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Uptake of neutral α- and β-amino acids by human proximal tubular cells

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Abstract

The transport characteristics of amino acids in primary cell cultures from the proximal tubule of human adults (AHKE cells) were examined, using α-aminoisobutyric acid (AIB) and β-alanine as representatives of α- and β-amino acids, respectively. The Na^+-gradient dependent influx of AIB occurred by a single, saturable transport system, whereas the Na^+-gradient dependent uptake data for β-alanine could be described in terms of two-independent transport components as well as one-transport one-leak model with identical kinetic constants for the high-affinity system. Competition experiments revealed that all the neutral amino acids tested reduced the uptake of AIB, whereas there was no effect of taurine, L-aspartic acid, and L-arginine. By contrast, the influx of β-alanine was only drastically reduced by β-amino acids, whereas the inhibition by neutral α-amino acids was relatively low. Nor did L-arginine and L-aspartic acid affect the uptake of β-alanine into AHKE cells. Comparison with the results obtained for normal (NHKE) and immortalized (IHKE) embryonic cells suggested an unaltered expression of the types of transport carriers for neutral α- and β-amino acids in the embryonic and AHKE cells. However, the uptake capacity of the above-mentioned transport proteins was relatively smaller in the embryonic kidney compared with the adult human kidney, which may explain, at least partly, the phenomenon of physiologic amino aciduria in neonates.

Keywords: α-Aminoisobutyric acid; β-Alanine; Proximal tubule; (Kidney cell)

1. Introduction

Renal proximal tubular epithelial cells have the capability to accumulate neutral amino acids against a concentration gradient. As a consequence approximately 99% of the filtered load of most of these amino acids is returned to the plasma by absorption along the proximal nephron in the mature kidney. By contrast, in the immature kidney from neonates the fractional excretion of most amino acids in relation to the glomerular load is higher, resulting in a physiologic aminoaciduria. The reason for this phenomenon is still not entirely clear. The enhanced urinary excretion of amino acids observed in neonates may be the result of a variety of factors (i) differences in the amount or type/isoform of the transport proteins at the apical membrane; (ii) differences in the properties of the cell membrane affecting the efflux and tubular transport of amino acid; and (iii) altered activity of the Na^+/K^+- and Na^+/H^+-exchanger (for further details, see [1,2]). One single explanation of the phenomenon seems less likely since the extent of the postnatal aminoaciduria varies from one amino acid to another [3].

Recently, we have characterized an immortalized, human embryonic kidney epithelial cell line (IHKE). We found that an IHKE cell line, immortalized by treatment with $NiSO_4$ [4], exhibited microvilli and various membrane enzymes specific for the proximal tubular epithelial cells. In addition, studies on the renal uptake of α-aminoisobutyric acid (AIB), as a representative for neutral α-amino acids, indicated that the immortalized tubular cells had retained the capability to transport AIB into the cells by transport component(s) with similar properties as those found in normal human embryonic kidney cells (NHKE) [5]. Furthermore, the IHKE cell line was also characterized with regard to the capability to transport β-amino acids like β-alanine and taurine [6]. These studies indicated the IHKE cell line to be a very promising model for future

Abbreviations: IHKE, immortalized human kidney epithelial cell line (embryonic); NHKE, normal human kidney epithelial cell line (embryonic, primary culture); AHKE, adult human kidney epithelial cell line (primary culture); Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; FCS, fetal calf serum.

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studies on transport and regulation of amino acid uptake in the human proximal tubule [5,6].

However, keeping in mind that the IHKE cell line is derived from the human embryonic kidney cells, the question arises whether the uptake characteristics of neutral amino acids by embryonic and by adult kidney cells are the same, since physiological aminoaciduria is only observed in neonates. The limited availability of adult human kidneys for research has previously been a major obstacle. However, we have recently solved this problem by obtaining macroscopically normal tissue from kidneys less than 1 h after removal by surgical nephrectomy for renal malignancy. Therefore, the aim of the present study is to describe the characteristics of neutral amino acid uptake in primary cell cultures obtained from proximal tubule cells of human adults and compare the results with those previously obtained for the embryonic kidney cell cultures. As representatives of neutral α- and β-amino acids, respectively, α-aminoisobutyric acid (AIB) and β-alanine were used.

2. Materials and methods

2.1. Materials

α-Amino[*methyl*-^{3}H]isobutyric acid (specific activity 8.9 Ci/mmol) and β-[3(n)-^{3}H]alanine (specific activity 92.6 Ci/mmol) were obtained from NEN. Fetal calf serum (FCS), growth medium and antibiotics were from Gibco, NY. All other non-radioactively labelled ligands, and buffers were obtained from Sigma.

2.2. Cell culture

Human renal tissue was obtained from kidneys less than 1 h after removal by surgical nephrectomy for renal malignancy. The specimens used were portions of kidney cortex cut from the non-involved pole, judged macroscopically to be normal. The specimens, having been kept sterile in culture medium at 4°C after surgery, were placed in a laminar flow hood. After removal of the fibrous capsule the renal outer cortex was cut into small explants (2 × 2 × 2 mm) that were placed on the growth surface of a petri dish suited for growing primary cultures (Primaria™, Falcon). Here, they were kept dry for 30 min to ensure adherence to the growth surface. Thereafter, a drop of culture medium was added to each explant and the petri dishes were left overnight in the incubator at 37°C and 5% CO_2 in air. Next morning, the Petri dishes were filled with medium and after approx. 6 days cells had grown out from the renal explants. The explants were then removed and after the remaining cells had reached near confluence, they were transferred to culture flasks known to inhibit growth of fibroblasts (25 ml, Primaria™, Falcon). When the cells had obtained confluence, the cell monolayers were subcultured in 35 mm single dishes (NUNC, Denmark) in order to perform the various experiments. In the first 6 days the cell medium consisted of 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium, complemented with epidermal growth factor (10^{-5} g/l), insulin ($5 \cdot 10^{-3}$ g/l), hydrocortisone ($1 \cdot 10^{-4}$ g/l), triiodothyronine (30 nM), transferrin ($5 \cdot 10^{-3}$ g/l), sodium selenite (30 nM), penicillin ($1 \cdot 10^5$ IU/l), streptomycin (0.1 g/l), 2 mM glutamine, 15 mM Hepes, and 14 mM $NaHCO_3$. Hereafter, the cell monolayers were grown in α-MEM containing 5% of FCS. 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 cultures were grown at 37°C, pH 7.4 in an atmosphere of 5% CO_2 in air and culture media were renewed every 3 days. The same culture medium (α-MEM) and conditions were used in regard to the NHKE and IHKE cell lines with the exception that the immortalized cells were grown in the presence of only 1% FCS [5]. The use of human tissue in the present study was approved by The Scientific Ethical Committee of Aarhus.

2.3. Marker enzyme assays

The activities of luminal-membrane marker enzymes were measured on crude cell homogenates from cell monolayer cultures. Maltase and leucine aminopeptidase activities were determined by the method of Dahlqvist [7] and with the use of the Sigma kit 251-AW, respectively, whereas the activity of alkaline phosphatase was measured as described by Bessey et al. [8].

2.4. Transport studies

Uptake studies were performed on monolayers in 35 mm single dishes (NUNC, Denmark) as soon as the cells had reached confluence. Transport of amino acid 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 aspirating 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 N NaOH was added to solubilize the cells. The radioactivity in the cells was determined by withdrawal of three samples of 0.1 ml, which were counted in a liquid scintillation counter (LKB-Wallac 1218 Rack-Beta) after addition of 3 ml scintillation fluid. Correction

for non-specific uptake was made in control experiments by estimating the amount of radiolabelled amino acid retained in the extracellular space or bound to the cell surface after incubation with ice-cold incubation medium. Thus, these parallel experiments were performed exactly in the same way as the other experiments with the only exception that the incubation medium, kept on ice, was added to the petri dishes for 10 s before withdrawal and washing the cells three times with ice-cold stop solution. 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 order to stabilize extracellular pH. 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 6.0) or 25 mM Hepes/KOH (pH 7.4) were used as buffers. The amount of total cell protein was determined by the method of Lowry et al. [9] with bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) as a standard. All solutions used in this study were sterilized before use.

2.5. Calculations

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

$$\text{Uptake} = \frac{V_{max1}[S]}{K_{m1} + [S]} + \frac{V_{max2}[S]}{K_{m2} + [S]}$$

where K_m represents the substrate concentration that gives half-maximal uptake, V_{max} denotes maximal rate and [S] indicates the concentration of substrate in the medium. Index 1 and 2 refer to the first and the second transport system, respectively. In the case where $K_{m2} \gg [S]$, the equation is simplified to:

$$\text{Uptake} = \frac{V_{max1}[S]}{K_{m1} + [S]} + P[S]$$

where P is a constant. This equation incorporates one transport system in parallel with a non-enzymatic leak mechanism. In the case of transport via a single pathway the same equation without the second fraction was used. Theoretical saturation curves were fitted to the experimental data by using a computer-analyzed statistical iteration procedure [10].

2.6. Statistics

Statistical significance was determined by Student's t-test with the level of significance at $P < 0.05$.

3. Results

3.1. Morphologic cell characteristics

The AHKE cells grown in monolayers showed a typical 'cobblestone' appearance and formed domes at confluence. Furthermore, transmission electron microscopy revealed a characteristic epithelial morphology with sparse microvilli scattered on the apical membrane (not shown).

3.2. Membrane enzymes

In order to confirm that the renal primary cell cultures were originating from the proximal tubule, a number of luminal-membrane enzyme markers were measured. As seen from Table 1, the AHKE cells possessed maltase, leucine aminopeptidase, as well as alkaline phosphatase which in the kidney is specific for the proximal tubule [11–13]. For comparison, corresponding values previously obtained in the normal (NHKE) and immortalized (IHKE) human embryonic kidney cells [5] are also given in Table 1.

3.3. Radioactive uptake of AIB and β-alanine

The uptake of AIB as a function of time by the AHKE cells is shown in Fig. 1. The sodium dependent uptake of amino acid exhibited a linear relationship within the first 5 min. Subsequently, the influx rate decreased and a maximal value of approximately 19 nmol/mg protein was observed after 60 min of incubation. In Na^+-free medium, prepared by substitution of sodium with choline, the uptake of amino acid was drastically reduced only accounting for approximately 10% of the sodium dependent uptake value at 60 min.

The concentration dependence of AIB uptake is illustrated in Fig. 2. which shows the initial influx of AIB over a range of 0.005 to 1 mM, both in the presence or absence of a sodium gradient. The Na^+-dependent uptake of AIB exhibits a hyperbolic dependence on amino acid concentration, consistent with predominant passage by a saturable transport component. In the absence of Na^+, the AIB

Table 1
Enzyme activities

	AHKE	NHKE	IHKE
Leucine aminopeptidase	45.7 ± 10.9	20.6 ± 8.5	34.1 ± 9.7
Alkaline phosphatase	3.9 ± 0.2	4.5 ± 1.7	3.3 ± 1.4
Maltase	25.3 ± 9.4	25.9 ± 7.6	32.0 ± 1.4

The various activities were determined as mentioned under Section 2. Enzyme activities for leucine aminopeptidase are expressed as micromoles of substrate converted/min per mg of cell protein. All other results are expressed as nmol of substrate converted/mg of cell protein per min. The results given are the mean ± S.D. of at least three experiments (n, 3–15).
The enzyme activities for NHKE and IHKE cells are from Ref. [5].

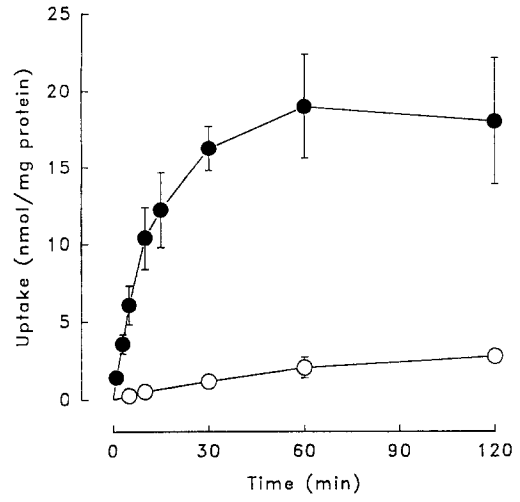

Fig. 1. Time-course of α-aminoisobutyric acid uptake by AHKE cells in the presence or absence of a Na^+-gradient. Monolayers of AHKE cells were incubated in Na^+-containing (●) or in Na^+-free EBSS (○) as described in Section 2. Media contained 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid and sufficiently 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.

Table 2
Effect of different amino acids on the uptake of AIB by AHKE cells

Inhibitor (concn.)	0.5 mM	5.0 mM
None	1.00	1.00
Glycine	0.71 ± 0.12 *	0.31 ± 0.06 *
L-Alanine	0.41 ± 0.12 *	0.06 ± 0.01 *
L-Leucine	0.83 ± 0.16	0.58 ± 0.03 *
L-Proline	0.77 ± 0.07 *	0.31 ± 0.03 *
L-Phenylalanine	0.90 ± 0.09	0.65 ± 0.04 *
L-Serine	0.50 ± 0.08 *	0.10 ± 0.01 *
L-Glutamine	0.52 ± 0.08 *	0.12 ± 0.02 *
L-Aspartic acid	0.97 ± 0.19	0.91 ± 0.05
L-Arginine	1.06 ± 0.05	1.03 ± 0.14
Taurine	0.98 ± 0.10	0.98 ± 0.14
AIB	0.54 ± 0.10 *	0.10 ± 0.01 *
Methyl-α-D-glucoside	1.04 ± 0.06	0.92 ± 0.10

The incubation medium consisted of Na^+-containing EBSS (pH 7.4), 0.6 μM α-[*methyl*-^{3}H]-amino-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 the uptake observed in the absence of test compound. Results are given as mean ± S.D. of 3–5 experiments.
* *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.

uptake was low and proportional to the concentration of amino acid in accordance with a simple diffusion behavior. The inset illustrates an Eadie-Hofstee plot of the saturable component after correction for Na^+-independent movement of amino acid. The straight-line relationship obtained suggests that the sodium dependent, carrier-mediated uptake of AIB by the AHKE cells occurs by means of a single transport system for which the following kinetic parameters were calculated: $K_m = 0.53 \pm 0.08$ mM and $V_{max} = 61.2 \pm 4.69$ nmol/mg protein per 5 min.

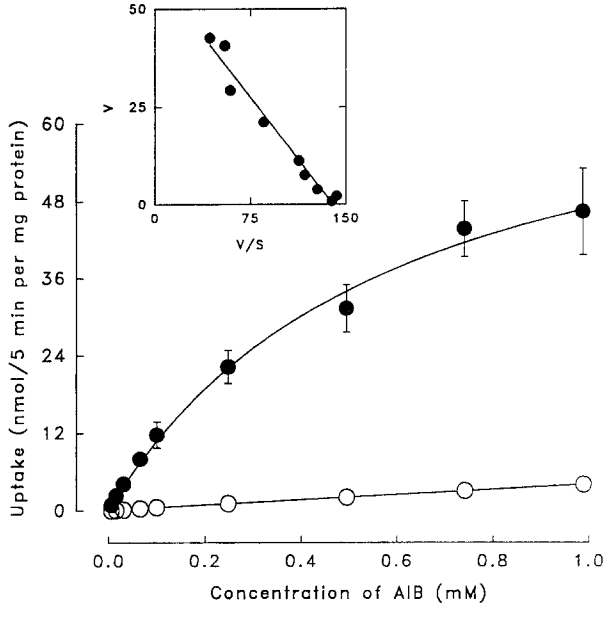

Fig. 2. Kinetics of α-aminoisobutyric acid uptake by AHKE cells in the presence or absence of a Na^+-gradient. Monolayers of AHKE cells were incubated in Na^+-containing (●) or Na^+-free EBSS (○), 1.0 μM α-amino[*methyl*-^{3}H]butyric acid and various concentrations of unlabelled α-aminoisobutyric acid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

Table 2 shows the effects of different neutral amino acids and methyl-α-D-glucoside on AIB uptake by the AHKE cells. As shown by the table, methyl-α-D-glucoside, being used as a test for sodium-depletion, had no measurable effect on the transport of AIB. All the neutral α-amino acids examined decreased the uptake of AIB even though the degree of inhibition varied from one amino acid to the other. Thus, the influx of AIB was drastically reduced by L-alanine, L-serine and L-glutamine whereas addition of glycine and L-proline produced a more moderate inhibition. L-leucine and L-phenylalanine only affected the transport of AIB at an inhibitor concentration of 5 mM indicating a relatively low affinity for the common transport system. L-aspartic acid and L-arginine had no inhibitory effect on the uptake of AIB. Nor did addition of taurine, a β-amino acid, reduce the influx of amino acid.

Fig. 3 depicts the time-course of amino acid uptake in similar experiments with β-alanine. The sodium dependent uptake of amino acid is linear up to 30 min after which the influx rate slowly declines. It is seen by comparing the uptake data with those in Fig. 1. that the Na^+-dependent uptake rate for β-alanine within the first 30 min was smaller than in the case of AIB. In addition, a maximal uptake value for β-alanine was never reached, even after 120 min of incubation. In the absence of sodium, amino

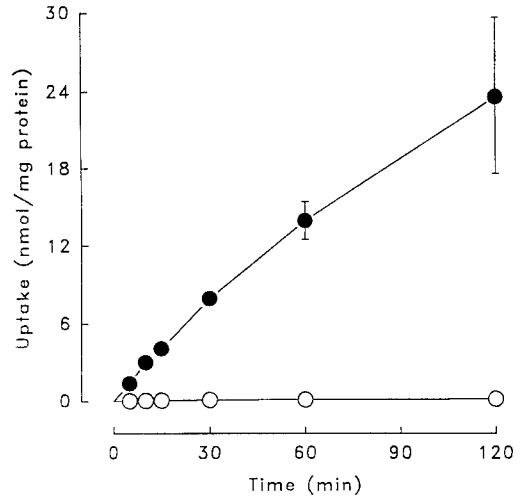

Fig. 3. Time-course of β-alanine uptake by AHKE cells in the presence or absence of a Na^+-gradient. Monolayers of AHKE cells were incubated in Na^+-containing (●) or in Na^+-free EBSS (○) as described in Section 2. Media contained 70 nM β-[3-^{3}H(n)]-alanine and unlabelled β-alanine to reach a total concentration of 50 μM (final concentration). The results shown are the mean values ±S.D. of three experiments.

Table 3
Effect of different amino acids on the uptake of β-alanine by AHKE cells

Inhibitor (concn.)	0.5 mM	5.0 mM
None	1.00	1.00
Glycine	0.88±0.12	0.58±0.08 *
L-Alanine	0.89±0.03	0.61±0.04 *
L-Leucine	0.96±0.08	0.73±0.08 *
L-Proline	0.95±0.10	0.62±0.08 *
L-Phenylalanine	1.07±0.12	0.81±0.12 *
L-Serine	0.89±0.06	0.64±0.02 *
L-Glutamine	0.95±0.06	0.85±0.05 *
L-Aspartic acid	1.04±0.14	1.07±0.12
L-Arginine	0.95±0.13	0.82±0.17
Taurine	0.18±0.07 *	0.14±0.05 *
β-Alanine	0.26±0.03 *	0.13±0.03 *
Methyl-α-D-glucoside	0.98±0.10	1.06±0.04

The incubation medium consisted of Na^+-containing EBSS, 70 nM β-[3-^{3}H(n)]-alanine and unlabelled β-alanine reaching 0.050 mM (final concentration) as well as test compounds at concentrations of 0.5 mM and 5.0 mM. The uptake of β-alanine at 30 min was normalized to the uptake observed in the absence of test compound. Results are given as mean± S.D. of 3–7 experiments.

* *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.

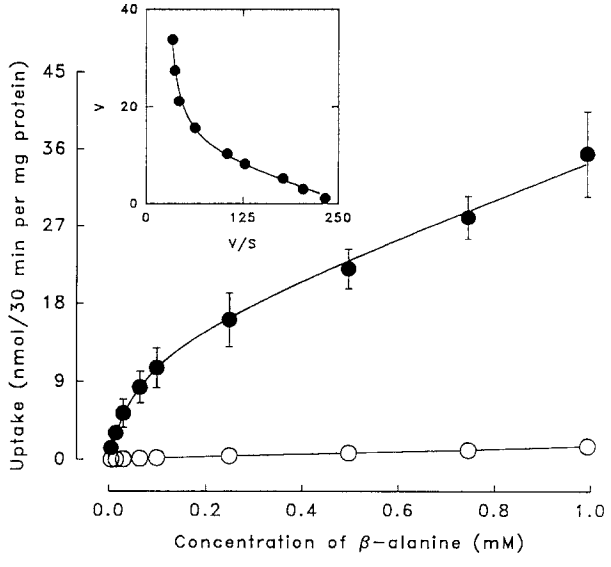

Fig. 4. Kinetics of β-alanine uptake by AHKE cells in the presence or absence of a Na^+-gradient. Monolayers of AHKE cells were incubated in Na^+-containing (●) or Na^+-free EBSS (○), 70 nM β-[3(n)-^{3}H]alanine and various concentrations of unlabelled β-alanine ranging from 0.005–1.0 mM. Inset shows an Eadie-Hofstee plot of data obtained in the presence of a Na^+-gradient alone by subtracting the uptake values in the absence of Na^+ from the total uptake in the presence of Na^+. *V* represents the rate of transport at substrate concentration S. 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.

acid influx was almost abolished, only amounting to approximately 0.9% of the Na^+-dependent uptake at 60 min.

Fig. 4 shows the uptake of β-alanine at various extracellular concentrations of amino acid over a range of 0.005 to 1.0 mM. The Na^+-dependent influx of β-alanine rose steeply as a function of amino acid at low concentrations, whereas there was a deflection above 0.1 mM. Nevertheless, the uptake curve did not approach a saturation. In the absence of sodium, β-alanine transport by AHKE cells was linearly proportional to extracellular amino acid concentration, suggesting simple diffusion properties. The inset illustrates an Eadie-Hofstee transformation of the β-alanine influx in the presence of a Na^+-gradient alone (corrected for a passive diffusion component) resulting in a curvilinear plot. Assuming the existence of more than one transport system, computerized calculations resulted in the following kinetic parameters for the high-affinity component: $K_m = 0.049 \pm 0.007$ mM and $V_{max} = 11.88 \pm 1.03$. However, the calculated constants for the low affinity system(s) could not be determined satisfactorily because of the lack of saturation of the transport component(s) over the concentration range studied. Calculations using the one-transport one-leak model resulted in exactly the same kinetic parameters for K_m and V_{max} as those given above and the standard deviation of the fit was identical to the one found for the two site-model.

To further characterize the sodium-dependent uptake of β-alanine, a series of competition experiments was performed. The results are shown in Table 3. The β-amino acids tested drastically reduced the uptake of amino acid already at an inhibitor concentration of 0.5 mM. By contrast, addition of the different neutral α-amino acids only produced incomplete inhibition at an inhibitor concentration of 5.0 mM where the effect was more pronounced for L-proline and short-chain amino acids than for L-phenylalanine and long-chain analogues. L-aspartic acid and L-arginine did not affect β-alanine transport in a statistical

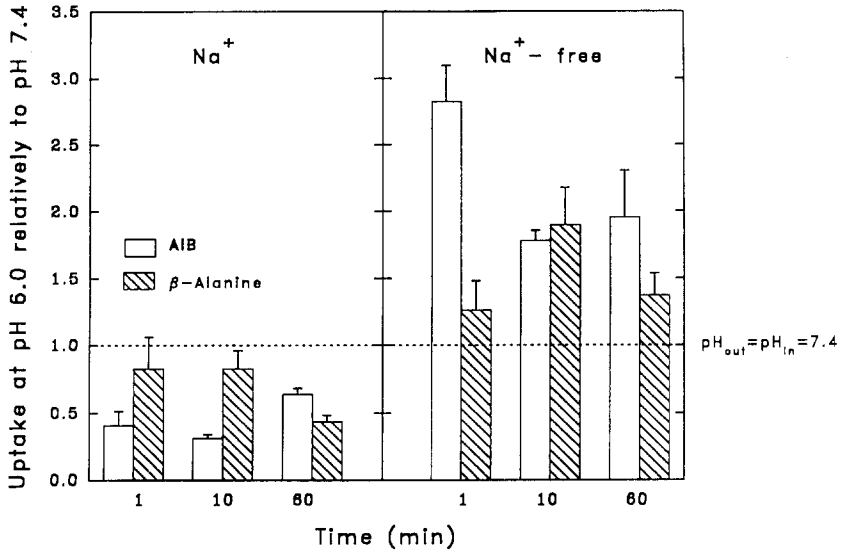

Fig. 5. Effect of an inwardly directed pH-gradient on the uptake of AIB and β-alanine. Monolayers of AHKE cells were incubated in Na^+-containing or Na^+-free EBSS, pH 6.0 or 7.4. Media contained 0.6 μM α-amino[*methyl*-^{3}H]isobutyric acid/70 nM β-[3-^{3}H(n)]-alanine and sufficiently unlabelled α-amino-isobutyric acid/β-alanine to reach a total concentration of 50 μM (final concentration). Uptake of amino acid in the absence of an inwardly directed pH-gradient ($pH_{in} = pH_{out} = 7.4$) is designated as 1.0 and illustrated by the dotted line. The uptakes obtained in the presence of an H^+-gradient (pH_{out} 6.0) are given as relative values of the uptakes measured in the absence of an H^+-gradient (pH_{out} 7.4). The results shown are the mean values ±S.D. of 3 experiments.

manner when compared with the test substrate *methyl*-α-D-glucoside as previously described for AIB.

Finally, we examined the effect of an inwardly directed H^+-gradient on the uptake of the two model substrates used in present study. Earlier studies had shown that both in the presence or absence of a Na^+-gradient the uphill transport of AIB and β-alanine can be driven by an inwardly directed pH-gradient in luminal membrane vesicles from the pars convoluta but not the pars recta of rabbit kidney [14–16]. However, in intact cells imposition of an H^+-gradient on the Na^+-gradient did not enhance the Na^+-dependent uptake of AIB and β-alanine in the AHKE cells (Fig. 5). In fact, the pH-gradient in some instances reduced the influx of amino acid. By contrast, the presence of an inwardly directed H^+-gradient in the absence of a Na^+-gradient increased the uptake of both amino acids into the cells to some extent.

4. Discussion

The present studies demonstrate that it is possible by relatively simple techniques to study amino acid transport in renal cells obtained as a primary culture from explants of human adult kidney. Thus, we have been able to isolate cell cultures which possessed enzyme markers and microvilli (although sparse), characteristic of highly specialized cells from the renal proximal tubule. This has given us the opportunity to examine the capability of the AHKE cells to actively take up neutral amino acids and compare the results with those previously obtained for human embryonic kidney cell cultures.

The uptake of AIB into AHKE cells occurred by a single, saturable Na^+-dependent and a by non-saturable, Na^+-independent transport process. Computerized analysis by use of Michaelis-Menten kinetics of the Na^+-gradient-dependent influx data (corrected for simple diffusion) revealed a K_m-value of 0.53 mM. Similar results were previously found for the NHKE and IHKE cell line (0.74 mM and 0.66 mM, respectively), but the transport capacity for AIB by the adult kidney cells was three times higher than that found for the normal embryonic kidney cells (19.5 nmol/mg protein per 5 min vs. 61.2 nmol/mg protein per 5 min) [5]. Thus, our results suggest that the affinity, but not the capacity for AIB by the transport system remains unchanged during maturation. A similar conclusion was previously reached for the IHKE cell line [5] where the uptake capacity for AIB (58.6 nmol/mg protein per 5 min) was very similar to that found for the AHKE cell line. In order to further characterize the Na^+-dependent transport system for AIB, a series of competition experiments was carried out. All the neutral α-amino acids tested reduced the uptake of AIB even though the degree of inhibition varied from one amino acid to the other. The acidic and basic amino acids had no influence on the uptake of AIB. Nor was there any effect of the β-amino acid taurine. This inhibition pattern for AHKE cells is similar to that previously reported for the NHKE cells [5], supporting the view that the same transport protein in both the AHKE and NHKE cells is responsible for the uptake of AIB even though there seems be a relatively increase in the uptake capacity during maturation.

We also characterized the uptake mechanism for β-

amino acids by the AHKE cells. In contrast to the influx of AIB, the Na^+-gradient-dependent uptake data for β-alanine seemed to fit a model of two transport systems as well as a one-transport one-leak model. Computerized calculations for both models resulted in a half-saturation constant of 0.049 mM for the high affinity system. In comparison, the corresponding value for the IHKE cell line was previously found to be 0.034 mM [6]. Thus, the K_m-values found for the two cell lines must be considered to be almost identical. By contrast, the capacity of the high affinity transport system for β-alanine by the immortalized embryonic cells was only approx. one fourth of the maximal uptake rate found for the AHKE cells. Addition of different unlabelled amino acids to the incubation medium revealed that the uptake of radioactive β-alanine was drastically reduced by the presence of β-amino acids, whereas the inhibition by neutral α-amino acids was relatively low. A similar uptake pattern for β-alanine and taurine was also demonstrated in the IHKE cell line [6]. L-Arginine and L-aspartic acid did not affect the uptake of β-alanine into the AHKE or IHKE cells [6].

We conclude on the basis of the above-mentioned findings that in the AHKE phenotype there is no evidence of additional transport systems and that the increased capacity for transport probably reflects an increase in the number of transport proteins in the brush-border membrane. A similar pattern has been reported for the uptake of taurine by rat renal brush-border membranes and of cystine in isolated dog proximal renal tubules [17,18]. A developmental increase in the uptake of amino acid has also been described for the transport of proline by luminal membrane vesicles from rat. However, in this case the Na^+-dependent 'overshoot phenomenon' of proline was smaller in young animals as compared with the adult, as a result of a faster dissipation of the sodium-gradient [19,20]. Likewise, previous studies concerning the renal Na^+-glucose uptake using rabbit brush-border membrane vesicles demonstrated an increase in V_{max} (but similar K_m-values) during maturation from fetus to adult that was a result of both a change in membrane permeability favouring Na^+-glucose influx and an increase in the transport carriers in the membrane [21]. In the present study we did not examine whether or not maturational changes in membrane potential and ionic conductances play a major role in development of the human kidney. It has also been reported that maturational changes in the metabolic rate of L-proline occur since the intracellular content of amino acid is relatively higher in the neonatal animal kidney [22]. However, in this study we have used AIB as a prototype for neutral α-amino acids. This amino acid is characterized by being non-metabolizable, thereby eliminating the dilemma of distinguishing between metabolism and transport mechanisms. Earlier experiments with confluent IHKE or NHKE cell monolayers, AIB, and p-amino-hippuric acid (PAH) strongly indicate that the influx data measured only represent uptake across the apical cell membranes [5]. The same kind of experiments with confluent AHKE cell monolayers showed similar results (results not shown).

It has previously been demonstrated that an inwardly directed pH-gradient can drive the uptake of AIB and β-alanine into luminal membrane vesicles from pars convoluta of rabbit kidney [14–16]. By contrast, a similar phenomenon was not observed in vesicles from the pars recta of the proximal tubule [14–16]. The effect of acidification of the extracellular medium on the uptake of amino acids into the AHKE cells was also examined in the present study. The imposition of an H^+-gradient on a Na^+-gradient did not enhance the Na^+-dependent uptake of amino acid but rather decreased it perhaps as the result of deterioration of the cell function at low pH. On the other hand, in the absence of sodium, the pH-gradient increased the transport of AIB and β-alanine in a statistically significant manner. A similar pattern for the uptake of AIB in embryonic NHKE/IHKE cells and for β-alanine in IHKE cells has previously been observed with the exception that addition of a proton gradient produced a marked increase in the Na^+-dependent uptake of AIB at short incubation times (1 min) in NHKE but not in IHKE cells [5,6]. Although it is difficult to explain the physiological role of acidification based on the results from cell cultures, it may seem that generally the pH effect on Na^+-dependent amino acid uptake does not improve as a function of the degree of kidney maturation in humans. However, since some of the results mentioned above are derived from different cell cultures, one has to be cautious in the interpretation of the data. Thus, it cannot be excluded that the AHKE, NHKE, and IHKE may differ from one another in regard to the part of the proximal tubule from which they are derived or consist of a mixture of cells from different segments of the proximal nephron. We measured the activity of various luminal-membrane enzyme markers in the AHKE cells in order to determine whether or not the cultured human renal cells have characteristics in common with the proximal tubule. The AHKE cells showed activities for leucine aminopeptidase, maltase, and alkaline phosphatase specific for the proximal tubule [11–13]. Previous studies on rabbit renal brush border membrane vesicles from the fetuses examined late in gestation period indicated an activity of leucine aminopeptidase in homogenates from adults 1.3 times higher than that of the fetal kidney. On the other hand, the specific activities for maltase and alkaline phosphatase were found to be factors of 4.1 and 2.4 higher, respectively [21]. The developmental profile of brush-border enzymes in the rabbit seems to be similar to those reported for rats [23,24]. The reason for the maturational discrepancy between rabbit, rat and human enzyme activities is not known at present but variations in species cannot be excluded.

In conclusion, even though it may be difficult to make direct comparisons of the different cell cultures, the observations reported here seem to indicate an unaltered expres-

sion of the types of transport carriers for neutral α- and β-amino acids in the embryonic and AHKE cells. Thus, the developmental changes in the amino acid uptake is not the result of additional transport systems appearing after birth. The relatively smaller uptake capacity in the embryonic kidney compared with the adult human kidney may explain, at least partly, the phenomenon of physiologic amino aciduria in neonates. Whether this increase in V_{max} is caused solely by an increase in the number of carrier proteins, or whether other factors changes in membrane permeability, or altered activity of the Na^+/K^+- and Na^+/H^+-exchanger have to be further examined in future studies with human kidney cells.

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