations of amino acid and 1 μCi/ml ^{3}H-labelled amino acid at room temperature (20°C). In experiments where alanine transport was measured 0.5 mM AOA was also present to inhibit alanine metabolism. The uptake was terminated by removing the transport medium and washing with 3×2 ml aliquots of ice-cold 137 mM NaCl, 10 mM Tris-Hepes (pH 7.4). Plates were drained and 0.5 ml 0.5% Triton X-100 added, cells were then removed with a cell scraper and resuspended in this medium. 200-μl samples were dissolved in 10 ml scintillation fluid for counting and protein was determined using the method of Bradford [8]. Na^+-independent uptake was performed using 137 mM choline chloride instead of NaCl in the transport medium.

In some experiments, cells were grown on permeable membrane supports (Transwell-COL; Costar, Cambridge, MA, USA) until confluent. Transport was measured by replacing the medium on either the apical or basolateral side with medium containing radioactive substrates as described above. Cells were removed from the support with a cell scraper and suspended in Triton as above.

FCS and antibiotic/antimycotic (10000 U penicillin G, 10 mg/ml streptomycin and 25 μg/ml amphotericin B) were obtained from Sigma (Poole, Dorset). Ham's F-12 medium and gentamycin (50 mg/ml) were bought from Gibco (Paisley, Scotland); ^{3}H-labelled amino acids were purchased from The Radiochemical Centre Amersham. All other chemicals were of analytical grade.

Results

The bovine renal epithelial cell line NBL-1 (otherwise known as MDBK) has general properties similar to those of the well-studied MDCK cell line. The cells do not express Na^+-dependent hexose transport activity and have very low activities of alkaline phosphatase and glutamyltransferase which are characteristic of proximal tubule brush border membranes. The cells, like MDCK cells [9] are probably not of proximal tubule origin.

Time courses of the uptake of alanine, glutamine, phenylalanine and leucine (0.1 mM) into confluent monolayers of NBL-1 cells in the presence and absence of Na^+ are shown in Fig. 1. A large proportion of the uptake of alanine and glutamine was Na^+-dependent. The uptake of phenylalanine and leucine showed no significant Na^+-dependence over the first three minutes, but a statistically significant stimulation of uptake in the presence of Na^+ was observed at times after 10 min in each case.

In order to determine whether accumulation of amino acids occurred, the cell internal volume was measured. Cell monolayers were incubated for 30 min with [^{14}C]urea which is commonly assumed to equilibrate across cell membranes by a carrier-independent mechanism. Measurements of equilibrium distribution of [^{14}C]urea gave an average value for the internal volume of 6.2 μl/mg protein. After 20 min, the uptakes of alanine and glutamine in the absence of Na^+ were approx. 1.5 nmol/mg in each case (Fig. 1) indicating a small (approx. 2-fold) accumulation. In the presence of Na^+, these amino acids accumulated more than 10-fold.

In contrast the amino acids phenylalanine and leucine showed an apparent 10-fold accumulation in the absence of Na^+. Since no driving force for net uptake is present in the absence of a Na^+ gradient, the apparent accumulation of amino acids under these conditions must be attributed to uptake into a non-osmotically active space and probably represents binding, although there is no direct evidence for this. This 'binding' component represents a major part of the uptake of the hydrophobic amino acids leucine and

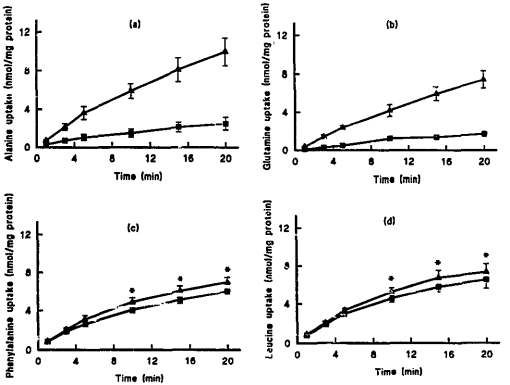

Fig. 1. Time courses of the uptake of various amino acids (0.1 mM) into confluent NBL-1 cell monolayers. Transport was measured in the presence and absence of Na^+ as described in Methods. ▲, $+Na^+$; ■, $-Na^+$. (a) Alanine; (b) glutamine; (c) phenylalanine; (d) leucine. Each point represents the mean ± S.E. of four separate monolayers in (a) and (c), three in (b) and five in (d). Significances in differences in values in the presence and absence of Na^+ were assessed by a paired Student's t-test. All the values were significantly different ($P < 0.01$) in (a) and (b); in (c) and (d) values marked * are significantly different ($P < 0.05$).

phenylalanine, but is much less significant for the hydrophilic amino acids alanine and glutamine.

Fig. 2(a) shows the concentration dependence of alanine uptake in the presence and absence of Na^+ in tye concentration range 0–3 mM alanine. The Na^+-dependent uptake of alanine (i.e. the uptake in the presence of Na^+ minus that in its absence) was saturable with a K_m value of 0.13 mM alanine and a V_{max} of 4.9 nmol/mg per 3 min. The uptake of alanine in this concentration range in the absence of Na^+ was also saturable (Fig. 2(b)). The results are consistent with Na^+-dependent and independent components of trans-

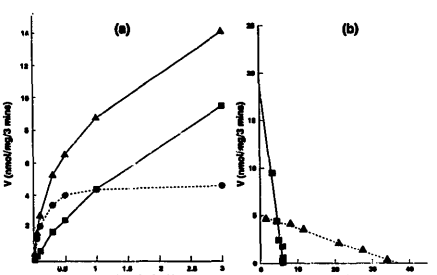

Fig. 2. Concentration dependence of alanine transport. The initial rate was measured after 3 min in the presence and absence of Na^+. (a) V vs. S plots of total uptake of alanine in the presence of Na^+ (▲); the absence of Na^+ (■); and the Na^+-dependent component of transport (i.e. transport in the presence of Na^+ minus that in the absence of Na^+; ●). (b) Eadie-Hofstee plots of Na^+-dependent transport (▲); and Na^+-independent transport (■).

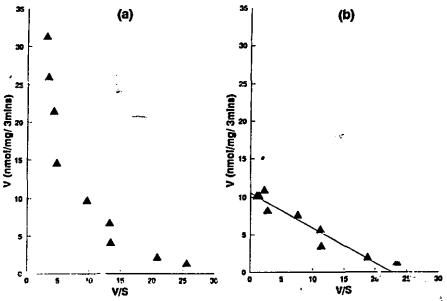

Fig. 3. Na^+-independent uptake of phenylalanine. Phenylalanine uptake was measured after 3 min in the absence of Na^+. The figure shows Eadie-Hofstee plots of (a) total uptake and (b) transport corrected for non-saturable uptake. The non-saturable component was estimated by non-linear regression to the equation $v = K \cdot S + V_{max} \cdot S/(K_m + S)$ where K is a constant. The value of K in this experiment was 2.1.

port each being mediated by a single separate transport system.

Na^+-dependent phenylalanine uptake could not be adequately characterised kinetically since the initial rate of uptake measured after 3 min in the presence of Na^+ was not significantly different from that in its absence at phenylalanine concentrations up to 0.2 mM. At 0.5 mM phenylalanine the Na^+-dependent rate was 0.52 nmol/mg per 3 min while the Na^+-independent rate was 6.3 nmol/mg per 3 min while at 1 mM phenylalanine the Na^+-dependent rate was 0.86 nmol/mg per 3 min against a Na^+-independent rate of 9.2 nmol/mg per 3 min.

Fig. 3 shows Eadie-Hofstee plots of the Na^+-independent uptake of phenylalanine. The Na^+-independent component did not fit simple saturation kinetics

TABLE I

Kinetic constants of amino acid transport

Cell monolayers were incubated with amino acids in the range 0.01–10 mM in sodium chloride or choline chloride containing medium as described in Methods. Initial rates were calculated from the uptake at 3 min. The Na^+-dependent rate was taken as the rate in the presence of sodium chloride minus that in the presence of choline chloride. Kinetic parameters were derived from non-linear regression to Michaelis-Menten equation after subtraction of any non-saturable component. Each value represents a separate experiment in which at least seven concentrations of substrate were used.

	Substrate	K_m (mM)	V_{max} (nmol/mg per 3 min)	Mean K_m (mM)	Mean V_{max} (nmol/mg per 3 min)
Sodium-dependent	Alanine	0.22 ± 0.06	3.2 ± 0.25	0.17	3.87
		0.13 ± 0.07	4.9 ± 0.07		
		0.16 ± 0.014	3.5 ± 0.08		
Sodium-dependent	Glutamine	0.29 ± 0.036	3.6 ± 0.21	0.275	3.05
		0.26 ± 0.058	2.5 ± 0.25		
Sodium-independent	Alanine	5.6 ± 0.83	16 ± 1.6	4.03	18.9
		3.8 ± 0.3	21 ± 1		
		2.7 ± 0.32	19.7 ± 1.6		
Sodium-independent	Glutamine	2.5 ± 0.18	11.4 ± 0.48	3.55	14.2
		4.6 ± 0.29	17 ± 0.72		
Sodium-independent	Phenylalanine	0.52 ± 0.14	10.9 ± 1.36	0.415	9.05
		0.31 ± 0.1	7.2 ± 0.89		
Sodium-independent	Leucine	1.2 ± 0.15	13 ± 0.9	0.85	10
		0.5 ± 0.1	7 ± 0.88		

(Fig. 3(a)). Regression analysis showed a better fit to a system incorporating saturation kinetics with a non-saturable component (Fig. 3(b)). In the case of leucine, no Na^+-dependent initial rate could be measured over 3 min although a significant Na^+-dependence was observed over longer time intervals (see Fig. 1); the Na^+-independent rate also contained a significant non-saturable component (not shown).

Table I shows kinetic parameters for the uptake of alanine, glutamine, leucine and phenylalanine. Kinetic parameters were derived from non-linear regression to the equation

$$v = V_{max} \cdot S/(K_m + S) + K \cdot S$$

where K is a diffusion constant. The Na^+-dependent transport of alanine and glutamine was saturable with K_m values in the range 0.2–1 mM and V_{max} values in the range 2–5 nmol/mg per 3 min. Na^+-independent transport was saturable in the case of alanine and glutamine but contained a non-saturable component in the case of leucine and phenylalanine. The K_m values for Na^+-independent transport of alanine and glutamine were much greater than those for phenylalanine and leucine.

In order to determine the properties of the systems responsible for the Na^+-dependent uptake of these amino acids the inhibition of Na^+-dependent alanine transport (0.1 mM) by a range of other amino acids and analogues (added at 5 mM) was examined. Table II shows that alanine uptake was not significantly inhibited by methyl AIB, glutamate or lysine under these conditions. Na^+-dependent alanine transport was however inhibited by leucine, phenylalanine, glycine, glu-

TABLE II

Inhibition of Na^+-dependent alanine transport by a range of amino acids

Transport of alanine (0.1 mM) was measured over 3 min in the presence of AOA to prevent alanine metabolism and in the presence and absence of Na^+ as described in Methods. The results shown are the rate in the presence of Na^+ minus the rate in the absence of Na^+ and are the means of data from taken from at least two cell monolayers in the presence of Na^+ and two in its absence. Duplicate results agreed to within 10%. The mean Na^+-dependent rate of alanine transport in the absence of inhibitors was 1.5 nmol/mg per 3 min.

Inhibitor (5 mM)	Percentage inhibition
Glutamine	88
Leucine	51
Glycine	35
Phenylalanine	32
BCH	26
Proline	12
Methyl AIB	< 5
Glutamate	< 5
Lysine	< 5

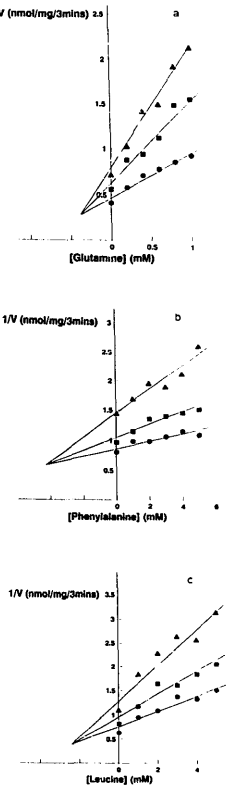

Fig. 4. Dixon plots of the inhibition of Na^+-dependent alanine transport by glutamine, leucine and phenylalanine. Initial rates of transport were measured over 3 min in the presence of various inhibitor concentrations in the presence and absence of Na^+. (a) Inhibition by glutamine: ▲, 0.1 mM alanine; ■, 0.15 mM alanine; ●, 0.25 mM alanine. (b) Inhibition by phenylalanine: ▲, 0.05 mM alanine; ■, 0.075 mM alanine; ●, 0.1 mM alanine. (c) Inhibition by leucine: ▲, 0.05 mM alanine; ■, 0.075 mM alanine; ●, 0.1 mM alanine.

tamine, proline and BCH. These results show that Na^+-dependent uptake of alanine is not mediated by the classical methyl AIB-sensitive System A.

Inhibition of the transport of one amino acid by another does not imply transport via the same carrier unless the inhibition can be shown to be competitive. Analysis of the type of inhibition in each case was performed using plots of $1/V$ vs. inhibitor concentration (Dixon plots [10]) at three substrate concentrations. In the case of simple competitive inhibition, these plots should be linear and should intersect above the x axis. In the case of simple non-competitive inhibition, the lines should intersect on the x axis [10]. Fig. 4 shows Dixon plots of the inhibition of the Na^+-dependent component of alanine transport by (a) glutamine, (b) phenylalanine, (c) leucine. Alanine was chosen as substrate, since alanine metabolism is completely inhibited by aminooxyacetate. Inhibition of alanine transport by glutamine (K_i 0.4 mM), phenylalanine (K_i 4.2 mM) and leucine (K_i 2.2 mM) was competitive in each case. In contrast, inhibition of alanine transport by glycine was non-competitive indicating uptake of glycine via a separate transport system (Fig. 5(a)). The K_i value for the inhibition of alanine transport by glutamine is similar to the K_m of Na^+-dependent glutamine transport (Table I). As discussed above K_m values for the Na^+-dependent uptakes of leucine and phenylalanine could not be determined.

These results are consistent with the postulate that Na^+-dependent uptakes of alanine, glutamine, phenylalanine and leucine are mediated by a common carrier in NBL-1 cells. This carrier does not catalyse the transport of glycine, lysine, glutamate or methyl AIB and is hence similar to the broad specificity transport system identified in bovine brush border membrane vesicles (System B^0). The carrier also presumably transports BCH which is an analogue of leucine.

A major difference between amino acid transport in bovine BBMV and NBL-1 cells is the much larger Na^+-independent uptake shown in cells. Interactions between the transport of various amino acids in the absence of Na^+ is shown in Table III. The L system inhibitor BCH added at 50-fold excess inhibits the uptake of alanine, leucine and phenylalanine, although this inhibition is not complete. Phenylalanine and leucine at the concentrations used are strong inhibitors of alanine transport while alanine at these concentrations is a relatively poor inhibitor of the uptake of phenylalanine and leucine. Lysine did not inhibit Na^+-independent alanine uptake (not shown).

TABLE III

Inhibition of Na^+-independent transport

The experiment was performed as in the legend to Table II, except that NaCl was replaced by choline Cl throughout.

Inhibitor (5 mM)	Percentage inhibition of transport		
Substrate (0.1 mM):	alanine	leucine	phenylalanine
Alanine	–	22	34
Leucine	68	–	73
Phenylalanine	77	70	–
BCH	49	55	57

Similar Dixon plot analysis (not shown) indicated that the inhibition of Na^+-independent transport of alanine was inhibited competitively by phenylalanine (K_i 1.3 mM), leucine (K_i 1.7 mM) and glutamine (K_i 3.2 mM). Competitive inhibition was also observed with glycine (Fig. 5(b)). The K_i for glutamine for inhibition of alanine transport is similar to the K_m for glutamine of Na^+-independent glutamine uptake. The K_i values for inhibition by of alanine transport by leucine and phenylalanine are considerably higher than the respective K_m values for leucine and phenylalanine transport, and this possibly relects association of these amino acids with the cells via other mechanisms. The results are consistent with the presence of a Na^+-independent L-type transport system for the uptake of alanine, glutamine, glycine, leucine and phenylalanine.

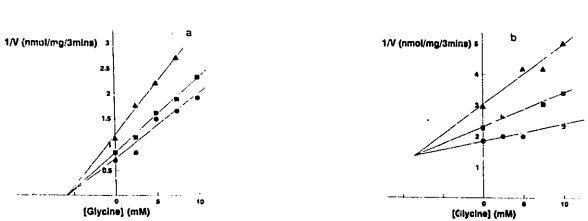

Fig. 5. Dixon plots of the inhibition of Na^+-dependent and Na^+-independent transport of alanine by glycine. (a) Na^+-dependent transport (rate in presence of Na^+ minus rate in the absence of Na^+). (b) Na^+-independent transport. Alanine concentrations were: ▲, 0.05 mM; ■, 0.075 mM; ●, 0.1 mM.

TABLE IV

Membrane localisation of transport systems in NBL-1 monolayers

Cells were grown on permeable membrane supports as described in Methods. Transport was measured over a 3-min time period. Results are means of determinations of two separate monolayers, which agreed within 10%.

Substrate (0.1 mM)	Inhibitor (5 mM)	Transporting membrane	Presence of sodium	Total uptake (nmol/mg per 3 min)	Na^+-dependent uptake (nmol/mg per 3 min)	% Inhibition
Alanine		Apical	+	0.7	0.58	
		Apical	−	0.12		
Alanine		Basolateral	+	1.5	1.335	
		Basolateral	−	0.165		
Alanine	Methyl AIB	Apical	+	0.64		< 10
	Methyl AIB	Basolateral	+	1.35		< 10
Alanine	Leucine	Apical	+	0.35		50
	Leucine	Basolateral	+	0.52		65
Phenylalanine		Apical	+	0.57	0.03	
		Apical	−	0.54		
Phenylalanine		Basolateral	+	1.03	0.21	
		Basolateral	−	0.82		
Phosphate [a]		Apical	+	3.7	3.48	
		Apical	−	0.22		
Phosphate [a]		Basolateral	+	0.98	0.884	
		Basolateral	−	0.096		

[a] Unpublished observations of Mr. C. Helps.

In order to determine whether amino acid transport systems were located mainly in the apical or the basolateral membranes of these cells, cells were grown to confluency on permeable membrane supports. Radioactive substrates were added either from the apical or the basolateral side and the initial rate of uptake of substrate into the cells was measured after 3 min (Table IV). Alanine transport from either side was Na^+-stimulated, insensitive to inhibition by methyl AIB and inhibited by leucine. The Na^+-dependent rate of transport was however higher from basolateral side than from the apical side. Phenylalanine transport was also Na^+-stimulated from both sides and again the Na^+-dependent rate was higher from the basolateral side. In contrast in similar experiments the rate of Na^+-dependent uptake of phosphate was higher when added from the apical side than from the basolateral side. These results provide no evidence for the presence of System A on either membrane. The broad-specificity System B^0 appears to occur on both apical and basolateral membranes, but the activity is 2-fold higher on the basolateral membranes under these conditions.

Discussion

The results in this paper are consistent with the presence of two separate transport systems for neutral amino acids in the NBL-1 cell line. One system: is Na^+-independent; transports alanine, glutamine, phenylalanine, leucine and glycine; is inhibited by BCH and shows properties similar to those of System L in many other cell types. This system has high activity in NBL-1 cells. The other system is Na^+-dependent. As shown by inhibition analysis, this system transports alanine, glutamine, phenylalanine and leucine. It is inhibited non-competitively by glycine and is not sensitive to inhibition by methyl AIB, lysine or glutamate. This system differs from the classical Systems A and ASC in that branched-chain and aromatic amino acids are transported. From the results presented, it appears that this transport system has very similar properties to those of System B^0 which has been characterised in bovine brush border membrane vesicles [1]. Alanine transport via System B^0 in bovine BBMV is also non-competitively inhibited by glycine which is transported on a separate transport system [11]. In addition to these two transport systems, a major proportion of the association of leucine and phenylalanine with the cells is non-saturable and may be due to non-speciific binding.

Leucine and phenylalanine competitively inhibit Na^+-dependent alanine uptake and the uptake of these amino acids is to some extent Na^+-dependent. The Na^+-dependent component of uptake represents only a small proportion of total uptake, particularly at short time intervals, and thus the kinetic parameters of Na^+-dependent transport could not be determined. The small overall apparent Na^+-dependence of leucine and phenylalanine uptake in these cells in comparison with bovine BBMV may arise from the fact that NBL-1 cells have a Na^+-independent transport system of hgh

activity for these amino acids and also that a considerable proportion of the apparent uptake is due to Na^+-independent binding. A large Na^+-dependent accumulation of hydrophobic amino acids in these cells would not be expected, since leucine or phenylalanine entering by Na^+-cotransport would rapidly equilibrate via the active System L. In these cells, it was not possible to use BCH to inhibit System L specifically since this compound inhibited Na^+-dependent amino acid transport also and presumably acts as a substrate for System B^0.

The apparent preferential localisation of the brush border-like transport System B^0 on the basolateral membrane when cells are grown on permeable filters is both surprising and paradoxical, particularly since Na^+-dependent phosphate transport activity is found mainly on the apical membrane. A preferential localisation of AIB and cycloleucine transport to the basolateral membrane has also been shown in LLC-PK_1 cells [12] although D-aspartate transport appeared to be an apical membrane activity in these cells [13]. Na^+-dependent alanine transport has been localised to the basolateral membrane of LLC-PK_1 cells using autoradiography [14] and in MDCK cells, methyl AIB transport is similarly a basolateral membrane activity [15]. Some evidence for the expression of an apical broad-specificity amino acid transport system in confluent MDCK cells exposed to prostaglandin E has been presented [16] but this system has not been adequately characterised.

It is of interest to consider whether the expression of the broad-specificity transport system characterised here is confined to NBL-1 cells or whether it occurs also in other renal cell lines. Although it is stated that the LLC-PK_1 and MDCK cell lines express classical System A, ASC and L activity, this conclusion is not well supported by the published evidence. In a characterisation of amino acid transport in MDCK cells it was observed that Na^+-dependent alanine transport was not inhibited by an excess of methyl AIB, but was considerably inhibited by leucine [4]. These findings are not typical of classical A and ASC transport activities. No kinetic analysis of the inhibitions observed was performed in this study.

Amino acid transport in LLC-PK_1 cells was characterised in a more thorough study [5]. Alanine transport was not inhibited by methyl AIB and it was concluded that System A activity was insignificant in these cells. Leucine uptake was partially Na^+-dependent. Na^+-dependent alanine transport was inhibited by leucine, phenylalanine and glycine, but not by lysine or glutamate. It was not determined whether inhibition was competitive or non-competitive. These results are again not characteristic of transport via System ASC which does not transport branched-chain or aromatic amino acids. However, the results closely resemble those for the uptake of amino acids into NBL-1 cells as shown above.

Finally, Silbernagl et al. [6] have shown that polarised monolayers of OK cells are depolarised in a Na^+-dependent manner by addition of a broad range of amino acids including alanine, glutamine, phenylalanine and valine. This indicates the presence of electrogenic Na^+-cotransport of these amino acids in these cells.

The above considerations are consistent with the possibility that the broad-specificity transport System B^0 defined in renal BBMV and in NBL-1 cells may be also distributed more widely in other renal epithelial cell lines.

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