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Transepithelial Taurine Transport in Caco-2 Cell Monolayers

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Abstract. Here we characterized transepithelial taurine transport in monolayers of cultured human intestinal Caco-2 cells by analyzing kinetic apical and basolateral uptake and efflux parameters. Basolateral uptake was Na^+- and Cl^-- dependent and was inhibited by β-amino acids. Uptake by this membrane showed properties similar to those of the apical TauT system. In both membranes, taurine uptake fitted a model consisting of a non-saturable plus a saturable component, with a higher half-saturation constant and transport capacity at the apical membrane (K_m, 17.1 μmol/L; V_{max}, 28.4 pmol·cm^{-2}·5 min^{-1}) than in the basolateral domain (K_m, 9.46 μmol/L; V_{max}, 5.59 pmol·cm^{-2}·5 min^{-1}). The non-saturable influx component, estimated in the absence of Na^+ and Cl^-, showed no significant differences between apical and basolateral membranes (K_D, 89.2 and 114.7 nL·cm^{-2} · 5 min^{-1}, respectively). Taurine efflux from the cells is a diffusive process, as shown in experiments using preloaded cells and in trans-stimulation studies (apical K_D,72.7 and basolateral K_D, 50.1 nL·cm^{-2}·5 min^{-1}). Basolateral efflux rates were significantly lower than passive influx rates. We conclude that basolateral taurine uptake in Caco-2 cells is mediated by a transport mechanism that shares some properties with the apical system TauT. Moreover, calculation of unidirectional and transepithelial taurine fluxes reveals that apical influx of this amino acid is higher than basolateral efflux rates, thereby enabling epithelial cells to accumulate taurine against a concentration gradient.

Key words: Taurine — Intestinal epithelium — Apical transport system — Basolateral transport system — TauT — Substrate efflux

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Introduction

Taurine is a sulfonic β-amino acid involved in many physiological processes such as osmoregulation, detoxification, antioxidation, membrane stabilization and retinal and cardiac function [7, 10]. Through its antioxidant capacity, taurine prevents the effects of docosahexaenoic acid on cellular lipid peroxidation and paracellular permeability in intestinal Caco-2 cells [16] and also participates in immune and inflammatory responses [15]. Taurine is an end-product of sulfur-containing amino-acid metabolism that mostly remains free as a zwitterion in body fluids, reaching plasma concentrations of around 100 μmol/L in humans [1]. In vertebrates, taurine homeostasis involves a fine balance between liver and brain biosynthesis and catabolism, and between intestinal absorption and renal reabsorption and excretion by the TauT system [10]. Of the two sources of this amino acid, the contribution of dietary taurine to the taurine body pool is greater than taurine biosynthesis, especially in newborn mammals. At this early stage of development, the demands for this amino acid are high, synthetic capacity is low and the mechanism of renal resorption is not fully established [14].

The capacity of the intestinal epithelium to absorb taurine depends on the properties of the transport mechanisms at the apical (AP) and basolateral (BL) membranes of the enterocyte. In the former, there is a high-affinity, low-capacity Na^+- and Cl^--dependent system, specific for β-amino acids identified as TauT [2, 13], which transports taurine against a concentration gradient. Little is known, however, about the transport of taurine across the basolateral membrane. In Ehrlich cells, uptake of this amino acid is mediated by a member of the TauT family and there is also an efflux pathway, activated by cell swelling, that modulates the cytosolic taurine concentration [9]. In cultured astrocytes, members of the chloride-channel family (ClC-2 and ClC-3) involved

in cell volume regulation are also permeable to taurine [21]. In the renal epithelium, taurine is transported by the conventional TauT system present at the apical membrane but efflux occurs across the basolateral domain through a voltage-dependent non-saturable mechanism [19].

In a previous study we have demonstrated in the human intestinal Caco-2 cell line, that taurine can prevent the effects of lipid peroxidation on paracellular permeability [16]. Moreover, taurine exhibits a 90- to 100-fold accumulation in human duodenal mucosa compared with its concentration in plasma, thus indicating a remarkable capacity of the intestinal epithelium to accumulate this amino acid [1]. Here we studied the kinetics of taurine transport across the intestinal epithelium and estimated the amount of this amino acid remaining in the epithelium and the amount delivered to the internal milieu. We first characterized basolateral taurine uptake and then estimated the transepithelial transport of this amino acid following the kinetics model described by Chen, Zhu & Hu [5]. This study was performed in isosmotic conditions because taurine transport is involved in cell volume regulation [18, 19]. Our results indicate that taurine is taken up from circulation by a member of the TauT family located at the basolateral membrane and that efflux from cells occurs at low rates through a non-mediated process.

Materials and Methods

Materials

Dulbecco's Modified Eagle's medium (DMEM), non-essential amino acids, penicillin, streptomycin, L-glutamine, trypsin, fetal bovine serum (FBS), D-glucose and HEPES, as well as other chemicals, were supplied by Sigma (St. Louis, MO). [1,2–^{14}C] taurine (specific activity 110 mCi/mmol) was purchased from ARC (St. Louis, MO). Tissue culture supplies, including Transwells, were obtained from Costar (Cambridge, MA).

Cell Culture

Caco-2 cells were kindly provided by Dr. David Thwaites at the School of Cell and Molecular Biosciences, University of Newcastle upon Tyne (UK) and cultured following Thwaites et al. [22]. The cells (passages 107–116) were routinely grown in plastic flasks at a density of 5×10^4 cells-cm^{-2} and cultured in DMEM supplemented with 4.5 g/L D-glucose, 1% (v/v) non-essential amino acids, 2 mmol/L L-glutamine, 10% (v/v) heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a modified atmosphere of 5% CO_2 in air.

For transport experiments, cells were seeded at a density of 4×10^5 cells·cm^{-2} onto polycarbonate filters (Transwells, 12 mm diameter) with a pore size of 0.4 μm. Growth medium was replaced twice weekly. Cell confluence was confirmed by microscopic observation and transepithelial electrical resistance (TER) measurements. Experiments were performed on monolayers 19–21 days post-seeding and 24 h after feeding.

Uptake Experiments

Transport experiments were performed following Thwaites et al. [22]. Monolayers grown on filters were gently washed by sequential transfer through four beakers containing 500 mL of modified Krebs buffer at room temperature. The composition of this buffer was (mmol/L): NaCl 137, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$ 1.0, NaH_2P0_4 0.3, D-glucose 10 and HEPES/Tris 10 (pH 7.4). The filters were then placed in culture wells containing 1.5 and 0.75 mL modified Krebs buffer in the basal and apical compartments respectively, and TER was measured using a Millicell-ERS voltohmmeter (Millipore, Bedford, MA). After TER determination, apical or basolateral medium (for apical and basolateral uptake experiments, respectively) was replaced with the same volume of modified Krebs buffer containing 0.2 μCi/mL [1,2–^{14}C] taurine and the appropriate concentration of unlabeled taurine. In some experiments, NaCl was replaced by an equimolar concentration of $NaNO_3$, choline chloride (ChoCl), KCl or KNO_3. Taurine uptake (37°C, 100 μmol/L substrate concentration) was linear for at least 30 min ($R^2 = 0.99$), indicating that substrate influx was not yet masked by efflux. Therefore, monolayers were incubated for 5 min, except for cellular substrate-loading experiments. Since taurine is a biochemically inert amino acid [7], a 5-min incubation was considered to be a valid measure of initial influx rates. After incubation, filters were washed 4 times in 500 mL ice-cold modified Krebs buffer to eliminate non-specific radioactivity fixation, removed from the insert and dissolved in scintillation cocktail to be counted in a Packard 1500 Tri-Carb counter (Downers Grove, IL). In addition, to establish apical-to-basolateral taurine transport, a sample of the basolateral compartment from apical substrate incubation experiments was also taken at the end of the experiment for radioactivity quantification. Intracellular taurine concentration was calculated assuming a monolayer volume of 2.14 μL, estimated following Hu & Borchardt [6], by multiplying the surface area of the filter by the height of the monolayer measured in transmission electronmicroscopy micrographs of known magnification (18.9 ± 0.8 μm, mean ± SEM of $n = 20$ micrographs).

Efflux Experiments

Cell monolayers were preloaded for 60 min at 37°C with 0.2 μCi/mL [1, 2–^{14}C] taurine and the appropriate unlabeled taurine concentration from either the basolateral or apical side for apical or basolateral efflux experiments, respectively. This strategy was followed to avoid the effect of non-specific substrate binding on the loading membrane [5]. The preloaded monolayers were then washed three times in 500 mL ice-cold modified Krebs buffer and placed in culture wells containing 0.75 and 1.5 mL modified Krebs buffer lacking the substrate in the apical and basolateral compartments, respectively, and incubated at 37°C for a range of periods. The amount of taurine released was determined by measuring radioactivity in aliquots from apical or basolateral media. Intracellular taurine concentration attained after the loading period was determined in three monolayers for each substrate concentration and processed as in uptake experiments. The time course of taurine efflux (cells preloaded with 100 μmol/L taurine from the apical or basolateral compartment) was maximal at 10 min incubation for both membrane domains. For this reason, and given that an incubation period of 5 min was applied in initial taurine influx experiments, efflux was also estimated after 5 min of incubation. To establish apical-to-basolateral taurine transport in steady-state condition, a sample of the basalolateral medium from apical substrate incubation experiments was taken after the loading period for radioactivity quantification.

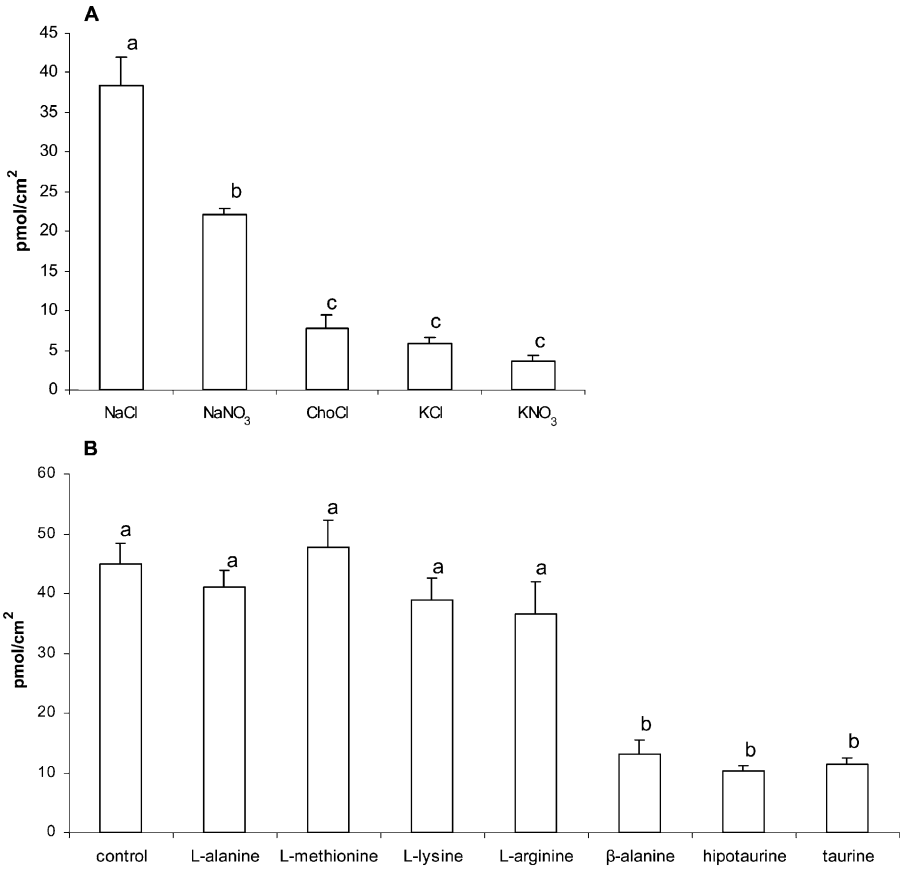

Fig. 1. Na^+-and Cl^--dependence (A) and cis-inhibition (*B*) of taurine uptake across Caco-2 cell apical membrane. Monolayers were incubated for 5 min at 37°C in the presence of 100 μmol/L taurine (0.2 μCi/mL [1,2-^{14}C] taurine) and equimolar concentrations of NaCl, $NaNO_3$, ChoCl (choline chloride), KCl or KNO_3 in the apical compartment and, for cis-inhibition experiments, in the presence of the substrate and 10 mmol/L of the unlabeled amino acid in NaCl-containing Krebs buffer. After the incubation period, the filters were removed from the inserts and the radioactivity was measured. Results are expressed as mean ± SEM of n = 3–4 monolayers. Mean values labeled with different letters are significantly different ($P < 0.05$).

Kinetic and Statistical Analysis

To estimate the kinetic parameters of apical and basolateral taurine uptake, the rates of mediated transport were analyzed following the strategy described in [20], assuming either a one-system or a two-system model by non-linear regression from plots generated by the Enzfitter statistical package (Biosoft, Cambridge, UK). The best fit was assigned to the fit with the lowest significantly different residual sums of squares ($P < 0.05$), following the criteria established by Motulsky & Ransnas [11]. In the case of apical and basolateral taurine efflux, the data were analyzed in the same way but we assumed either a simple diffusion mechanism or a one-system plus diffusion model.

Results are given as means ± SEM. Analysis of variance was followed by Scheffe's multiple comparison test to detect significant differences between conditions. Student's t-test was used to compare mean values from the two groups. P values < 0.05 were considered significant.

Transepithelial Taurine Transport

Transepithelial taurine transport was estimated following the simplified kinetics model described by Chen et al. [5], which evaluates apical and basolateral uptake and efflux rates in an integrated approach. In this model, net apical uptake is considered to be equal to apical influx minus apical efflux, as shown in equation (1)

$$J_{net,u,AP} = J_{u,AP} - J_{e,AP} = \frac{V_{max,u,AP} \cdot C_0}{K_{m,u,AP} + C_0} + K_{D,u,AP}(C_0 - C_i) - K_{D,e,AP}(C_i - C_0) \quad (1)$$

in which $V_{max,u,AP}$ and $K_{m,u,AP}$ are the kinetic parameters of mediated apical taurine uptake and $K_{D,u,AP}$ and $K_{D,e,AP}$, the passive diffusion constants of non-mediated taurine uptake and efflux, respectively. C_0 is apical taurine concentration and C_i the intracellular concentration achieved at C_0. Similarly, basolateral efflux is considered to be equal to basolateral efflux minus basolateral uptake:

$$J_{net,e,BL} = J_{e,BL} - J_{u,BL} = K_{D,e,BL}(C_i - C_r) - \left[\frac{V_{max,u,BL} \cdot C_r}{K_{m,u,BL} + C_r} + K_{D,u,BL}(C_r - C_i)\right] \quad (2)$$

in which $V_{max,u,BL}$ and $K_{m,u,BL}$ are the kinetic constants of mediated basolateral taurine uptake, and $K_{D,u,BL}$ and $K_{D,e,BL}$, the passive diffusion constants of non-mediated taurine uptake and efflux, respectively. C_i is the intracellular concentration attained as a result of monolayer incubation with C_0 present in the apical compartment and C_r, which stands for the receiver concentration, defined as the taurine concentration attained in the basolateral compartment at the end of the 5 min incubation period. On the basis of the assumption that passive diffusion occurs only for a downward concentration gradient, the relative values attained for C_i and C_r with respect to C_0 may lead to the simplification of Eqs. 1 and 2 (*see* Eqs. 3 and 4 in the Results section).

Results

Apical Taurine Uptake

The participation of the TauT system in taurine uptake across the apical membrane of Caco-2 cells [4, 17] was confirmed from Na^+ and Cl^- dependence and cis-inhibition experiments. Replacement

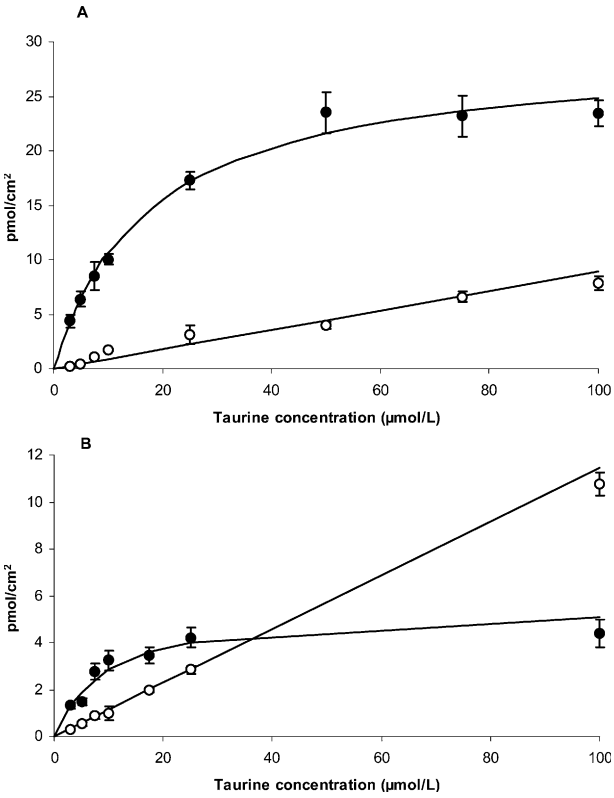

Fig. 2. Substrate concentration dependence of taurine uptake across Caco-2 cell apical (*A*) and basolateral (*B*) membrane. Monolayers were incubated for 5 min, as reported in the legend to Fig. 1, in the presence of increasing unlabeled taurine concentrations (3–100 μmol/L) in the apical or basolateral compartment. The non-mediated component (o) was estimated under Na^+- and Cl^--free conditions (NaCl replaced by KNO_3) and subtracted from total taurine uptake. The best fit for the Na^+-dependent component (•) was obtained by considering a model of a single transport system, for both apical and basolateral taurine uptake. R^2 for the non-mediated component: apical, 0.973 and basolateral 0.998. Results are expressed as mean ± SEM of n = 3–4 monolayers. Only SEM that exceed size of the symbols are shown.

of Na^+ and Cl^- significantly reduced taurine uptake in this membrane (Fig. 1*A*). Moreover, the presence of 10 mmol/L L-alanine, L-methionine, L-arginine or L-lysine on the *cis* side did not modify uptake, while the inclusion of other β-amino acids, such as β-alanine and hypotaurine, and taurine itself, significantly reduced the transport of this amino acid (Fig. 1*B*).

The kinetic parameters of apical taurine uptake were calculated from initial taurine influx rates at a range of substrate concentrations (3–100 μmol/L). The non-mediated component was estimated in Na^+- and Cl^--free conditions (NaCl replaced by KNO_3) and subtracted from total taurine uptake. The best fit for the Na^+-dependent component (Fig. 2*A*) was obtained by considering a model of a single transport system (Table 1).

Basolateral Taurine Uptake

The basolateral taurine influx was Na^+- and Cl^--dependent (Fig. 3*A*) but in the absence of Na^+, uptake was higher with KCl and choline chloride than with KNO_3 substitution. Cis-inhibition experiments (Fig. 3*B*) showed a distinct profile from apical transport, as indicated by the significant inhibition (about 40 %) of α-amino acids such as L-methionine, L-lysine and L-alanine on taurine uptake. For these experiments a lower substrate concentration was used (3 μmol/L) because the basolateral K_m for taurine is lower than that corresponding to apical uptake (*see* below). The kinetic parameters obtained for basolateral uptake of this amino acid were calculated in the same way as for the apical membrane. The best fit for the Na^+-dependent transport component (Fig. 2*B*) was obtained by considering a single transport system with a half-saturation constant and transport capacity lower than that calculated for the apical membrane (Table 1). Our results also show that the non-mediated uptake component is identical to that in the apical membrane (Table 1).

Apical and Basolateral Taurine efflux

Cell monolayers were preloaded, from the apical side for the study of basolateral efflux and from the basolateral side for apical efflux, with increasing concentrations of taurine (10–2000 μmol/L). After the preloading period (60 min), the intracellular taurine concentrations calculated indicated that the substrate was accumulated against a concentration gradient (Table 2 and abscissa of Fig. 4*A* and *B*). The higher concentration obtained with apical loading is consistent with the higher V_{max} of the apical system compared to the basolatera system (Table 1). The kinetic analysis of taurine efflux from preloaded cells shows the best fit for the two membrane domains by considering a simple diffusion component (Fig. 4*A* and *B*). Comparison of influx and efflux diffusion constants (Table 1) shows no statistical differences for the apical membrane, while in the basolateral domain efflux rates were significantly lower than influx rates.

To further study the possible contribution of a mediated mechanism in taurine efflux, trans-stimulation experiments were performed in cells preloaded (100 μmol/L) either from the basolateral or apical side and incubated in the presence of 10 mmol/L β-alanine at the apical or basolateral compartment, respectively. Our results (Fig. 5) indicate no significant effect of this treatment, thus ruling out the participation of a mediated mechanism.

Transepithelial Taurine Transport

To calculate transepithelial taurine transport from the equations shown in the Materials and Methods sec-

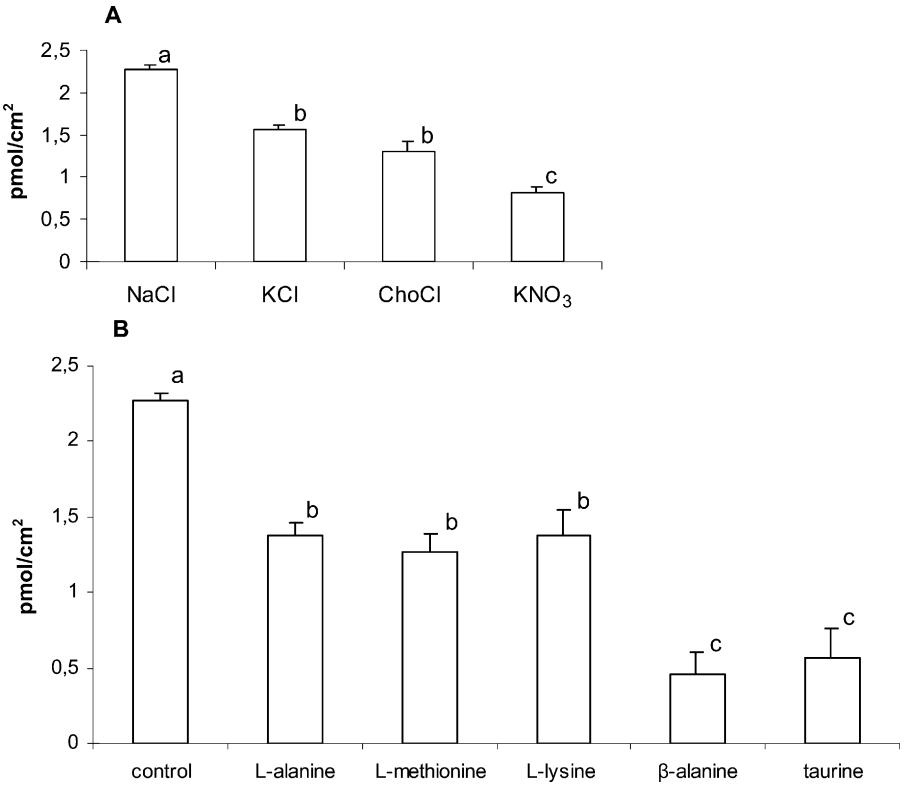

Fig. 3. Na^+-and Cl^--dependence (*A*) and cis-inhibition (*B*) of taurine uptake across Caco-2 cell basolateral membrane. Monolayers were incubated for 5 min, as reported in the legend to Fig. 1, in the presence of the substrate (3 μmol/L taurine) and equimolar concentrations of NaCl, KCl, ChoCl (choline chloride) or KNO_3 in the basolateral compartment and, for cis-inhibition experiments, in the presence of the substrate and 10 mmol/L of the unlabeled amino acid in NaCl-containing Krebs. Results are expressed as mean ± SEM of $n = 3$–4 monolayers. Mean values labeled with different letters are significantly different ($P < 0.05$).

Table 1. Kinetic constants for taurine uptake and efflux across Caco-2 cell apical (AP) and basolateral (BL) membrane

	K_m uptake (μmol/L)	V_{max} uptake (pmol·cm^{-2}·5 min^{-1})	K_D uptake (nL·cm^{-2}·5 min^{-1})	K_D efflux (nL·cm^{-2}·5 min^{-1})
AP	17.1 ± 0.8*	28.4 ± 0.8*	89.2 ± 9.98	72.7 ± 3.31*
BL	9.46 ± 0.6	5.59 ± 0.3	114 ± 10.6	50.1 ± 5.4

Kinetic constants were estimated as described in the Materials and Methods section. Results are expressed as mean ± SEM of $n = 3$ monolayers. *$P < 0.05$ for differences between apical and basolateral membrane and **$P < 0.05$ for differences between uptake and efflux K_D.

Table 2. Taurine concentration in Caco-2 cells as a result of substrate loading from the apical or basolateral compartment and taurine concentration in basolateral compartment after apical taurine incubation (receiver concentration)

C_0 (μmol/L)	C_i Apical (μmol/L)	C_i Basolateral (μmol/L)	C_r, Receiver Concentration (μmol/L)
10	75.7 ± 3.24	66.2 ± 14.1	8.05 ± 1.21
100	538 ± 56.6	223 ± 24.9*	65.5 ± 8.4
200	1018 ± 38.6	289 ± 26.5*	85.4 ± 10.9
2000	9117 ± 446	1539 ± 42.2*	912 ± 100

Intracellular taurine concentration (C_i) was measured in cells incubated for 60 min with increasing taurine concentrations (C_0) present in the apical or basolateral compartment in NaCl-containing Krebs buffer. For calculations, a mean monolayer volume of 2.14 μL was considered (*see* Materials and Methods). The receiver concentration is the taurine concentration attained in the basolateral compartment as a result of monolayer incubation for 60 min with C_0 present in the apical compartment. Results are expressed as mean ± SEM of $n = 3$ monolayers. *$P < 0.05$ between membrane domains.

tion, the intracellular (C_i) and receiver (C_r) taurine concentrations attained in the presence of a range of substrate concentrations in the apical compartment (C_0) was first considered. Our results on intracellular taurine concentrations (Table 3) indicate that after 5 min of incubation the substrate did not accumulate against a concentration gradient. Therefore, $C_0 > C_i$ and Eq. 1 of net apical uptake can be simplified to:

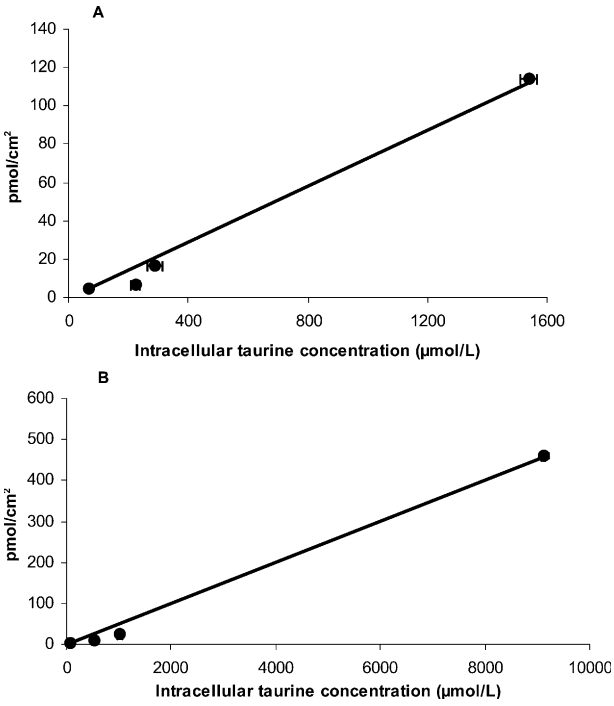

Fig. 4. Substrate concentration dependence of taurine efflux across Caco-2 cell apical (*A*) and basolateral (*B*) membrane. Cell monolayers were preloaded for 60 min at 37°C with increasing taurine concentrations (10–2000 µmol/L) in NaCl-containing Krebs buffer. For the study of apical efflux, cells were preloaded from the basolateral side and for the basolateral efflux experiments from the apical side. Intracellular taurine concentrations attained (abscissa and Table 2) were measured in three monolayers for each substrate concentration, processed as in uptake experiments and are expressed as mean ± SEM. After the loading period, the substrate was allowed to efflux from the cells at 37°C for 5 min. The amount of taurine released was quantified by measuring radioactivity in aliquots from apical or basolateral media. The best fit was obtained for the two membrane domains by considering a simple diffusion component (apical and basolateral, $R^2 = 0.99$). Efflux results are expressed as mean ± SEM of $n = 3$ monolayers. Only SEM that exceed the size of symbols are shown.

$$J_{\text{net,u,AP}} = J_{\text{u,AP}} - J_{\text{e,AP}} = \frac{V_{\text{max,u,AP}} \cdot C_0}{K_{\text{m,u,AP}} + C_0} + K_{\text{D,u,AP}}(C_0 - C_i) \quad (3)$$

Our results on the basolateral domain indicate that substrate concentrations reached in the basolateral compartment (C_r) as a result of 5 min monolayer incubation in the presence of a range of apical substrate concentrations (C_0) were very low (in the nmol/L range, Table 3) thus, $C_i > C_r$ and Eq. 2 of net basolateral uptake can be simplified to:

$$J_{\text{net,u,AP}} = J_{\text{e,BL}} - J_{\text{u,BL}} = K_{\text{D,e,AP}}(C_i - C_r) - \frac{V_{\text{max,u,BL}} \cdot C_r}{K_{\text{m,u,BL}} + C_r} \quad (4)$$

The results of net apical uptake and basolateral efflux calculated from Eqs. 3 and 4, taking into account the kinetic parameters of Table 1 and C_i and C_r values of Table 3, are also shown in Table 3. Our results also show that the estimated net uptake rate is always higher than net efflux rate. The apical-to-basolateral taurine transport (receiver concentration) in steady-state condition was also determined from apical preloading experiments (60 min incubation). Higher values than for a 5 min incubation (Table 2 versus Table 3) were observed, as expected.

Discussion

We have shown that taurine is accumulated by cultured intestinal epithelial cells as a result of active transport mechanisms in apical and basolateral membranes. Taurine uptake across the apical membrane of our Caco-2 preparation has the characteristics of the TauT system in the small intestine and other tissues [4, 9, 12, 17]. This system shows Na^+- and Cl^--dependence, specificity for β-amino acids and a half-saturation constant in the micromolar range [4, 17, 23]. The V_{max}, normalized to mg protein (0.39 mg protein·cm^{-2}) was similar to the values reported by Brandsch et al. [4] and Satsu, Miyamoto and Shimizu [18].

The basolateral-mediated taurine uptake (ionic dependence, substrate specificity and half-saturation constant) has properties similar to those described for the apical TauT system and also for the basolateral β-amino acid transporter of the renal epithelium [3]. However, apical and basolateral taurine uptake in Caco-2 cells differ. Firstly, differences in basolateral taurine uptake in the presence of KCl, choline chloride or KNO_3 may reflect a lower Na^+ dependence compared to that in the apical system. In the renal epithelium, basolateral uptake of this amino acid has lower Na^+ requirements than apical uptake, with a Na^+: taurine stoichiometry of 1:1 in the basolateral membrane and 3:1 in the apical membrane [3]. Secondly, the *cis*-inhibition profile of basolateral taurine uptake by α-amino acids differs, which may be attributed to distinct specificity with respect to apical transporter, to a higher sensitivity to competition with neutral amino acids for the Na^+-gradient or to depolarization induced by cationic amino acid transport. A similar inhibition profile has been described for taurine transport in the rat intestine, which was explained by competition for the Na^+-gradient and not by the taurine carrier [12].

Non-mediated taurine uptake across the apical membrane was very low (only 2% and 13% of total flux for taurine concentrations of 3 and 50 µmol/L respectively), which is consistent with the results described in intestinal HT-29 cells [4]. The kinetics of taurine efflux from the cells and trans-stimulation experiments indicate that this amino acid leaves the

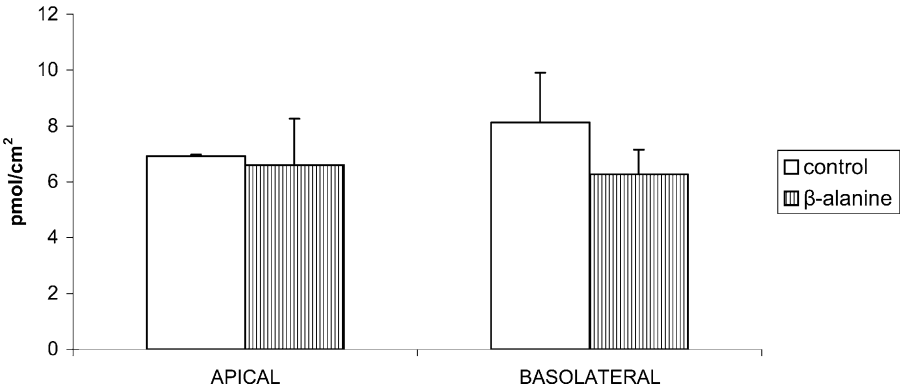

Fig. 5. Trans-stimulation of taurine efflux across Caco-2 cell apical and basolateral membrane. Cell monolayers were loaded, as reported in the legend to Fig. 4, with 100 μmol/L taurine from either the basolateral or apical side for apical or basolateral efflux experiments, respectively. After the loading period, the substrate was allowed to efflux from the cells at 37 °C for 5 min in the absence or in the presence of 10 mmol/L β-alanine at the apical or basolateral compartment, respectively. The amount of taurine released was quantified by measuring radioactivity in aliquots from apical or basolateral media. Results are expressed as mean ± SEM, $n = 3$ monolayers. $^*P < 0.05$ versus control cells.

Table 3. Intracellular and receiver taurine concentrations and estimated net apical taurine influx and efflux rates

C_0 (μmol/L)	C_i (μmol/L)	C_r (nmol/L)	$J_{net,u,AP}$ (pmol·cm^{-2}·5 min^{-1})	$J_{net,e,BL}$ (pmol·cm^{-2}·5 min^{-1})
3	1.77 ± 0.16	0.53 ± 0.08	4.48	0.09
5	3.60 ± 0.35	0.61 ± 0.09	6.71	0.18
7.5	5.08 ± 0.68	1.00 ± 0.18	9.10	0.25
10	6.20 ± 0.25	2.03 ± 0.38	11.1	0.31
50	14.5 ± 0.97	13.4 ± 1.8	25.0	0.72
100	20.6 ± 2.37	20.6 ± 2.5	32.2	1.02

Intracellular taurine concentration (C_i) was measured in cells incubated for 5 min with increasing taurine concentrations (C_0) present in the apical compartment in NaCl-containing Krebs buffer. For calculations, a mean monolayer volume of 2.14 μL was considered (*see* Materials and Methods). C_r is the receiver concentration, i.e., the concentration of taurine attained in the basolateral compartment as a result of monolayer incubation during 5 min with C_0 present in the apical compartment. Results are expressed as mean ± SEM of $n = 3$ monolayers. Net apical uptake ($J_{net,u,AP}$) and net basolateral efflux ($J_{net,e,BL}$) were calculated from Eqs. 3 and 4 (*see* Results section).

cell by a non-mediated process. Trans-stimulation experiments were performed with β-alanine on the trans-side because both amino acids share several transport mechanisms in different tissues [14]. Our results also reveal that the basolateral membrane has a lower permeability for taurine than the apical domain. In reptilian renal epithelium, taurine efflux across the apical membrane is also non-mediated, whereas basolateral efflux is stimulated by extracellular taurine [3]. In Caco-2 cells, other amino acids such as L-methionine and L-phenylalanine also flow out across the basolateral membrane via non-mediated mechanisms [5, 6].

The calculations of transepithelial taurine transport show that the net flux across the apical membrane is 50-fold higher than basolateral efflux for the 3 μmol/L apical substrate concentration and around 35-fold for the other concentrations. This observation indicates that, in isosmotic conditions, the limiting factor for taurine efflux is the diffusion across the basolateral membrane. Apical uptake of taurine occurs mainly through TauT (78–98%) in a wide range of concentrations. In contrast, uptake through the basolateral membrane is low since less than 1% of the taurine leaving the cell is recovered by mediated transport. Therefore, the net taurine efflux is determined by the passive movement of the substrate across the basolateral membrane. In reptilian renal cells, the highest basolateral permeability to taurine results in net transepithelial reabsorption [3]. Similarly, Chen et al. [5] and Hu and Borchardt [6] observed in Caco-2 cells that the K_D for the basolateral membrane for L-methionine and L-phenylalanine was higher than the K_D for the apical membrane, thus favoring transepithelial fluxes in terms of net substrate absorption. However, in our model, the low permeability of the basolateral membrane to taurine efflux in isosmotic conditions would favor the maintenance of the high intracellular taurine concentration, as reported for the human duodenal mucosa [1].

The high capacity of the cells to accumulate the substrate was also observed in steady-state condition.

After a 60 min incubation, the cells maintained an intracellular taurine concentration around 10 times higher than that observed in the basolateral compartment (receiver concentration). These results further support the contribution of the mediated apical influx and low basolateral efflux to the maintenance of high intracellular concentrations during long incubation periods. The high taurine concentration attained by the mucosa indicates that this amino acid is involved in functions in the intestinal wall, such as the protection of the epithelium from lipid peroxidation [16].

In renal epithelium, taurine transport participates in various physiological processes [8]. In proximal tubular cells, taurine is efficiently reabsorbed because of the high capacity of the apical transporter, while distal tubular cells have a high basolateral uptake capacity for taurine accumulation from the interstitial compartment, indicating that in this region taurine may be involved in the regulation of cell volume in response to hyperosmotic stress. In contrast, intestinal Caco-2 cells respond to high external osmolarity by increasing the activity of the apical TauT system [18]. These results indicate a distinct regulatory mechanism of taurine transport in response to osmotic stress in the intestine when compared to the kidney. Given that adaptation to hyposmotic conditions is mediated by an increase in basolateral taurine efflux in cultured A6 kidney cells [19], a role of this pathway in the regulation of cellular volume in the intestinal epithelium cannot be ruled out. In conclusion, a high mucosal concentration of taurine may contribute to cell physiology, thereby preventing deviation from homeostasis induced by a luminal environment with marked changes during both digestive and interdigestive periods.

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