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Biochimica et Biophysica Acta 1190 (1994) 279–288

BBA Biochimica et Biophysica Acta

A comparative study on the uptake of α-aminoisobutyric acid by normal and immortalized human embryonic kidney cells from proximal tubule

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(Received 27 July 1993)

Abstract

We investigated whether an immortalized human kidney epithelial cell line (IHKE), compared with normal embryonic cells (NHKE), can be used as a representative system with which to characterize the transport of neutral amino acids in the proximal tubule of the human kidney. The IHKE cell line, immortalized by treatment with $NiSO_4$, exhibited microvilli and enzyme markers specific for highly specialized tubule cells. The Na^+-dependent uptake of α-aminoisobutyric acid (AIB) by IHKE and NHKE cells occurred by means of a single transport system with identical half-saturation constants, but the capacity for uptake was higher in the immortalized cells. Proton-dependent influx of AIB was also mediated by a single transport component with similar uptake characteristics in both types of cells. Imposition of an H^+-gradient to a Na^+-gradient reduced the sodium dependent uptake of AIB with the exception of short incubation time (1 min), where addition of a proton gradient produced a marked increase in the Na^+-dependent influx of AIB in NHKE but not in IHKE cells. Competition experiments revealed that the Na^+-dependent uptake at 50 μM AIB was reduced by neutral α-amino acids in the two cell lines. L-Glutamate, L-aspartate, L-arginine and the β-amino acid taurine had no effect. Only in the IHKE cell line did addition of 5 mM L-lysine produce a slight inhibition. Except for L-proline all of the neutral and acidic amino acids tested reduced the H^+-dependent uptake of AIB in the IHKE cell line. By contrast, addition of L-aspartate did not influence the transport of AIB in NHKE cells. L-Arginine, but not L-lysine decreased the influx in both cell lines. We conclude that the IHKE cell line has retained the capability to accumulate AIB by transport protein(s) similar to those present for neutral α-amino acids in NHKE cells.

Key words: α-Aminoisobutyric acid transport; Sodium ion dependent uptake; Proton dependent influx; Proximal tubule; (Human kidney cells)

1. Introduction

Characterization of transport processes in the renal proximal tubule with the aid of continuous epithelial cell lines is under intense study at present. Examples of such cell lines are the LLC-PK_1 and the OK cell line, which are derived from pig [1] and opossum kidney [2], respectively. Especially the LLC-PK_1 cells, which retain several characteristics of the proximal tubule, have been thoroughly investigated for their capability to transport sugar, phosphate and amino acid [3–8]. However, in contrast to proximal tubule cells the LLC-PK_1 cells have no, or only very few parathyroidea hormone (PTH) receptors and the Na^+-coupled phosphate transport is not controlled by PTH or cyclic AMP. Furthermore, a cystine/lysine transport system cannot be detected in this cell line. In these respects, the OK cell line may be a better model system since the transport components and parathyroid hormone receptors have been shown to be more representative of proximal tubular epithelial cells than those found in the LLC-PK_1 cells [9–11]. On the other hand, D-glucose, in contrast to experiments with other well-known in vivo/in vitro techniques, is primarily transported via an Na^+-independent system in the OK cells.

In order to characterize and understand the transport mechanisms of filtered solutes in the human kidney under normal and pathological conditions, it would be appropriate to use a human cell line. Normal human kidney epithelial cells (NHKE) can be purchased from therapeutic midtrimester abortions, but they are difficult to obtain on a regular basis and have a very

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SSDI 0005-2736(93)E0343-9

short life-span in culture. Recently, Tveito and co-workers were able to obtain a human embryonic kidney cell line (IHKE) from the proximal tubule, which had been immortalized after treatment with $NiSO_4$ [12]. This has given us the opportunity to investigate whether this new human cell line (IHKE), as compared with normal cells (NHKE), is an appropriate model for examination of the different transport systems concerning organic compounds (e.g., amino acids, sugars, mono-, di-, tri-carboxylic acids, etc.).

In recent years, we have characterized the renal transport of a number of neutral amino acids [13–17] by the use of luminal membrane vesicles from proximal tubule of rabbit kidney. Thus, initially, the aim of the present study was to compare the normal (NHKE) cell line with the immortalized (IHKE) cell line in regard to the uptake of neutral α-amino acids. To study the renal handling of neutral amino acids α-aminoisobutyric acid (AIB) was used as a prototype. This amino acid is characterized by being non-metabolizable, thus eliminating the problem of distinguishing between transport mechanisms and metabolism. Furthermore, we show the presence of brush-border membranes for the immortalized cells and the activities of marker enzymes from NHKE and IHKE cell monolayer cultures are determined.

2. Materials and methods

Materials

α-Amino[*methyl*-^{3}H]isobutyric acid (spec. act. 13.4 Ci/mmol) and *p*-amino[*glycyl*-2-^{3}H]hippuric acid (spec. act. 1.66 Ci/mmol) were purchased from New England Nuclear. [C^{14}]Urea (spec. act. 55.3 mCi/mmol) and [^{3}H]inulin (spec. act. 1.43 Ci/mmol) were obtained from Amersham. Fetal calf serum (FCS), growth medium and antibiotics were from Gibco, NY. All other non-radiactively labelled ligands, Hepes and Mes were obtained from Sigma, St. Louis, MO, USA.

Cell culture

NHKE and IHKE cells were kindly provided by Dr. Aage Haugen, Department of Toxicology, National Institute of Occupational Health, Oslo, Norway and grown in α-MEM containing 5% and 1% of FCS, respectively. The α-MEM was supplemented with epidermal growth factor (10^{-5} g/l), insulin ($5 \cdot 10^{-3}$ g/l), hydrocortisone ($1 \cdot 10^{-4}$ g/l), transferrin ($5 \cdot 10^{-3}$ g/l), 2 mM glutamine, penicillin ($5 \cdot 10^{4}$ IU/l) and streptomycin ($5 \cdot 10^{-2}$ g/l). The conditions of the culture were 37°C (pH 7.4) and atmosphere 5% CO_2 in air.

Marker enzyme assays

The activities of marker enzymes were measured on crude cell homogenates from NHKE and IHKE cell monolayer cultures. $Na^+ + K^+$-stimulated ATPase activity was assayed as reported by Skou [18] and used as a basolateral-membrane marker. Alkaline phosphatase and leucine aminopeptidase activities were measured as described by Bessey et al. [19] and with the use of the Sigma kit 251-AW, respectively, and used as luminal-membrane markers. Activity of another luminal marker, maltase, was determined by the method of Dahlqvist [20].

Electron microscopy

Cells were grown on 25 mm tissue culture inserts (Nunc) till they reached a confluent state. Cell monolayers were fixed in situ in 2% glutaraldehyde, prepared in 0.1 M cacodylate buffer (pH 7.2) supplemented with 0.1 M sucrose. The cell cultures were dehydrated in ascending concentrations of ethanol and embedded in Lowicryl K4M. After photopolymerization the monolayers were sectioned perpendicular to the growth substratum on an Ultratome III ultramicrotome (LKB). The sections were contrasted with uranyl acetate and lead citrate and studied in a Zeiss EM 10 B transmission microscope, operated at 80 kV.

Transport studies

Uptake studies were performed on monolayers in 35 mm single dishes (Nunc, Denmark) as soon as the cells had obtained confluence. Transport of AIB was measured by the following techniques: After removal of medium, each dish was washed twice at 37°C with 2 ml Earle's Balanced Salt Solution (EBSS), containing 5 mM D-glucose (pH 7.4) and incubated for 90 min with the same solution in order to minimize any trans-effects due to the intracellular pool of amino acids. They were then incubated with 0.75 ml of incubation medium containing EBSS (pH 7.4, 37°C), radiolabelled ligand and other constituents as required. The incubation was terminated by aspiration of the incubation media and immediately washing the cells three times with 2 ml ice-cold EBSS (pH 7.4). After removal of the last volume of the ice-cold stop solution, 0.6 ml of 1% SDS in 0.2 M NaOH was added in order to solubilize the cells. The radioactivity in the cells was determined by taking three samples of 0.1 ml, which were counted in a liquid scintillation counter (LKB-Wallac 1218 RackBeta) after addition of 3 ml scintillation fluid. Correction for non-specific uptake was made in control experiments by estimating the amount of radiolabelled AIB retained in the extracellular space or bound to the cell surface after incubation with ice-cold incubation medium. For sodium-free assays, Na_2HPO_4, NaCl, and $NaHCO_3$ were replaced by choline phosphate, choline chloride and choline bicarbonate, respectively (Na^+-free EBSS). All uptake studies were performed in an atmosphere of 5% CO_2 in air and the various kinds of EBSS were supplemented with 15 mM Hepes/KOH

(pH 7.4). In experiments where the effect of an inwardly directed H^+-gradient was examined, $NaHCO_3$/choline bicarbonate was replaced by NaCl/choline chloride. Furthermore, 25 mM Mes/KOH (pH 4.5) or 25 mM Hepes/KOH (pH 7.4) were used as buffers.

To determine Na^+- or H^+-gradient-dependent uptake of AIB, the Na^+- or H^+-independent uptake was subtracted from total uptake. In competition experiments the uptake values were not corrected for the influx in the absence of the driving cation-gradient because of the difficulties in achieving a sufficient amount of NHKE cells. However, preliminary experiments with both cell lines demonstrated that inhibition data based on the Na^+-gradient-dependent uptake values only varied slightly, if at all, compared to the non-corrected data. Furthermore, the pH-dependent uptake of AIB by the NHKE cells only indicated a slight, if detectable, additional reduction in the uptake of amino acid when inhibitor concentration was raised from 0.5 mM to 5.0 mM, suggesting that a maximal inhibition, corresponding to the basal uptake level had already been reached at the lower concentration. The amount of total cell protein was determined by the method of Lowry et al. [21] with bovine serum albumin (Sigma) as a standard. All solutions used in this study were sterilized before use.

Calculations

Cell water. Intracellular fluid volume was estimated by subtracting the extracellular fluid volume ([^{3}H]inulin space) from the total water ([^{14}C]urea space) as previously reported by Elsas and co-workers [22].

Kinetics. The results of the saturation experiments were analysed by use of Michaelis-Menten kinetics. The results were analysed according to the following equation:

$$\text{Uptake} = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

where K_m represents the substrate concentration that gives half-maximal uptake, V_{max} denotes maximal uptake and [S] indicates initial concentration of substrate. Theoretical saturation curves were fitted to the experimental data by computer-analyzed statistical iteration procedures [23].

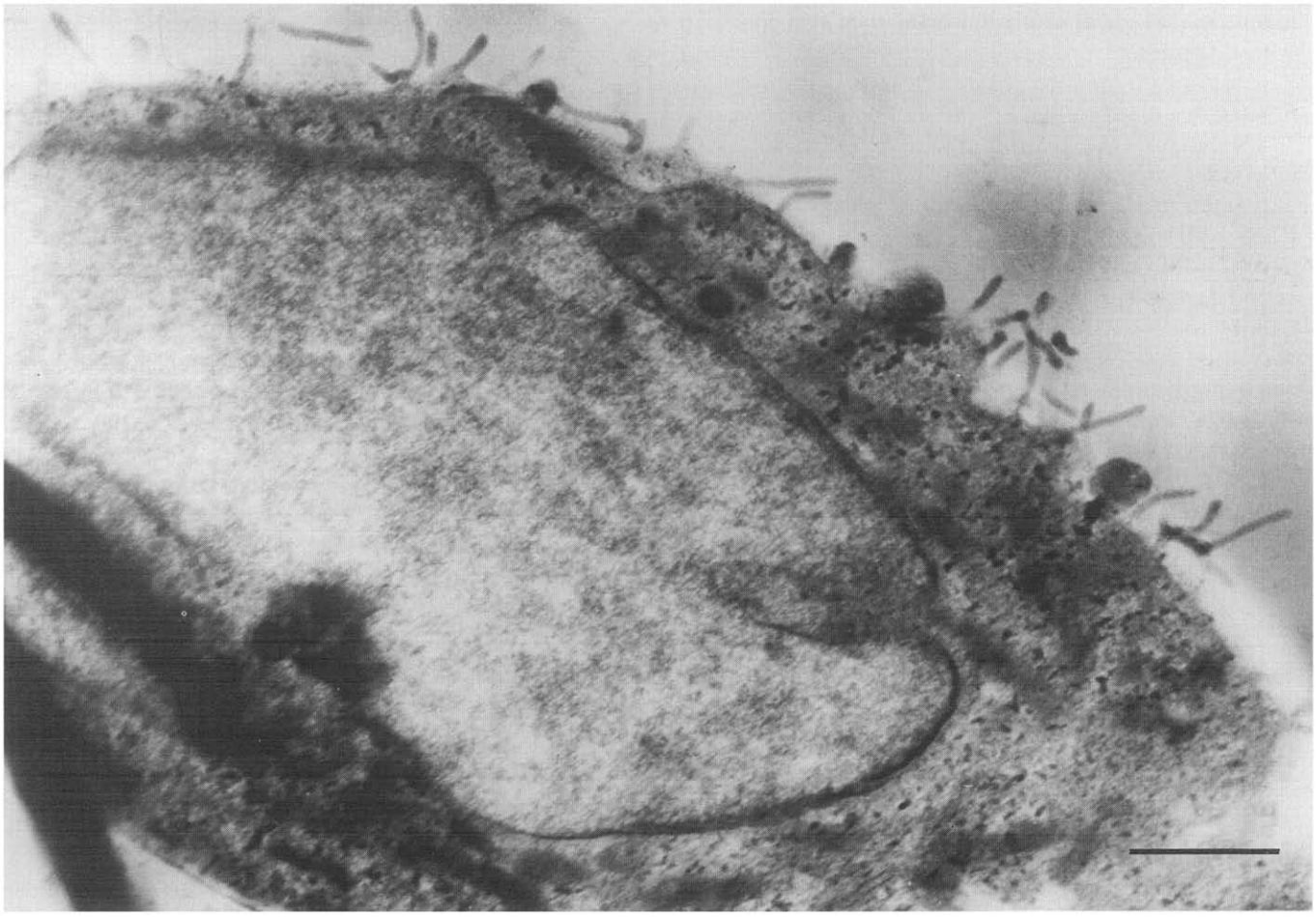

Fig. 1. Electron micrograph of vertical section through monolayer of IHKE cells exhibiting microvilli on the apical surface. The bar equals 1 μm.

3. Results

Electron microscopy

Tveito and co-workers [12] previously demonstrated by transmission electron microscopy that the IHKE cells possess a number of characteristics, considered to be specific for epithelial cells (e.g., tonofilaments (keratin filaments) and desmosomes). However, the above mentioned ultrastructural examination did not provide any information as to the presence of a brush-border membrane in this immortalized cell line. Fig. 1 shows the apical surface of thinly sliced IHKE cells, investigated by transmission electron microscopy. The proximal tubule cell line presents microvilli, indicative of highly specialized epithelial cells.

Luminal and basolateral membrane enzymes

Since leucine aminopeptidase, maltase and alkaline phosphatase are exclusively confined to the luminal membrane of proximal tubule in the kidney, we measured the activities of these enzyme markers in the IHKE and NHKE cells. Table 1 shows that all three enzymes were present, confirming that the cells originate from the proximal tubule. Activity of the $Na^+ + K^+$-stimulated ATPase, localized in the basolateral membrane of renal epithelial cells, was also found in both cell lines.

Radioactive uptake of AIB

The time-course for the uptake of radioactive AIB by confluent monolayers of IHKE and NHKE cells is illustrated in Fig. 2. In the presence of Na^+-containing EBSS, AIB uptake was linear for at least the first 5 min in both cell lines. However, the initial uptake of amino acid occurred more rapidly by the IHKE cells than by the NHKE cells. The uptake rate subsequently decreased and reached a maximal value of approx. 17 nmol/mg protein after 60 min by the IHKE cell line. By contrast, the uptake of AIB by NHKE cells still increased after 2 h of incubation. In Na^+-free medium, the influx of AIB in both cell types was drastically reduced. Thus, the uptake of AIB was less than 10% compared to the uptake in the presence of sodium after an incubation period of 60 min.

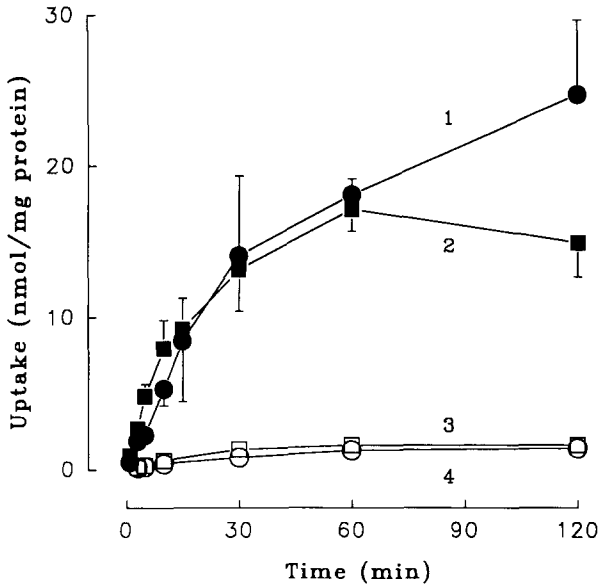

Fig. 2. Time-course of α-aminoisobutyric acid uptake by IHKE or NHKE cells in the presence or absence of Na^+-gradient. Monolayers of IHKE/NHKE cells were incubated in Na^+-containing (curve 2/curve 1) or in Na^+-free EBSS (curve 3/curve 4) as described under Materials and methods. Media contained 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and sufficiently unlabelled α-aminoisobutyric acid to reach a total concentration of 50 μM (final concentration). The results shown are the mean values ±S.D. of three (NHKE) or four (IHKE) experiments.

Table 1
Enzyme activities

	IHKE	NHKE
Leucine aminopeptidase	34.1 ± 9.7 *	20.6 ± 8.5 *
Alkaline phosphatase	3.3 ± 1.4 *	4.5 ± 1.7 *
Maltase	32.0 ± 1.9 *	25.9 ± 7.6 *
Na^+/K^+-ATPase	2.4 ± 1.3 *	1.1 ± 0.6 *

The various activities were determined as mentioned under Materials and methods. Enzyme activities for leucine aminopeptidase are expressed as micromoles of substrate converted per min per milligram of cell protein. All other results are expressed as nanomoles of substrate converted per milligram of cell protein per min. The results given are the mean values ± S.D. of at least three experiments ($n = 3$–15). * *t*-test: The means of the samples are not different at the 0.05 level.

To examine whether the uptake of AIB occurred across the luminal or basolateral membranes of the monolayers, a series of experiments were done with [^{3}H]AIB and *p*-amino[^{3}H]hippuric acid (PAH). The latter is characterized by only being transported across the basolateral membrane of the proximal tubule cell [24,25]. We found that after 60 min of incubation in Na^+-containing EBSS, the accumulation ratio of PAH into the IHKE and NHKE cells was only 0.038 and 0.005, respectively. By contrast, the accumulation of AIB intracellularly was 15.8 and 10.9 times the amino acid concentration in the incubation medium for the immortalized and normal kidney cells. These results strongly suggest that the data presented in this study represent AIB influx across the luminal membrane in the two cell lines.

We have previously demonstrated the existence of an H^+-cotransport system for AIB in luminal membrane vesicles from the pars convoluta of rabbit kidney, which is capable of driving the uphill transport of AIB in the absence of other energy sources [17]. Therefore, a number of experiments were performed in order to measure the effect of an inwardly directed proton gradient on the cellular uptake of amino acid. As

illustrated in Fig. 3, imposition of an H^+-gradient ($pH_{out} < pH_{in}$) in the absence of a sodium gradient led to a marked enhancement in the uptake of amino acid by the IHKE cells. Another series of experiments indicated the same phenomenon for the NHKE cell line (results not shown). By contrast, addition of an H^+-gradient to a Na^+-gradient (both inwardly directed) resulted in a reduction of the sodium-dependent influx of AIB by the IHKE and NHKE cell line with the exception of the uptake measured at 1 min, where the influx of amino acid in the NHKE but not in the IHKE cells is markedly stimulated (Fig. 4).

The concentration dependence of AIB uptake was studied at concentrations ranging from 0.005–1.0 mM. The kinetic experiments in Fig. 5A were performed at 5 min in the presence or absence of sodium. The figure shows that influx of the amino acid by the IHKE and NHKE cell line was a curvilinear function of external AIB concentration in the Na^+-containing medium (curve 1 and 2). In the Na^+-free medium, the uptake of AIB by the two cell lines exhibited simple diffusion properties, being proportional to medium concentration of AIB under these conditions (curve 3 and 4). To further characterize the Na^+-gradient dependent component of AIB transport, uptake observed in the absence of sodium was subtracted from that found in the presence of sodium. The corrected data are given in Fig. 5B. In the inset is shown Eadie-Hofstee analyses

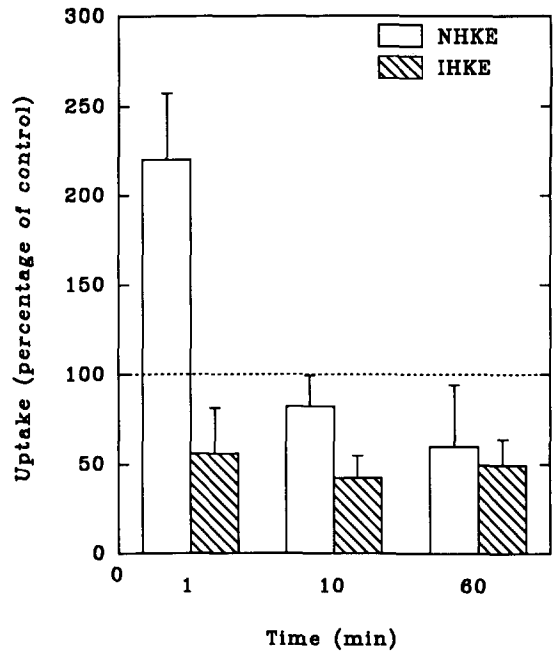

Fig. 4. Effect of an inwardly directed pH-gradient on the uptake of α-aminoisobutyric acid in the presence of Na^+-containing EBSS. Monolayers of IHKE and NHKE cells were incubated in Na^+-containing EBSS, pH 4.5 or pH 7.4. Media contained 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and sufficiently unlabelled α-aminoisobutyric acid to reach a total concentration of 50 μM (final concentration). Uptake of AIB in the absence of an inwardly directed pH-gradient (control) is designated as 100%. The results shown are the mean values ±S.D. of three (IHKE) or four (NHKE) experiments.

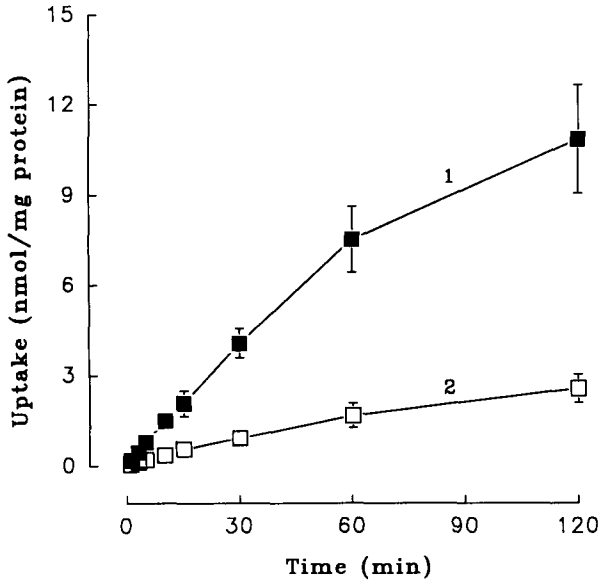

Fig. 3. Time-course of α-aminoisobutyric acid uptake by IHKE cells in the presence or absence of H^+-gradient. Monolayers of IHKE cells were incubated in Na^+-free EBSS (pH 4.5) (curve 1) or in Na^+-free EBSS (pH 7.4) (curve 2) as described under Materials and methods. Media contained 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and sufficiently unlabelled α-aminoisobutyric acid to reach a total concentration of 50 μM (final concentration). The results shown are the mean values ±S.D. of three experiments.

which indicates that the influx of AIB occurs by means of a single transport system in both human cell lines. Computerized calculations gave a K_m value of 0.74 ± 0.13 mM and a V_{max} value of 19.5 ± 1.8 nmol/mg protein per 5 min for the normal cells and a K_m value of 0.66 ± 0.14 mM and a V_{max} value of 58.6 ± 6.4 nmol/mg protein per 5 min for the immortalized cells. Thus, our data clearly suggest that the sodium-dependent uptake of AIB by IHKE and NHKE cells occurred by means of a transport component with identical half-saturation constants but with a higher uptake capacity for the immortalized cells.

Similar experiments were performed in order to further characterize the effect of a pH-gradient on the uptake of AIB. The kinetic experiments in Fig. 6A were done in the presence (curve 1 and 2) or absence (curve 3 and 4) of an inwardly directed H^+-gradient. The H^+-gradient-dependent components of amino acid influx calculated as the difference between curves 1 and 3 and curves 2 and 4 from Fig. 6A are plotted in Fig. 6B. The kinetic analysis by computer resulted in a single transport system with the following kinetic parameters for the IHKE cells: $K_m = 0.50 \pm 0.13$ mM and $V_{max} = 4.8 \pm 0.6$ nmol/mg protein per 5 min and for the NHKE cells: $K_m = 1.13 \pm 0.20$ mM and $V_{max} =$

8.2 ± 0.9 nmol/mg protein per 5 min. As seen from the figure the data (mean values ± S.D.) of the two curves are not significantly different from each other, demonstrating that the effect of an H^+-gradient is nearly the same in the two cell lines. These data are also illustrated in the inset as an Eadie-Hofstee graphical representation, where the curves are computer drawn regression lines.

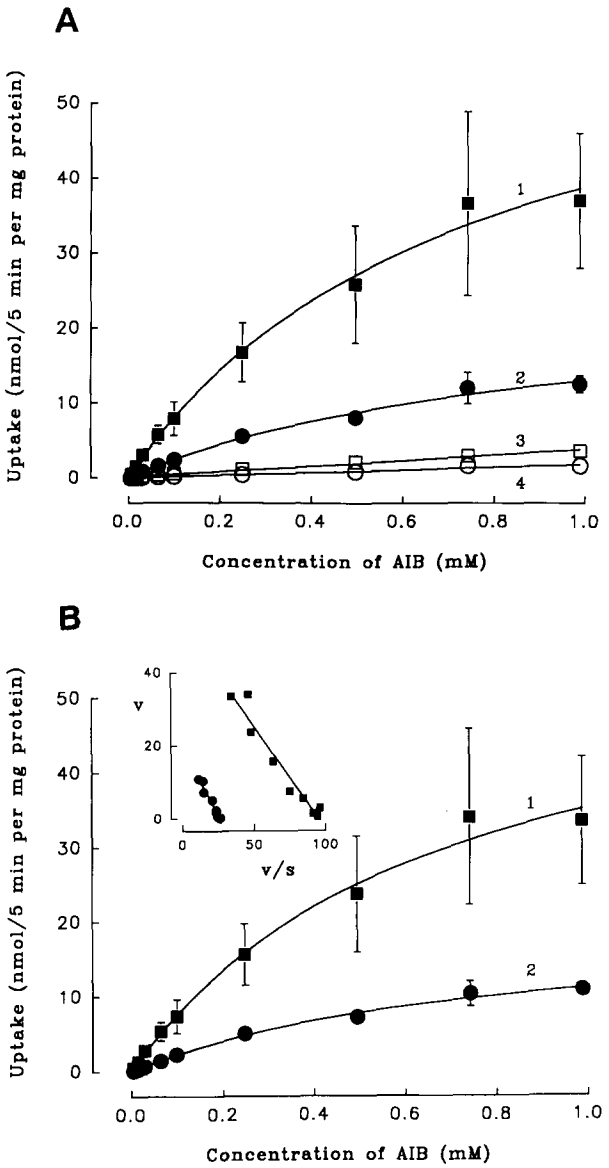

Fig. 5. Kinetics of α-aminoisobutyric acid uptake by IHKE or NHKE cells in the presence or absence of Na^+-gradient. (A) Monolayers of IHKE/NHKE cells were incubated in Na^+-containing (curve 1/curve 2) or Na^+-free EBSS (curve 3/curve 4), 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and various concentrations of unlabelled α-aminoisobutyric acid ranging from 0.005–1.0 mM. (B) Subtracting the uptake values in the absence of Na^+ from the total uptake in the presence of Na^+ resulted in the uptake values in the presence of a Na^+-gradient alone (IHKE (curve 1), NHKE (curve 2)). Inset shows an Eadie-Hofstee plot of data obtained in the presence of a Na^+-gradient alone. V represents the rate of transport at substrate concentration S. The results given are the mean values ± S.D. of 3 (NHKE) or 4 (IHKE) experiments. In those cases where vertical bars are absent, S.D. were smaller than graphical representation of mean.

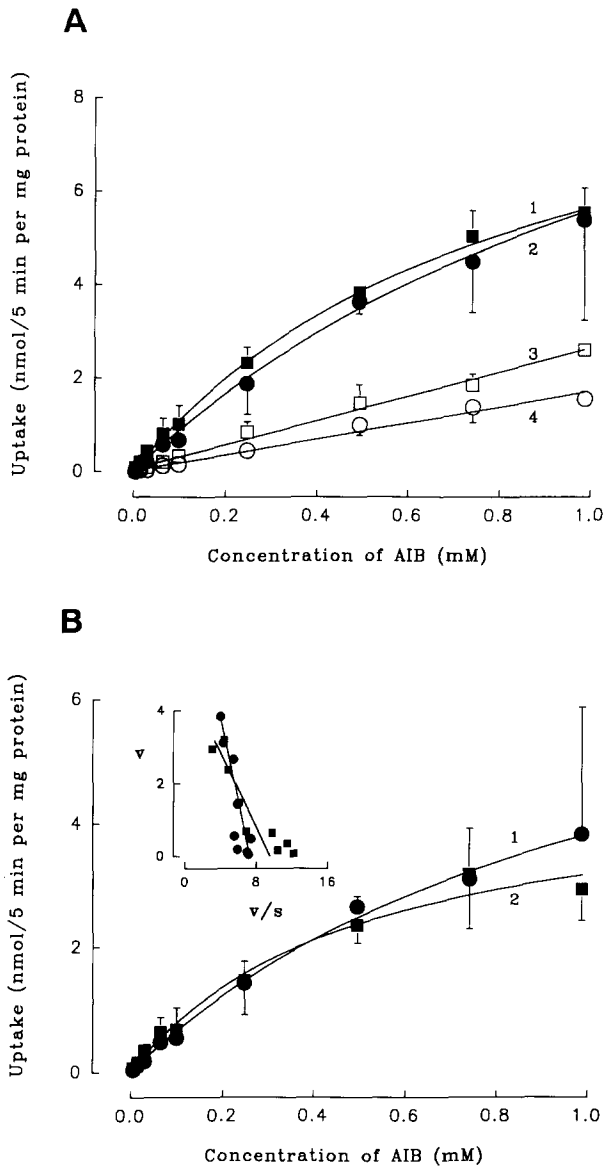

Fig. 6. Kinetics of α-aminoisobutyric acid uptake by IHKE or NHKE cells in the presence or absence of H^+-gradient. (A) Monolayers of IHKE/NHKE cells were incubated in Na^+-free EBSS (pH 4.5) (curve 1/curve 2) or Na^+-free EBSS (pH 7.4) (curve 3/curve 4), 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and various concentrations of unlabelled α-aminoisobutyric acid ranging from 0.005–1.0 mM. (B) Subtracting the uptake values in the absence of an H^+-gradient from the uptake in presence of an H^+-gradient resulted in the uptake values in the presence of an H^+-gradient alone (IHKE (curve 1), NHKE (curve 2)). Inset shows an Eadie-Hofstee plot of data obtained in the presence of an H^+-gradient alone. The results given are the mean values ± S.D. of three experiments. In those cases where vertical bars are absent, S.D. were smaller than graphical representation of mean.

We also examined the effect of different amino acids in the extracellular fluid on the uptake of AIB. In these experiments, the concentrations of the inhibitors were 0.5 mM and 5.0 mM. It is seen from Table 2 that

Table 2
Effect of different amino acids on the sodium-dependent uptake of AIB

Inhibitor (concn.)	IHKE		NHKE	
	0.5 mM	5.0 mM	0.5 mM	5.0 mM
None	1.00	1.00	1.00	1.00
Glycine	0.61 ± 0.10 *	0.26 ± 0.03 *	0.74 ± 0.07	0.55 ± 0.08 *
L-Alanine	0.39 ± 0.14 *	0.07 ± 0.01 *	0.42 ± 0.04 *	0.12 ± 0.04 *
D-Alanine	0.93 ± 0.22	0.46 ± 0.03 *	0.99 ± 0.12	0.61 ± 0.06 *
L-Valine	0.78 ± 0.19	0.52 ± 0.05 *	0.97 ± 0.14	0.66 ± 0.12 *
L-Leucine	0.78 ± 0.10 *	0.50 ± 0.06 *	0.98 ± 0.13	0.67 ± 0.11 *
L-Isoleucine	0.76 ± 0.03 *	0.76 ± 0.04 *	1.08 ± 0.02	0.74 ± 0.06 *
L-Proline	0.75 ± 0.09 *	0.30 ± 0.03 *	0.77 ± 0.03	0.39 ± 0.02 *
L-Phenylalanine	0.49 ± 0.09 *	0.59 ± 0.04 *	0.96 ± 0.16	0.64 ± 0.15 *
L-Tryptophan	0.74 ± 0.17 *	0.68 ± 0.07 *	0.82 ± 0.01	0.63 ± 0.05 *
L-Methionine	0.31 ± 0.13 *	0.06 ± 0.01 *	0.48 ± 0.09 *	0.10 ± 0.02 *
L-Serine	0.58 ± 0.18 *	0.11 ± 0.01 *	0.57 ± 0.13 *	0.21 ± 0.02 *
L-Threonine	0.77 ± 0.15 *	0.39 ± 0.04 *	n.d.	n.d.
L-Asparagine	0.63 ± 0.16 *	0.15 ± 0.02 *	n.d.	n.d.
L-Glutamine	0.40 ± 0.08 *	0.13 ± 0.02 *	0.56 ± 0.04 *	0.17 ± 0.05 *
L-Aspartate	1.04 ± 0.08	0.91 ± 0.04	0.98 ± 0.09	0.93 ± 0.12
L-Glutamate	0.97 ± 0.14	0.96 ± 0.10	0.96 ± 0.10	1.06 ± 0.03
L-Lysine	0.86 ± 0.11	0.77 ± 0.05 *	0.98 ± 0.15	0.83 ± 0.04
L-Arginine	0.92 ± 0.17	0.96 ± 0.02	0.88 ± 0.10	0.90 ± 0.12
L-Histidine	0.55 ± 0.13 *	0.24 ± 0.02 *	0.68 ± 0.04 *	0.30 ± 0.02 *
Taurine	1.07 ± 0.13	1.02 ± 0.07	0.89 ± 0.11	1.01 ± 0.17
AIB	0.50 ± 0.13 *	0.11 ± 0.01 *	0.66 ± 0.07 *	0.22 ± 0.03 *
Methyl α-D-glucoside	1.08 ± 0.16	0.98 ± 0.06	0.95 ± 0.12	0.95 ± 0.10

Incubation medium consisted of Na^+-containing EBSS (pH 7.4), 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and unlabelled α-aminoisobutyric acid reaching 0.050 mM (final concentration) as well as test compounds at concentrations of 0.5 mM and 5.0 mM. The uptake of AIB at 5 min was normalized to uptake observed in the absence of test compound. Results are given as mean values ± S.D. of 3–5 experiments. n.d., not determined.

* *t*-test: The mean value of the sample is different from the mean value of methyl α-D-glucoside (non-specific inhibition) at the 0.05 level.

Table 3
Effect of different amino acids on the proton-dependent uptake of AIB

Inhibitor (concn.)	IHKE		NHKE	
	0.5 mM	5.0 mM	0.5 mM	5.0 mM
None	1.00	1.00	1.00	1.00
Glycine	0.95 ± 0.16	0.45 ± 0.04 *	0.95 ± 0.15	0.77 ± 0.11 *
L-Alanine	0.39 ± 0.04 *	0.13 ± 0.04 *	0.77 ± 0.08	0.78 ± 0.08 *
D-Alanine	0.73 ± 0.05 *	0.42 ± 0.04 *	0.99 ± 0.11	0.81 ± 0.05 *
L-Valine	0.16 ± 0.01 *	0.08 ± 0.01 *	0.56 ± 0.11 *	0.55 ± 0.12 *
L-Leucine	0.11 ± 0.02 *	0.07 ± 0.01 *	0.59 ± 0.16 *	0.62 ± 0.25 *
L-Isoleucine	0.18 ± 0.08 *	0.07 ± 0.00 *	0.47 ± 0.04 *	0.57 ± 0.11 *
L-Proline	1.05 ± 0.15	1.04 ± 0.15	0.95 ± 0.06	0.98 ± 0.07
L-Phenylalanine	0.11 ± 0.03 *	0.08 ± 0.03 *	0.60 ± 0.07 *	0.51 ± 0.11 *
L-Tryptophan	0.11 ± 0.04 *	0.08 ± 0.02 *	0.52 ± 0.13 *	0.47 ± 0.08 *
L-Methionine	0.13 ± 0.02 *	0.08 ± 0.02 *	0.61 ± 0.04 *	0.59 ± 0.13 *
L-Serine	0.37 ± 0.05 *	0.12 ± 0.01 *	0.84 ± 0.12	0.69 ± 0.17 *
L-Asparagine	0.63 ± 0.11 *	0.21 ± 0.04 *	n.d.	n.d.
L-Glutamine	0.25 ± 0.04 *	0.11 ± 0.04 *	0.79 ± 0.13	0.59 ± 0.13 *
L-Aspartate	0.76 ± 0.08 *	0.36 ± 0.05 *	0.96 ± 0.13	0.84 ± 0.18
L-Glutamate	0.24 ± 0.04 *	0.11 ± 0.05 *	0.84 ± 0.09	0.73 ± 0.07 *
L-Lysine	0.99 ± 0.06	0.92 ± 0.13	0.90 ± 0.06	0.94 ± 0.12
L-Arginine	0.96 ± 0.13	0.74 ± 0.06 *	0.88 ± 0.08	0.88 ± 0.03 *
L-Histidine	0.32 ± 0.02 *	0.06 ± 0.01 *	0.68 ± 0.17 *	0.55 ± 0.04 *
Taurine	0.96 ± 0.14	0.90 ± 0.11	0.93 ± 0.13	0.96 ± 0.21
AIB	0.57 ± 0.08 *	0.51 ± 0.02 *	0.87 ± 0.20	0.54 ± 0.01 *
Methyl α-D-glucoside	0.94 ± 0.04	0.99 ± 0.05	1.06 ± 0.20	0.97 ± 0.04

Incubation medium consisted of Na^+-free EBSS (pH 4.5), 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and unlabelled α-aminoisobutyric acid reaching 0.050 mM (final concentration) as well as test compounds at concentrations of 0.5 mM and 5.0 mM. The uptake of AIB at 5 min was normalized to uptake observed in the absence of test compound. Results are given as mean values ± S.D. of 3–5 experiments. n.d., not determined.

* *t*-test: The mean value of the sample is different from the mean value of methyl α-D-glucoside (non-specific inhibition) at the 0.05 level.

in the presence of a sodium gradient, L-alanine drastically reduced the uptake of AIB at both concentrations of the inhibitor in the two cell lines. The uptake of AIB was only inhibited by D-alanine at 5 mM, indicating the existence of a common transport system for the two amino acids but with a relatively low affinity for D-alanine. All the other neutral α-amino acids tested decreased the influx of AIB even though the degree of inhibition varied from one amino acid to the other. Addition of 5 mM L-lysine only produced a slight inhibition in the IHKE cell line, whereas L-glutamate, L-aspartate and L-arginine did not significantly change the influx of AIB in the two kinds of cells. Nor was there any inhibition by the β-amino acid taurine. Addition of methyl α-D-glucoside had no effect on the transport process for AIB, excluding the presence of non-specific inhibition caused by sodium depletion. Table 3 shows the effect of different amino acids on the H^+-dependent uptake of AIB in the absence of Na^+. All of the acidic and neutral α-amino acids tested, with the exception of L-proline, reduced the influx of AIB in the IHKE cells. By contrast, addition of L-aspartate did not influence the transport of AIB in NHKE cells. Moreover, even though the remaining acidic and neutral α-amino acids showed the same tendency as in IHKE cells the degree of inhibition was less pronounced for the NHKE cells than for the IHKE cells. Thus, the competition experiments for NHKE cells only indicated a slight, if detectable, additional reduction in the uptake of amino acid when inhibitor concentration was raised from 0.5 mM to 5.0 mM, suggesting that a basal influx level had already been reached at the lower concentration. The reason for this phenomenon remains unknown. L-Arginine, but not L-lysine decreased the influx of AIB at a high concentration of inhibitor in both cell lines. The β-amino acid taurine and methyl α-D-glucoside did not affect the transport of AIB.

4. Discussion

Primary human kidney cells have a short life-span in culture. Therefore, a number of model systems have been developed by oncogene transfection of normal human kidney cells e.g., the 293 cells [26], and the ST-1i and the STt-4i cell lines [27]. In this study we demonstrate a different type of modified cell line for examination of transport systems in proximal tubule of the human kidney. In contrast to the above-mentioned cell lines, this new IHKE cell line is based on human embryonic kidney cells, immortalized by exposure to heavy metal ($NiSO_4$). No tumorigenicity was induced by this treatment [12]. Furthermore, these IHKE cells retained a brush-border membrane and enzyme markers, which is specific for highly specialized epithelial cells in the proximal tubule (Table 1).

Amsler and co-workers have previously reported that transport of AIB by the LLC-PK_1 cell line was most active in sparse, growing cultures, but became stepped down at confluence [28]. Likewise, we found that the linear relationship between uptake and amount of cell protein was lost if the human cell cultures were more than confluent (results not shown). On the other hand, our aim was only to measure the uptake of amino acid across the luminal membrane of the human kidney cells. Consequently, uptake studies were performed as soon as the cells had reached a confluent state, and the IHKE and NHKE cell lines were investigated with respect to the cellular polarity of the AIB uptake. Our results strongly suggest that the data obtained in this study represent influx of amino acid across the luminal membranes of the monolayers. This is in accordance with studies of AIB transport in segment-specific luminal membrane vesicles from proximal tubule of rabbit kidney [17].

The present paper describes the transport characteristics of AIB uptake by the IHKE cell line as compared to NHKE cells. Initially, we examined the effect of an inwardly directed Na^+-gradient on the influx of amino acid across the brush-border membrane. We found that AIB accumulates against a concentration gradient and that the influx of AIB in both cell lines is the result of a single, saturable Na^+-dependent and a non-saturable, Na^+-independent transport process. The half-saturation values (IHKE: $K_m = 0.66$ mM and NHKE: $K_m = 0.74$ mM) were very similar, but the maximal uptake for the IHKE cell line was three times that of the NHKE cell line. These results suggest that even though there has been an increase in the number of transport carriers in the immortalized cells as compared to the normal cells, it is probably the same functional transport protein in the two cell lines which is responsible for the uptake of AIB. To support this thesis further and to characterize the transport mechanism(s) of AIB by the NHKE and the IHKE cell lines, a series of competition experiments were performed. The results obtained (Table 2) show that the inhibition pattern was exactly the same for the two cell lines with the exception of L-lysine. However, the difference in the effect of L-lysine may simply reflect uncertainty in the determination of the basal AIB influx. The uptake of AIB was inhibited by all the neutral L-amino acids tested. Addition of D-alanine also reduced the Na^+-dependent influx of AIB, but with a lower affinity than for L-alanine. We found no effect of L-glutamate, L-aspartate and L-arginine on the uptake of AIB. A number of studies have demonstrated that the Na^+-dependent influx of taurine is mediated by a β-amino-acid-specific transport process in the proximal tubule [15,29]. In accordance with expectations, taurine had

no influence on the uptake of the α-amino acid AIB by the IHKE or NHKE cell line.

Similar experiments were carried out by Rabito and Karish [30], who studied the transport of AIB by the immortalized epithelial cell line from juvenile pig kidney, the LLC-PK_1 line. They found a K_m value of 4.47 mM for the Na^+-dependent transport component and we have obtained similar results for this pig cell line (results not published). By contrast, our competition experiments regarding the human cell lines differ from the observations reported by Rabito and Karish [30] on the LLC-PK_1 cell line. According to these authors addition of acidic or basic amino acids to the incubation medium resulted in a marked stimulation of the transport of AIB (between 200 to 500%). However, preliminary results obtained by us with the LLC-PK_1 cell line do not confirm this phenomenon (results not published).

We also examined the effect of an inwardly directed H^+-gradient on the transport of AIB by the two human cell lines. We found that in the absence of other energy sources an inwardly directed H^+-gradient was able to stimulate the uptake of AIB, whereas imposition of an H^+-gradient on top of a Na^+-gradient reduced the sodium-dependent uptake of amino acid. Only at short incubation time (1 min) addition of a proton gradient produced a marked increase in the Na^+-dependent influx of AIB in NHKE but not in IHKE cells. It is rather difficult to interpret this observation at present. However, it should be taken into consideration that at present we do not know which part of the proximal tubule the two cell lines represent. It could even be that the IHKE and NHKE cells are a mixture of pars convoluta and pars recta. This may partly explain the H^+-phenomenon since imposition of an inwardly directed H^+-gradient in luminal membrane vesicles from rabbit kidney has been shown to increase the Na^+-dependent uptake of AIB in pars convoluta, but reducing the influx of amino acid in pars recta. By contrast, an inwardly directed H^+-gradient in the absence of a Na^+-gradient is capable of stimulating the uptake of AIB in the convoluted part but has no effect in the straight part of the proximal tubule [17]. Another aspect may be a toxic effect of the acidic pH (4.5) on the human cell lines at longer incubation periods affecting the Na^+-dependent uptake of AIB. However, preliminary experiments revealed the same phenomenon at pH 6.0 (results not shown). Thus even though it is well known that the luminal pH in proximal tubule can be as low as 6.2 to 6.4 under some conditions in vivo [31], the present study does not provide us with an answer to whether or not an inwardly directed H^+-gradient has a physiological role in the reabsorption of neutral α-amino acids in the proximal tubule. The competition experiments showed that apart from L-proline all of the neutral and acidic amino acids tested reduced the H^+-gradient dependent uptake of AIB in the IHKE cell line. By contrast, addition of L-apartate did not influence the transport of AIB in NHKE cells. L-arginine, but not L-lysine decreased the influx in both cell lines.

Tveito and co-workers have demonstrated that the NHKE cell line exhibited dome formation [12], which is characteristic of differentiated kidney epithelial cells. By contast, none of the tranfected human cell lines (293 cells [26], the ST-1i and the STt-4i cell line [27]) and the IHKE cell line formed domes. Furthermore, treatment of the NHKE cells with nickel resulted in chromosome changes such as ploidy ($3n$) and other abnormalities [12]. But regardless of this, we can conclude that the IHKE cell line, immortalized by treatment with $NiSO_4$, has retained the capability to accumulate AIB by transport carrier(s) similar to those present for neutral α-amino acids in NHKE cells. In addition, we found expression of enzyme markers characteristic of a brush-border membrane. It thus seems highly probable that the IHKE cell line with its apparently unlimited life-span can be a very valuable tool for the characterization of the transport mechanism of filtered solutes in the human proximal tubule at the cellular level.

5. Acknowledgements

This study was supported in part by the Danish Medical Research Council, Aarhus University Research Foundation, the Danish Foundation for the Advancement of Medical Science, the Novo Nordisk Foundation, the Carlsberg Foundation, Michaelsen Fonden and the Danish Biotechnology Programme. The authors are grateful to Dr. Steen Mollerup, Department of Toxicology, National Institute of Occupational Health, Oslo, Norway, for his assistance in the preparation of NHKE cells.

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