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Taurine and β-alanine transport in an established human kidney cell line derived from the proximal tubule

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Abstract

The transport mechanisms of taurine and β-alanine by an immortalized human embryonic kidney epithelial cell line (IHKE) were examined. The uptake of these β-amino acids was characterized by two Na^+-dependent transport components, whereas an inwardly directed H^+-gradient only stimulated amino acid influx to a small extent and in the absence of sodium. Competition experiments revealed that taurine and β-alanine drastically reduced the uptake of one another by the high-affinity Na^+-dependent transport system. However, some α-amino acids could also compete with the β-amino acids, but with a low affinity. Examinations of the effect of different anions on the Na^+-dependent uptake of taurine at a low amino acid concentration (240 nM) revealed a specific requirement for Cl^-, whereas Cl^- had no measurable effect at a higher concentration (1.0 mM) of taurine. In addition, activation of taurine transport as a function of Na^+ and Cl^- concentration indicated a probable coupling ratio of 3 Na^+/1 Cl^-/1 taurine for the high-affinity carrier. Finally, cellular regulation of taurine transport was indicated by the finding that pretreatment with taurine containing media decreased the activity of the taurine transporter(s).

Key words: Taurine; β-Alanine; Sodium ion dependent uptake; Anion dependence; Stoichiometry; Proximal tubule; (Human); (Kidney cell)

1. Introduction

During the past two decades, there has been an increasing focus on the biological role of the β-amino acid taurine. As an example taurine has been shown to be an essential nutrient for cats where dietary defiency of this amino acid leads to a number of different malfunctions (for further details, see [1]). Likewise, taurine defiency in humans results in retinal dysfunction [2], indicating that the amino acid may also play a major role in primates, especially during development [1]. It is well known that the kidney is important in the regulation of the total body pool of taurine by being able to alter the transport activity across the cell membranes of proximal tubule in response to changes in the dietary intake of taurine or of its precursors [3–8]. Nevertheless, despite numerous reports concerning the renal transport mechanism for taurine in mammals, only a single, preliminary study has been published regarding the human uptake process for this β-amino acid. The picture that has emerged by the use of brush border membrane vesicles from rat [4,6,9–11] and rabbit [12,13] or from studies on the continuous LLC-PK_1 cell line from pig kidney [5,7], is the existence of an electrogenic, high-affinity transport component, which is dependent on both Na^+ and Cl^-, and specific for β-amino acids. In addition, a low-affinity, sodium-dependent and a proton-gradient-dependent transport system has also been described in segment-specific brush border membrane vesicles from proximal tubule of rabbit kidney [12]. A similar uptake pattern for the renal handling of the structurally related β-alanine in the proximal tubule has been demonstrated [14–17]. Recently, examinations of taurine transport by LLC-PK_1 cells grown on filters revealed that Na^+-dependent uptake of this amino acid occurs by two transport systems at the apical surface of the cells [7].

All the above-mentioned results are based on renal tissue from various animals. Preliminary data for pri-

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mate renal tubular cells (BS1) and human tubular cells grown in continuous culture showed that the influx of taurine is Na^+-dependent and linear up to 60 minutes [18]. In this study, it was found that contrary to the above-mentioned specificity for β-amino acids reported for other species, addition of 10 mM L-alanine decreases the uptake of 10 μM taurine to 30%, suggesting a difference in taurine transport pathways among species. Thus, the mechanism of taurine uptake needs to be further characterized in detail in the human kidney. In the present study the influx of taurine and β-alanine has been examined by a human embryonic kidney epithelial cell line (IHKE), which has been immortalized after treatment with $NiSO_4$ [19]. As previously shown [20] IHKE cells possess enzyme markers and a brush border membrane characteristic of highly specialized epithelial cells from the renal proximal tubule. Moreover, it has been demonstrated that the IHKE cell line, compared with normal human embryonic kidney cells (NHKE), has retained the capability and specificity to accumulate α-aminoisobutyric acid, a representative of neutral α-amino acids [20]. In the current study, the uptake of taurine and the structurally related β-alanine by the IHKE cell line is described in regard to cation and pH dependence, number of transport components, and competition with other amino acids. In addition, the taurine influx is further characterized with respect to anion dependence, stoichiometry and a regulatory effect of the taurine medium concentration on transport activity.

2. Materials and methods

2.1. Materials

[1,2-^{3}H]Taurine (specific activity 28.0 and 8.9 Ci/mmol) was purchased from Amersham and β-[3(n)-^{3}H]alanine (specific activity 92.6 Ci/mmol) was obtained from NEN. Fetal calf serum (FCS), growth medium and antibiotics were from Gibco, NY. All other non-radioactively labelled ligands, Hepes and Mes were obtained from Sigma.

2.2. Cell culture

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 1% 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.

2.3. 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 taurine 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 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 taurine retained in the extracellular space or bound to the cell surface after incubation with ice-cold incubation medium. In 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 and either 25 mM Mes-KOH (pH 4.5 or 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. [21] with bovine serum albumin (Sigma, St. Louis, MO, USA) as a standard. All solutions used in this study were sterilized before use.

2.4. 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. Theoretical saturation curves were fitted to the experimental data by using a computer-analyzed statistical iteration procedure [22].

To determine the ion/taurine coupling ratio the 'activation method' was used [23], where one measures the stimulation of taurine flux at different concentrations of activator (Na^+ or Cl^-). The data were analyzed by the Hill equation [24]:

$$\text{Flux} = V_{max}[A]^n/(K_{0.5}^n + [A]^n)$$

The equation assumes the existence of n essential cooperative site(s) for the activator A per taurine site. According to this equation a plot of flux/$[A]^n$ against flux for the correct value of n will yield a straight line with slope $1/K_{0.5}^n$. The value of n was also determined by non-linear regression analysis of the flux data according to the Marquardt-Levenberg algorithm [25].

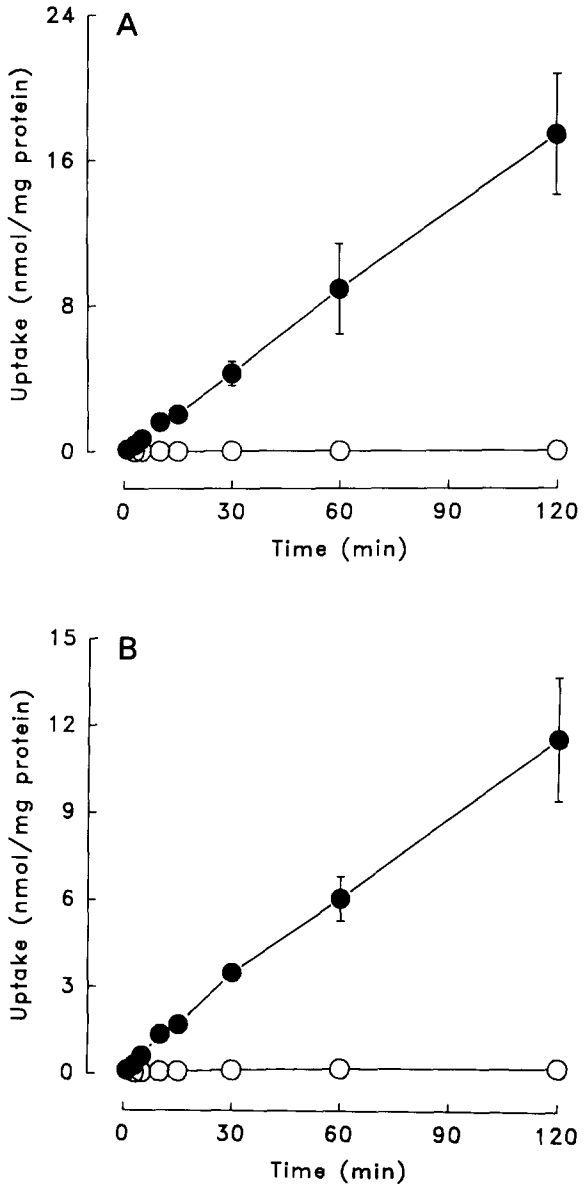

Fig. 1. Time-course of taurine and β-alanine uptake by IHKE cells in the presence or absence of a Na^+-gradient. Monolayers of IHKE cells were incubated in Na^+-containing (●) or in Na^+-free EBSS (○) as described under Materials and methods. (A and B) Media contained 440 nM [1,2-^{3}H]taurine/80 nM β-[3(n)-^{3}H]alanine and sufficiently unlabelled taurine/β-alanine to reach a total concentration of 50 μM (final concentration). The results shown are the mean values ± S.D. of four (taurine) and three (β-alanine) experiments. In those cases where vertical bars are absent, S.D. were smaller than graphical representation of the mean.

Table 1
Effect of an inwardly directed pH-gradient on the uptake of taurine and β-alanine

Amino acid	Na^+	pH_{out}	10 min	120 min
Taurine	+	7.4	0.930 ± 0.129 [b]	14.531 ± 1.852 [c,d]
	+	6.0	0.792 ± 0.092	8.392 ± 0.533 [c]
	+	4.5	0.256 ± 0.029 [b]	0.608 ± 0.149 [d]
	÷	7.4	0.011 ± 0.002 [f]	0.035 ± 0.006 [h]
	÷	6.0	0.011 ± 0.005	0.037 ± 0.010
	÷	4.5	0.021 ± 0.003 [f]	0.075 ± 0.010 [h]
β-Alanine	+	7.4	0.965 ± 0.112 [a,b]	11.170 ± 1.230 [c,d]
	+	6.0	0.742 ± 0.048 [a]	7.396 ± 0.607 [c]
	+	4.5	0.343 ± 0.02 [b]	0.886 ± 0.171 [d]
	÷	7.4	0.043 ± 0.006 [c,f]	0.125 ± 0.016 [g,h]
	÷	6.0	0.059 ± 0.010 [e]	0.197 ± 0.006 [g]
	÷	4.5	0.110 ± 0.026 [f]	0.296 ± 0.046 [h]

Monolayers of IHKE cells were incubated in Na^+-containing or Na^+-free EBSS (pH 4.5, 6.0 and 7.4). Media contained 240 nM [1,2-^{3}H]taurine/80 nM β-[3(n)-^{3}H]alanine and sufficiently unlabelled taurine/β-alanine to reach a total concentration of 50 μM (final concentration). The results shown are the mean values (nmol/mg protein) ± S.D. of 3–6 experiments.
[a–h] t-test: The means of the samples are different at the 0.05 level.

3. Results

Fig. 1A illustrates the time course of 50 μM taurine in monolayers of IHKE cells. The uptake of amino acid in the presence of a Na^+-gradient was linear as a function of time, at least up to 120 min. The influx of taurine was drastically reduced when the Na^+-gradient was replaced by a choline gradient. Thus, the uptake of amino acid at 120 min was 266-fold higher in the presence of sodium than in the presence of choline. Exactly the same kind of experiments was carried out for β-alanine and the results are shown in Fig. 1B. The uptake of β-alanine exhibited the same pattern as that of taurine although the Na^+-dependent influx only appeared to be strictly linear with time up to 30 min. For this amino acid the ratio between uptake in the presence or absence of sodium was 50 at 120 min.

It has previously been found that an inwardly directed H^+-gradient can drive the uphill transport of

taurine and β-alanine in brush border membrane vesicles from the pars convoluta of rabbit kidney [12,14,15]. Therefore, the effect of imposition of an inwardly directed H^+-gradient (pH_{out} 4.5 or 6.0) on the Na^+-dependent influx of taurine and β-alanine was also examined. The presence of both an H^+-gradient and a Na^+-gradient reduced the uptake of amino acid compared with the influx in the presence of a Na^+-gradient alone (Table 1). In the absence of Na^+, the pH-gradients resulted in a significant, but relatively small stimulation of β-alanine transport into the IHKE cells. A similar effect was observed for taurine, but only at pH 4.5. Consequently, since changes in pH may affect cellular functions in many different ways, further experiments concerning a pH-effect on amino acid uptake were not performed in the present study.

Fig. 2A depicts the uptake of taurine as a function of taurine concentrations in the presence of a NaCl gradient or in the presence of a choline chloride gradient. For comparison, the same kind of data for β-alanine are given in Fig. 2C. The sodium-dependent uptake of taurine was biphasic, rising steeply at low medium concentrations and increasing at a slower rate at higher medium concentrations. The uptake data for β-alanine showed the same tendency as for taurine, and saturation of the capacity of the carrier(s) was never observed. For both taurine and β-alanine, the influx of amino acid in the presence of choline chloride shows simple diffusion properties, being proportional to medium concentration of amino acid. The difference between the two curves in each figure (Fig. 2A and C), which may be considered to represent Na^+-gradient-

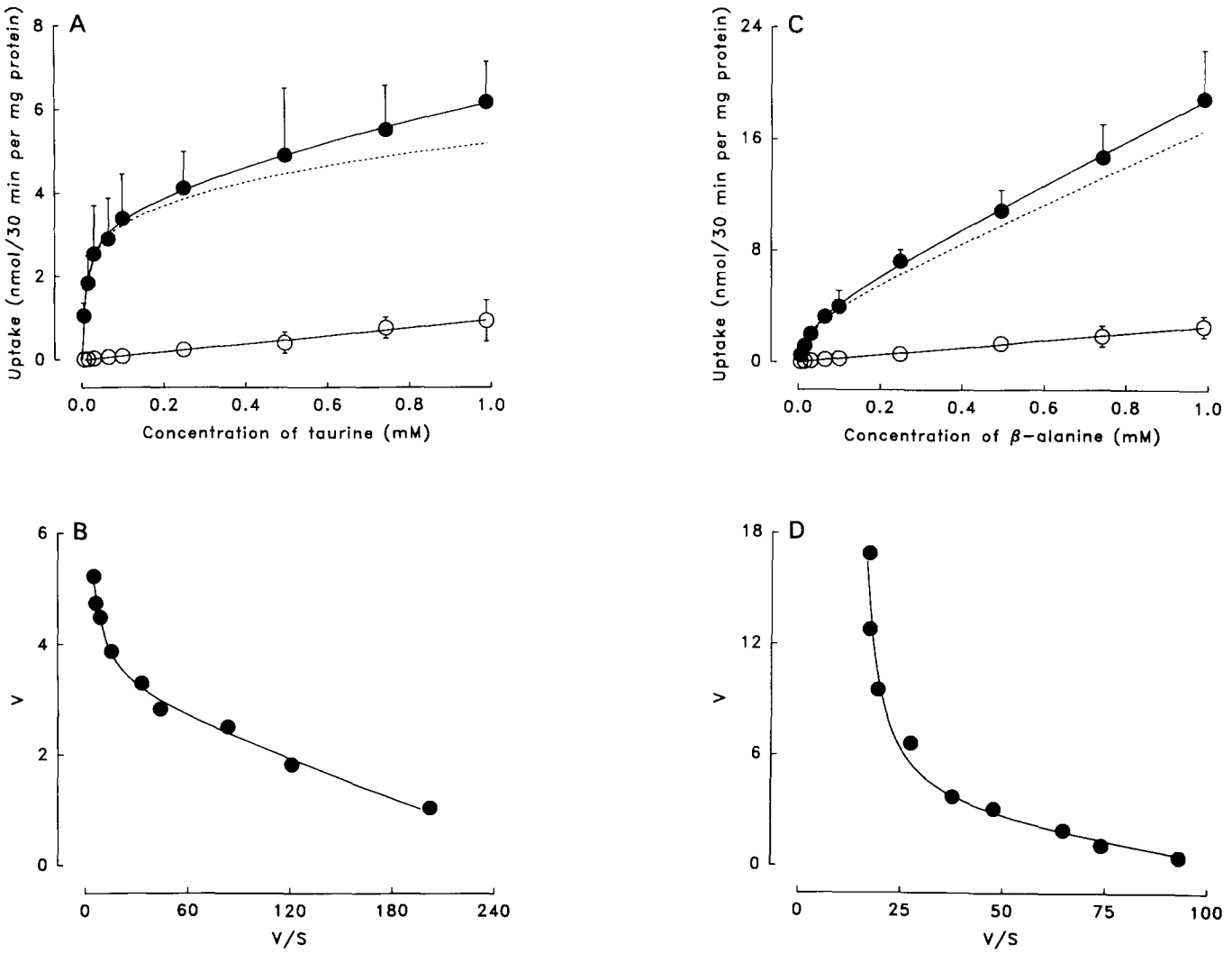

Fig. 2. Kinetics of taurine and β-alanine uptake by IHKE cells in the presence or absence of a Na^+-gradient. (A and C) Monolayers of IHKE cells were incubated in Na^+-containing (●) or Na^+-free EBSS (○), 500 nM [1,2-^{3}H]taurine/ 80 nM β-[3(n)-^{3}H]alanine and various concentrations of unlabelled taurine/β-alanine ranging from 0.005–1.0 mM. Subtracting the uptake values in the absence of Na^+ from the total uptake in presence of Na^+ resulted in the uptake values in the presence of a Na^+-gradient alone (the dotted line). (B and D) Eadie-Hofstee plot of data obtained for taurine/β-alanine 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 four(taurine) and three(β-alanine) experiments. In those cases where vertical bars are absent, S.D. were smaller than graphical representation of the mean.

Table 2
Effect of different amino acids on the uptake of taurine by IHKE cells

Inhibitor (conc.)	0.5 mM	5.0 mM
None	1.00	1.00
Glycine	0.85 ± 0.08 *	0.60 ± 0.05 *
L-Alanine	0.97 ± 0.11	0.69 ± 0.08 *
L-Leucine	0.99 ± 0.08	0.86 ± 0.08 *
L-Proline	0.87 ± 0.05	0.56 ± 0.06 *
L-Phenylalanine	1.09 ± 0.14	0.78 ± 0.08 *
L-Serine	0.87 ± 0.11	0.58 ± 0.06 *
L-Glutamine	0.98 ± 0.11	0.81 ± 0.15 *
L-Aspartic acid	1.03 ± 0.08	1.07 ± 0.13
L-Arginine	1.02 ± 0.11	0.72 ± 0.06 *
Taurine	0.14 ± 0.01 *	0.04 ± 0.00 *
β-Alanine	0.41 ± 0.07 *	0.07 ± 0.02 *
Methyl-α-D-glucoside	1.02 ± 0.12	1.01 ± 0.06

Incubation medium consisted of Na^+-containing EBSS, 210 nM [1,2-^{3}H]taurine and unlabelled taurine reaching 0.050 mM (final concentration) as well as test compounds at concentrations of 0.5 mM and 5.0 mM. The uptake of taurine at 30 min was normalized to uptake observed in the absence of test compound. Results are given as mean values ± 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.

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

Inhibitor (conc.)	0.5 mM	5.0 mM
None	1.00	1.00
Glycine	0.81 ± 0.07	0.60 ± 0.13 *
L-Alanine	0.62 ± 0.13 *	0.45 ± 0.02 *
L-Leucine	1.03 ± 0.12	0.76 ± 0.03 *
L-Proline	0.78 ± 0.03	0.47 ± 0.06 *
L-Phenylalanine	1.08 ± 0.15	0.70 ± 0.07 *
L-Serine	0.63 ± 0.05 *	0.52 ± 0.03 *
L-Glutamine	1.04 ± 0.14	0.78 ± 0.19
L-Aspartic acid	1.05 ± 0.11	0.92 ± 0.05
L-Arginine	0.93 ± 0.06	0.89 ± 0.19
Taurine	0.34 ± 0.04 *	0.31 ± 0.06 *
β-Alanine	0.39 ± 0.07 *	0.27 ± 0.04 *
Methyl-α-D-glucoside	0.89 ± 0.10	1.00 ± 0.05

Incubation medium consisted of Na^+-containing EBSS, 80 nM β-[3(n)-^{3}H]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 uptake observed in the absence of test compound. Results are given as mean values ± 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.

dependent uptake of the β-amino acid influx, is illustrated in the figures as the dotted line. The corrected data are shown as Eadie-Hofstee plots in Fig. 2B and D. It is apparent from these plots that the uptake of taurine and β-alanine in the IHKE cells occurs by more than one transport system. Assuming the existence of two transport components, the computer calculations resulted in the following kinetic parameters for taurine: $K_{m1} = 0.011 \pm 0.002$ mM, $K_{m2} = 1.03 \pm 0.69$ mM, and $V_{max1} = 3.20 \pm 0.24$ and $V_{max2} = 4.11 \pm 1.17$ nmol/mg protein per 30 min and for β-alanine: $K_{m1} = 0.034 \pm 0.009$ mM, $K_{m2} = 31.16 \pm 1.46$ mM, and $V_{max1} = 3.19 \pm 0.63$ and $V_{max2} = 439.81 \pm 124.70$ nmol/mg protein per 30 min. However, the kinetic constants for the low affinity systems should be taken with considerable reservations as indicated in Fig. 2A and C.

The substrate specificity of the β-amino acid influx by addition of different unlabelled amino acids to the incubation medium was also examined. The results of these competition experiments for taurine are given in Table 2. Addition of the β-amino acids taurine and β-alanine drastically reduced the uptake of amino acid. Likewise, all the neutral amino acids tested and L-arginine at high concentration (5 mM) decreased the transport of taurine into the IHKE cells, whereas L-aspartic acid had no effect. However, the decrease even at this high concentration was moderate for all α-amino acids tested. Note also that methyl-α-D-glucoside, which was used to test for non-specific inhibition caused by sodium-depletion, had no effect on the influx of taurine in these experiments. Corresponding data on the substrate specificity of β-alanine uptake is shown in Table 3. Generally, the same pattern of inhibition was observed, but it is seen by comparison with the data in Table 2 that in contrast to taurine, the influx of β-alanine was decreased by L-alanine and L-serine even at low inhibitor concentration. This seems to be in accordance with the previously shown difference in the high affinity K_m-values for the uptake of taurine and β-alanine. Furthermore, L-arginine had no effect at all on the transport of β-alanine in the IHKE cell line.

Since it is well known from previous studies [5,9,10,12–14,26] that the high-affinity Na^+-dependent β-amino acid transport system is dependent on both Na^+ and Cl^- ions, the anion dependence of taurine transport was also investigated in the human kidney cell line. It is seen from Table 4 that, at low taurine concentration i.e under conditions where the high-af-

Table 4
Effect of anions on the uptake of taurine by IHKE cells

Anion	Solute uptake relative to that in Cl^- medium	
	240 nM	1.0 mM
Cl^-	1.00	1.00
NO_3^-	0.25 ± 0.03	1.01 ± 0.05
SCN^-	0.52 ± 0.04	1.35 ± 0.16
Gluconate	0.11 ± 0.01	0.69 ± 0.03

Incubation medium consisted of modified Na^+-containing EBSS, 240 nM [1,2-^{3}H]taurine and unlabelled taurine reaching 240 nM or 1.0 mM (final concentration). In these experiments, chloride was replaced by the anions listed above. The uptake of taurine at 30 min was normalized to uptake observed in the presence of NaCl. Results are given as mean values ± S.D. of 3–6 experiments.

finity transporter prevails, the Na^+-dependent influx was highest in the presence of Cl^-, and that the anions NO_3^-, SCN^- and gluconate could only partly substitute for Cl^-. By contrast, at 1 mM of taurine, the uptake of amino acid appeared to be more dependent on the diffusion potentials, caused by addition of the anions, than on a specific requirement for Cl^-. This is similar to the effect of anions previously reported for the Cl^--independent uptake of D-glucose [27]. Thus, the results demonstrate that only the high-affinity transporter is stimulated by direct interaction with Cl^-, whereas a similar effect is not observed for the low-affinity transport component.

An attempt to estimate the stoichiometric properties of the taurine transporters in the IHKE cells was also made by use of the 'activation method' [23]. According to the rationale of this method, the uptake of amino acid was measured at different medium concentrations of activator (Na^+ or Cl^-). Fig. 3 illustrates the influx of taurine as a function of $[Na^+]$ at low amino acid concentration, representing the high-affinity transport system. Choline replaced sodium isosmotically to obtain the various sodium concentrations in the range of 5 to 140 mM. It is seen that the uptake values exhibit a sigmoidal dependence on sodium concentration, indicating that a coupling ratio of more than one Na^+ per molecule taurine is involved in the translocation process. A further analysis of the data by linear transformation of the Hill equation (see Section 2.4)

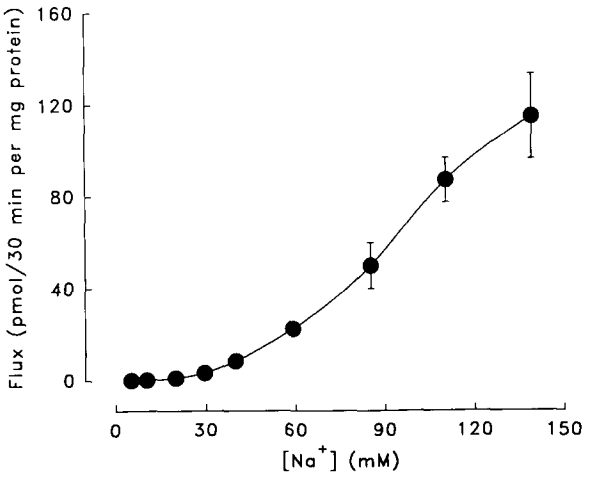

Fig. 3. Effect of increasing extracellular Na^+ concentrations on taurine transport. The IHKE cells were pre-incubated in 140 mM choline chloride, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 25 mM Hepes-KOH (pH 7.4) and 5 mM D-glucose for 90 min. The composition of the transport buffers was exactly the same, apart from D-glucose (absent), 520 nM [1,2-^{3}H]taurine and the varying concentrations of NaCl (5–140 mM). Choline replaced sodium isosmotically to obtain the various sodium concentrations studied. All uptake values were corrected for uptake in the absence of a Na^+ gradient. Results are given as mean values ±S.D. of five experiments. In those cases where vertical bars are absent, standard deviations are smaller than the graphical representation of the mean.

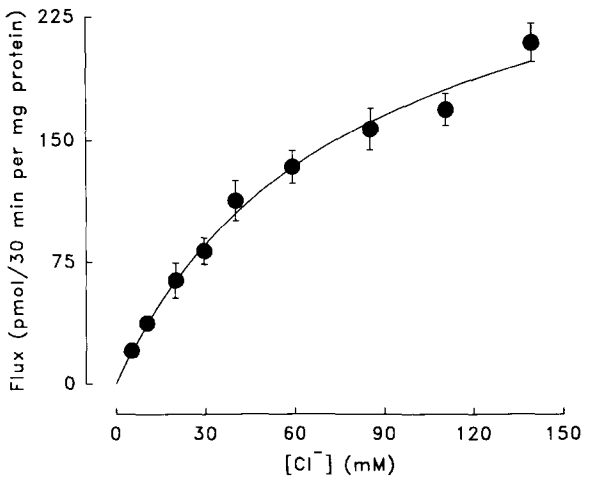

Fig. 4. Effect of increasing extracellular Cl^- concentrations on taurine transport. The IHKE cells were pre-incubated in 140 mM sodium D-gluconate, 5.4 mM potassium D-gluconate, 1.8 mM calcium D-gluconate, 0.8 mM magnesium D-gluconate, 25 mM Hepes-KOH (pH 7.4) and 5 mM D-glucose for 90 min. The composition of the transport buffers was exactly the same, apart from D-glucose (absent), 560 nM [1,2-^{3}H]taurine and the varying concentrations of NaCl (5–140 mM). D-Gluconate replaced chloride isosmotically to obtain the various chloride concentrations studied. All uptake values were corrected for uptake in the absence of a Cl^- gradient. Results are given as mean values ± S.D. of four experiments. In those cases where vertical bars are absent, standard deviations are smaller than the graphical representation of the mean.

suggested that the best value for n, resulting in a straight line relationship, was 2.8. However, the correlation coefficient (r) was only −0.43. Therefore, the data were reanalyzed by non-linear regression, which gave an n value of 3.0 ± 0.2. Thus, the results of Fig. 3 imply the involvement of 1 amino acid and 3 Na^+ ions for the high-affinity transport of taurine. A series of similar experiments was performed in order to further characterize the low-affinity, Na^+-dependent transport component involved in the influx of taurine. However, at 1 mM of amino acid, the uptake data at low concentrations of Na^+ were very scattered, making it impossible to draw any conclusion (results not shown). Taurine uptake by the high-affinity system versus Cl^--concentration is illustrated in Fig. 4. The curve indicates a Michaelis-Menten type dependence, suggesting a 1:1 stoichiometry. This is supported by linear transformation, where an n value of 1 resulted in the best fit of the data to a straight line ($r = -0.98$). The non-linear regression analysis of the Cl^--dependent uptake of taurine as a function of Cl^- concentration gave a value of 0.9 ± 0.1. Thus, the coupling ratio between Na^+, Cl^-, and taurine at low amino acid concentrations in the IHKE cells is most likely 3:1:1.

Finally, the effect of exposure of the cells to different medium concentrations of taurine on the taurine transporter(s) was examined. To do so, a series of experiments was performed in which taurine was added

to the cell medium reaching a final concentration of 0, 50 or 500 μM. After 24 h, the cell medium was removed and the Na^+-dependent uptake of radioactively labelled taurine (50 μM) was measured after 30 min of incubation according to Materials and methods. On the assumption that 50 μM taurine reflects a physiological level of amino acid, the uptake at this medium concentration was designated as 100%. It was found that the presence of 500 μM taurine in the medium resulted in a decrease in taurine transport to $84.3 \pm 6.9\%$ of the control value ($n = 6$). On the other hand, omission of taurine in the medium before the transport experiment increased the uptake of amino acid to $142.9 \pm 25.2\%$ ($n = 6$). These findings suggest that the activity of the taurine membrane carrier(s) in the IHKE cell line is very sensitive to variations in the extracellular taurine concentration.

4. Discussion

The present study characterizes the proximal tubular uptake of taurine and β-alanine by an immortalized, human embryonic kidney epithelial cell line. It was found that in the absence of sodium, the transport of β-amino acids into the cells was almost abolished. Thus, the uptake of taurine by human renal cells is clearly sodium-dependent even though, as also observed by continuous microperfusion of rat tubules [28], and in uptake studies on luminal membrane vesicles from rat [10] and rabbit [12] and LLC-PK_1 cells [7], the amino acid influx is a relatively slow process compared with that of α-amino acids. Thus, the uptake was still linear for taurine after 120 min of incubation. Similar results were obtained for the structurally related β-alanine. It has previously been demonstrated that both in the presence or absence of a Na^+-gradient the transport of taurine and β-alanine can be driven by an inwardly directed H^+-gradient in luminal membrane vesicles from pars convoluta of rabbit kidney. By contrast, no such effect was observed in vesicles from pars recta [12,14,15]. Experiments regarding the IHKE cell line revealed that only in the absence of a Na^+-gradient a small, but significant increase in the influx was observed for β-alanine at pH 4.5 and 6.0, and for taurine at pH 4.5 (Table 1), whereas a low extracellular pH reduced the uptake of amino acid in the presence of inwardly directed Na^+-gradient. Thus, the physiological meaning of acidification seems unclear, since one will always expect the presence of a higher extracellular than intracellular concentration of sodium.

Analysis of data, obtained at different concentrations of taurine in the medium, indicated that the sodium-gradient-dependent influx of amino acid occurs via a dual transport component with a K_m value of 0.011 mM for the high-affinity system. The existence of two transport components at the apical membrane has also been demonstrated in the LLC-PK_1 cell line [7], and brush-border membrane vesicles from rabbit [12] and reptilian kidney [29]. However, in a number of other studies only a single, Na^+-dependent transport system has been described for taurine but, as previously pointed out by Benyajati and Bay [29], the main cause for this discrepancy seems to be attributable to differences in the range of taurine concentrations studied and in the kind of kinetic analysis used. The K_m value for the high-affinity component in the IHKE cells is identical to the one found for LLC-PK_1 cells [7] and is also within the range of the half-saturation constants reported for membrane preparations from rabbit [12] and rat kidney [9,11]. The kinetics of β-alanine influx also suggests the existence of more than one transport system. The high-affinity K_m value was higher than the value for taurine, which is in good agreement with previous reports concerning membrane vesicles from rabbit kidney [12,14,15]. The half-saturation constant of 0.034 mM for β-alanine is comparable to the values obtained by brush border membrane vesicles from both dog [16] and rabbit [17] kidney.

It is generally agreed that the renal uptake of taurine occurs by a specific β-amino acid transporter [10–12,17,30,31]. However, in a preliminary report by Vadgama and Kopple [18], an overlap of taurine transport with α-amino acid transport pathways was described for human proximal tubular cells. Thus, they found that addition of 10 mM L-alanine to 10 μM taurine resulted in 70% inhibition of the β-amino acid uptake. Table 2 suggests that in the human cell line used in this study some α-amino acids can be transported by the high-affinity β-amino acid transport system but that the affinity for these amino acids is relatively small compared with taurine. This conclusion is supported by the fact that methyl-α-D-glucoside had no effect on the taurine transport, indicating that the inhibitory effect of the different amino acids is caused by a direct involvement in the transport process rather than by sodium-depletion from the operation of other transport systems. Similar results were obtained for β-alanine (Table 3) in which case the uptake, as expected, was more sensitive to the presence of α-amino acids than was taurine. The difference in response to addition of L-arginine, which reduced the uptake of taurine but not that of β-alanine (Tables 2 and 3), may just be a result of uncertainty in the determination of β-amino acid uptake. A relatively small, but nonetheless significant inhibition of taurine uptake by α-amino acids was also reported for the LLC-PK_1 cell line although the effect of a possible reduction in the Na^+-gradient by the inhibiting amino acids was not examined[5]. It should be noted from Tables 2 and 3 that L-proline and α-amino acids with 2 or 3 carbon atoms are more efficient inhibitors of the β-amino acid

uptake than are L-phenylalanine and α-amino acids with 4 or more carbon atoms.

The effect of replacing Cl^- with SCN^-, NO_3^-, and gluconate indicated a specific requirement for Cl^- by the high-affinity taurine transporter, whereas Cl^- had no effect on the low-affinity transport component (Table 4). This is in good agreement with earlier studies in renal brush-border membrane vesicles from rabbit [12] where a similar conclusion was reached both for the pars convoluta and the pars recta of proximal tubule.

Based on the activation method, the data presented in this study indicate involvement of multiple Na^+ as well as of one Cl^- when taurine is transported across the cell membrane by the high-affinity carrier. Even though the method does not distinguish between catalytic and energetic coupling, the stoichiometry must be at least 2 Na^+/1 Cl^-/1 amino acid (zwitterion at pH 7.4), since it has been demonstrated in studies on vesicles prepared from proximal tubule, that the translocation of amino acid is a positive electrogenic process [10–12] and that Cl^- in dog [16] and rabbit [13] renal brush-border membrane vesicles is cotransported with β-amino acids. The current study provides evidence for a coupling ratio of 3 Na^+/1 Cl^-/1 amino acid. The stoichiometry of three sodium ions contrasts with previous reports regarding LLC-PK_1 cells and different brush-border membrane preparations. The latter have primarily indicated a stoichiometry of 2 Na^+/1 taurine in short-circuited rabbit membranes [12], whereas Wolff and Kinne [13], voltage-clamping the membrane potential by an outwardly directed K^+-diffusion potential, found that the Na^+ coupling ratio decreased from 3 to 2 with increasing concentrations of Na^+. It was suggested that the higher Na^+ uptake produced a faster dissipation of the membrane potential, thereby giving a false impression of different coupling ratios. A K^+-diffusion potential was also used in experiments with luminal membrane vesicles from rat [9] and garter snake [29] in which the uptake data suggested that translocation of 1 molecule of taurine by the high-affinity transport system involves 2 and 3 sodium ions, respectively. However, uptake studies with garter snake vesicles have also indicated a 2 Cl^-/1 amino acid stoichiometry [32]. For the LLC-PK_1 cell line Jones and coworkers [7] have demonstrated that at the apical membrane taurine is reabsorbed against a concentration gradient along with two sodium ions in the proximal tubule. In addition, they found a coupling ratio of 1 Na^+/1 taurine at the basolateral cell membrane. The authors also presented data, which seems to confirm that the net transepithelial transport occurs from the luminal to the contraluminal cell surface in the mammalian kidney. This is in contrast to some marine fish species, where the transport of taurine is in the opposite direction in order to regulate cell volume osmotically [33,34], whereas ophidian reptiles, depending on the filtered load of amino acid, showed both net secretion and net reabsorption of taurine [35]. As previously shown [20] experiments with PAH (*p*-aminohippuric acid) strongly suggested that when the IHKE cell monolayers were confluent, the uptake data only represent influx across the luminal cell membranes. A coupling ratio of 3 Na^+/1 taurine at the apical side of the IHKE cells would, all things considered equal, enhance the energetic cost of the re-absorption of taurine compared with a stoichiometry of 2 Na^+/1 amino acid. Nevertheless, in the light of the large body of evidence indicating a 2 Na^+/1 taurine coupling ratio and the difficulties involved in transport studies with continuous cell lines, one has to be cautious to draw firm conclusions on stoichiometry.

As previously described, substantial evidence indicates that the activity of taurine transporter(s) is regulated in response to alterations in the dietary intake of taurine or its precursors [3–8]. Therefore, an attempt was made to confirm this phenomenon for the IHKE cell line. It was found that low- and high-medium concentrations of taurine increased and decreased, respectively, the activity of the taurine transporter(s). A similar pattern has been demonstrated for the LLC-PK_1 and MDCK cell lines [7]. Thus, the IHKE cell line seems to be a very suitable model for future studies on the regulation of taurine transport in the human proximal tubule.

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References

[1] Sturman, J.A. (1993) Physiol. Rev. 73, 119–147.
[2] Geggel, H.S., Ament, M.E., Heckenlively, J.R., Martin, D.A. and Kopple, J.D. (1985) N. Engl. J. Med. 312, 142–146.
[3] Rozen, R and Scriver, C.R. (1982) Proc. Natl. Acad. Sci. USA 79, 2101–2105.
[4] Chesney, R.W., Gusowski, N. and Friedman, A.L. (1983) Kidney Int. 24, 588–594.
[5] Jones, D.P., Miller, L.A. and Chesney, R.W. (1990) Kidney Int. 38, 219–226.
[6] Chesney, R.W., Gusowski, N. and Dabbagh, S. (1985) J. Clin. Invest. 76, 2213–2221.
[7] Jones, D.P., Miller, L.A. and Chesney, R.W. (1993) Am. J. Physiol. 265, F137–F145.
[8] Friedman, A.L., Albright, P.W., Gusowski, N., Padilla, M. and Chesney, R.W. (1983) Am. J. Physiol. 245, F159–F166.
[9] Zelikovic, I., Stejskal Lorenz, E., Lohstroh, P., Budreau, A. and Chesney, R.W. (1989) Am. J. Physiol. 256, F646–F655.
[10] Chesney, R.W., Gusowski, N., Dabbagh, S., Theissen, M., Padilla, M. and Diehl, A. (1985) Biochim. Biophys. Acta 812, 702–712.

[11] Rozen, R, Tenenhouse, H.S and Scriver, C.R. (1979) Biochem. J. 180, 245–248.
[12] Jessen, H. and Sheikh, M.I. (1991) Biochim. Biophys. Acta 1064, 189–198.
[13] Wolff, N.A. and Kinne, R. (1988) J. Membr. Biol. 102, 131–139.
[14] Jessen, H. and Sheikh, M.I. (1991) Biochem. J. 277, 891–894.
[15] Jessen, H., Jørgensen, K.E., Røigaard Petersen, H. and Sheikh, M.I. (1989) J. Physiol. (Lond.) 411, 517–528.
[16] Turner, R.J. (1986) J. Biol. Chem. 261, 16060–16066.
[17] Hammerman, M.R. and Sacktor, B. (1978) Biochim. Biophys. Acta 509, 338–347.
[18] Vadgama, J.V. and Kopple, J.D. (1989) FASEB J. 3, A1060.
[19] Tveito, G., Hansteen, I.L., Dalen, H. and Haugen, A. (1989) Cancer Res. 49, 1829–1835.
[20] Jessen, H., Røigaard, H., Riahi-Esfahani, S. and Jacobsen, C. (1994) Biochim. Biophys. Acta 1190, 279–288.
[21] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[22] Jacobsen, C., Frich, J.R. and Steensgaard, J. (1982) J. Immunol. Methods 50, 77–88.
[23] Turner, R.J. and Moran, A. (1982) J. Membr. Biol. 67, 73–80.
[24] Turner, R.J. and Moran, A. (1982) J. Membr. Biol. 70, 37–45.
[25] Marquardt, D.W. (1963) J. Soc. Industr. Appl. Math. 11, 431–441.
[26] Røigaard Petersen, H., Jacobsen, C., Jessen, H., Mollerup, S. and Sheikh, M.I. (1989) Biochim. Biophys. Acta 984, 231–237.
[27] Ullrich, K.J. (1979) Annu. Rev. Physiol. 41, 181–195.
[28] Dantzler, W.H. and Silbernagl, S. (1976) Pflugers Arch. 367, 123–128.
[29] Benyajati, S. and Bay, S.M. (1992) Pflugers Arch. 421, 168–175.
[30] Chesney, R.W. (1985) Adv. Pediatr. 32, 1–42.
[31] Chesney, R.W., Scriver, C.R. and Mohyuddin, F. (1976) J. Clin. Invest. 57, 183–193.
[32] Benyajati, S. and Bay, S.M. (1993) Proc. Int. Union. Physiol. Sci. 32st. Glasgow, 93.7/P.
[33] Schrock, H., Forster, R.P. and Goldstein, L. (1982) Am. J. Physiol. 242, R64–R69.
[34] King, P.A., Kinne, R. and Goldstein, L. (1985) J. Comp. Physiol. B. 155, 185–193.
[35] Benyajati, S. and Dantzler, W.H. (1986) Am. J. Physiol. 250, R712–R720.