Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta

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A cDNA clone highly related to the rat brain taurine transporter has been isolated from a human placental cDNA library. Transfection of this cDNA into HeLa cells results in a marked elevation of taurine transport activity. The activity of the cDNA-induced transporter is dependent on the presence of Na^+ as well as Cl^-. The Na^+/Cl^-/taurine stoichiometry for the cloned transporter is 2:1:1. The transporter is specific for taurine and other β-amino acids, including β-alanine, and exhibits high affinity for taurine (Michaelis–Menten constant $\approx 6\ \mu M$). The clone consists of a coding region 1863 bp long (including the termination codon), flanked by a 376 bp-long 5′ non-coding region and a 625 bp-long 3′ non-coding region. The nucleotide sequence of the coding region predicts a 620-amino acid protein with a calculated M_r of 69853. Northern-blot analysis of poly(A)$^+$ RNA from several human tissues indicates a complex expression pattern differing across tissues. The principal transcript, 6.9 kb in size, is expressed abundantly in placenta and skeletal muscle, at intermediate levels in heart, brain, lung, kidney and pancreas and at low levels in liver. Cultured human cell lines derived from placenta (JAR and BeWo), intestine (HT-29), cervix (HeLa) and retinal pigment epithelium (HRPE), which are known to possess Na^+- and Cl^--coupled taurine transport activity, also contain the 6.9 kb transcript. Somatic cell hybrid and *in situ* hybridization studies indicate that the cloned taurine transporter is localized to human chromosome 3 p24→p26.

INTRODUCTION

Taurine is a β-amino acid (2-aminoethanesulphonic acid) which is essential in fetal nutrition and development and is present in abundant quantities in several tissues in the fetus [1–3]. However, the capability of the developing fetus for endogenous biosynthesis of taurine is extremely low in many animals, including man [4,5]. Thus transplacental transfer of taurine from the mother is the primary source of this important amino acid in the fetus. We demonstrated a few years ago that the maternal-facing brush-border membrane of the human placental syncytiotrophoblast possesses an active high-affinity transport system for taurine [6,7], an observation confirmed in other laboratories [8–10]. The placental brush-border membrane taurine transporter specifically recognizes taurine and other β-amino acids and its catalytic activity is dependent on Na^+ as well as Cl^-. The Na^+/Cl^-/taurine stoichiometry for this transporter has been shown to be 2:1:1 [7,10,11]. We have also shown that the human placental choriocarcinoma cell line JAR expresses the taurine transporter [12]. This placental cell line has proved to be very useful in studies involving regulation of the activity of the placental taurine transporter by intracellular second messengers. The taurine transporter in JAR cells is inhibited by activation of protein kinase C [12] and by calmodulin antagonists [13] but is unaffected by agents that elevate cyclic AMP levels [14,15].

The taurine transporter has been recently cloned from MDCK (Madin–Darby canine kidney) cells [16] and from rat brain [17], mouse brain [18] and human FRTL-5 thyroid cells [19]. A comparison of the nucleotide sequences of the taurine transporter cDNAs with those of the other cloned transporters indicates that the taurine transporter belongs to a gene family that encodes Na^+- and Cl^--coupled transporters [20–22]. The substrates of this family of transporters include taurine, betaine, glycine, γ-aminobutyrate (GABA), proline, 5-hydroxytryptamine, dopamine and noradrenaline. Degenerate oligonucleotides encoding highly conserved sequences of two of these transporters, namely the GABA transporter and the noradrenaline transporter, have been useful in the identification and subsequent cloning of several members of this transporter family [20,21,23]. Recently, we employed this strategy successfully to isolate the cDNA encoding the human 5-hydroxytryptamine-transporter from a placental cDNA library [24]. Here we report the use of a similar approach in the cloning of the cDNA encoding a taurine transporter from the human placenta.

MATERIALS AND METHODS

Materials

[2-^{3}H]Taurine (specific radioactivity 25.6 Ci/mmol), β-[3-^{3}H]-alanine (specific radioactivity 92.6 Ci/mmol), [γ-^{32}P]ATP, [α-^{32}P]dCTP, [α-^{35}S]dATP, [^{3}H]dATP and [^{3}H]dCTP were purchased from DuPont–New England Nuclear (Boston, MA, U.S.A.). The human placental choriocarcinoma cell lines (JAR and BeWo), the human colon carcinoma cell line HT-29 and the human cervical carcinoma cell line HeLa were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The human retinal pigment epithelial cell line (HRPE) was kindly provided by M.A. Del Monte, W.K. Kellogg Eye Center,

Abbreviations used: GABA, γ-aminobutyrate; hTAUT, human taurine transporter; poly(A)$^+$, polyadenylated; 1 × SSPE, 0.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA.

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Ann Arbor, MI, U.S.A. The human placental cDNA library was generously provided by Albert S. Chang, Baylor College of Medicine (The Woodlands, TX, U.S.A.).

RNA isolation and PCR

Poly(A)$^+$ RNA isolated from JAR human placental choriocarcinoma cells by the guanidinium isothiocyanate/caesium chloride method [25] was reverse-transcribed using the SuperScript Preamplification System (Gibco–BRL) according to the manufacturer's protocol. The resulting cDNA was amplified by PCR (30 cycles of 94 °C, 1 min; 42 °C, 2 min; 72 °C, 3 min, with 10 min extension time on cycles 1 and 30) with *Taq* polymerase (Promega). The following degenerate oligonucleotides were used as primers in the amplification: 5′-CCGCTCGAGAA(C/T)GT(G/C)TGGCG(G/C)TT(C/T)CC(A/G/C/T)TA-3′ (upstream primer) and 5′-GCTCTAGAGCTG(A/G)GTIGC(A/G)GC(A/G)TC(A/G)A(T/G)CCA-3′ (downstream primer). These primers were designed to encode highly conserved amino acid sequences near the transmembrane domains I (NVWRFPY) and VI (WIDAATQ) of GABA [26] and noradrenaline [27] transporters and the underlined sequences indicate addition of 5′ restriction sites for cloning.

cDNA cloning and sequencing

The PCR products were digested with *Xba*I and *Xho*I, gel-purified and ligated into *Xba*I–*Xho*I-digested pBluescript SKII$^-$ (Stratagene). After partial sequencing of the individual plasmid clones, a single clone was identified which had a cDNA insert encoding an amino acid sequence similar to that of the rat brain taurine transporter [17]. A synthetic 21-mer (5′-GAGGGACACAATTACAACGGA-3′) derived from this cDNA insert was 3′-end-labelled with [γ-^{32}P]ATP and polynucleotide kinase [28] and used to screen a human placental cDNA library in λZAPII (Stratagene). Screening was by MagnaGraph (Micron Separations, Westboro, MA, U.S.A.) filter hybridization at 57 °C according to the manufacturer's instructions, but with the use of heparin sulphate (0.5 mg/ml) to prevent non-specific hybridization. Sequencing of the sense and antisense cDNA strands was by the dideoxy chain-termination method, using the Sequenase 2.0 kit (U.S. Biochemicals).

Transfection and uptake measurement in HeLa cells

The reconstituted cDNA in the vector pBluescript SK(−) contains the start site codon 3′ to the plasmid-encoded T7 RNA polymerase promoter. This plasmid (1 μg) was introduced into HeLa cells (100000–200000 cells per well of a 24-well plate) by liposome-mediated transfection (Lipofectin; Gibco–BRL). The HeLa cells had been previously infected with recombinant vaccinia virus (VTF$_{7-3}$) encoding T7 RNA polymerase, as described previously [24,29]. Uptake measurements were performed 12 h after transfection by incubating the cells for the desired time at 37 °C with [^{3}H]taurine or β-[^{3}H]alanine in 0.5 ml of Krebs/Ringer/Hepes (KRH) buffer (10 mM Hepes/Tris, 120 mM NaCl, 4.7 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 10 mM D-glucose, pH 7.4). Basal uptake activity in these cells was assessed in parallel transfections with the plasmid vector. Na^+-dependence of uptake was determined by using KRH buffer in which NaCl was iso-osmotically replaced by choline chloride. Cl^--dependence of uptake was assessed by using KRH buffer in which the chloride salts were replaced iso-osmotically by sodium gluconate, potassium gluconate and calcium gluconate. The number of Na^+ and Cl^- ions involved in the transport of one taurine molecule was determined by the 'activation' method [30]. After incubation, uptake was terminated by addition of 1 ml of ice-cold KRH buffer and the cells were washed twice with the same buffer. After this step, the cells were solubilized with 0.5 ml of 1% SDS, and the radioactivity associated with the cells was determined by liquid-scintillation counting.

Northern-blot analysis

Poly(A)$^+$ RNA, isolated from cultured human cell lines (JAR, BeWo, HT-29 and HRPE) using the FastTrack mRNA isolation kit (Invitrogen), was size-fractionated on a denaturing formaldehyde–agarose gel and transferred to a nylon membrane (Hybond N$^+$; Amersham). Total RNA was used in the case of HeLa cells. A hybridization-ready blot containing poly(A)$^+$ RNA from multiple human tissues was obtained from Clontech (human MTN blot). The PCR cDNA fragment (705 bp) corresponding to the nucleotide sequences 563–1267 of the full-length cDNA was radiolabelled with [α-^{32}P]dCTP using the oligolabelling kit (Pharmacia) and employed as a probe in Northern-blot analysis of the RNA blots. The blots were prehybridized at 42 °C for 12 h in solution containing 50% formamide, 5× SSPE, 10× Denhardt's solution, 2% SDS and 100 μg/ml freshly denatured sheared salmon sperm DNA. The cDNA probe was then added and hybridization continued for 24 h. The blots were rinsed with 2× SSPE and 0.05% SDS twice at room temperature (22 °C), followed by a high-stringency wash (0.1× SSPE, 0.1% SDS, 65 °C, 1 h) and then exposed to autoradiographic film. To ensure the presence of RNA in each lane after loading and transfer, the same blots were used for Northern-blot analysis of the β-actin mRNA which was performed by stripping of the blots followed by rehybridization with random-primed human β-actin cDNA (Clontech).

Chromosomal localization

Chromosomal localization of the gene for the cloned taurine transporter was obtained by somatic-cell hybrid analysis as well as by *in situ* hybridization to human metaphase and prometaphase chromosomes. Mapping panel no. 1 consisting of mouse–human and Chinese hamster–human hybrids was obtained from the National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository and used in somatic-cell hybrid analysis. Southern-blot hybridization to restriction fragments of DNA derived from these hybrid cells was performed as described previously [31]. *In situ* hybridization to human chromosomes and emulsion autoradiography were carried out by the method of Harper and Saunders [32]. Chromosomes were G-banded using Wright's stain, and G-banded chromosomes were analysed for silver grain localization. Two different cDNA probes, one corresponding to the full-length human placental taurine transporter (hTAUT) cDNA (2864 bp long) and the other corresponding to a 612 bp-long sequence in the 3′ non-coding region of the hTAUT cDNA were labelled by nick-translation either in the presence of [^{32}P]dCTP (Southern-blot hybridization) or in the presence of [^{3}H]dATP and [^{3}H]dCTP (*in situ* hybridization) and used in these experiments.

Data analysis

The experiments were carried out in triplicate and the results are expressed as means ± S.E.M. Computer analysis of experimental data by linear and non-linear regression methods was performed using the *Fig. P* 6.0 program (Biosoft, Cambridge, U.K.).

RESULTS AND DISCUSSION

Cloning of the hTAUT cDNA

Screening of a human placental cDNA library (λZAPII) with a synthetic 21-mer probe specific for the JAR cell taurine transporter identified two hybridizing clones which, after plaque rescreening, were obtained as individual plasmids by *in vivo* excision. Sequencing and restriction analysis of these two clones revealed that they were homologous to each other. However, neither of these clones represented the full-length cDNA. One of the clones contained the start site but lacked sequences at the 3′ end encompassing the termination site, whereas the other clone contained the termination site but lacked sequences at the 5′ end encompassing the start site. The two clones exhibited identical overlapping sequences. A full-length cDNA with start and termination sites was constructed from these two partial clones after digestion of the cDNAs with *Kpn*I and ligation of the appropriate fragments. The resulting cDNA (hTAUT) was sequenced at the ligation site to select the clone with correct orientation.

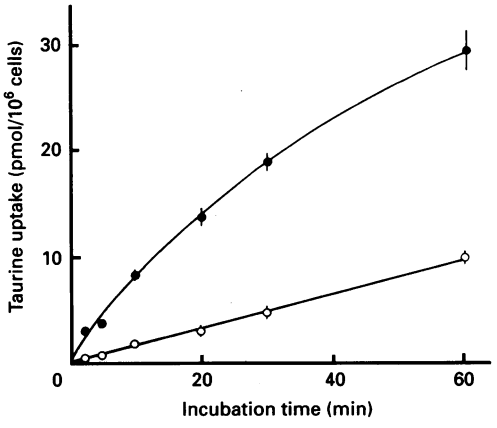

Figure 1 Uptake of taurine in HeLa cells transfected with pBluescript SK(−) vector alone or with hTAUT cDNA

Cells were transfected with either vector alone (○) or with vector carrying the hTAUT cDNA insert (●). Uptake of taurine (50 nM) was determined in these transfected cells in uptake medium containing NaCl.

Identity of the clone as a Na^+- and Cl^--coupled taurine transporter

To demonstrate that the reconstructed full-length cDNA clone indeed represents a Na^+- and Cl^--coupled taurine transporter, the cDNA, oriented for sense transcription under the control of the T7 promoter in pBluescript SK(−), was introduced into HeLa cells that had been infected with a recombinant vaccinia virus to express T7 RNA polymerase. Control experiments were carried out in a similar manner by transfecting with the plasmid vector alone. HeLe cells transfected with the vector lacking the cDNA insert showed time-dependent accumulation of taurine (Figure 1), indicating that these cells possess endogenous taurine-transport activity. Introduction of the hTAUT cDNA into the cells resulted in a marked increase in the taurine-transport activity (Figure 1). The initial uptake rate in cDNA-transfected cells was 4–6-fold greater than the rate in vector-transfected cells.

Table 1 describes the Na^+- and Cl^--dependence of taurine and β-alanine uptake in control and hTAUT cDNA-transfected HeLa cells. Uptake of both substrates from a Na^+-free medium (choline chloride) or from a Cl^--free medium (sodium gluconate) was negligible compared with uptake from an NaCl-containing medium. This requirement for Na^+ and Cl^- was seen for the endogenous transport activity as well as for the transport activity induced by the hTAUT cDNA.

The Na^+ and Cl^- stoichiometry for taurine uptake in control and hTAUT cDNA-transfected HeLa cells was determined. To determine the Na^+ stoichiometry, the uptake rates of taurine at a fixed concentration (100 nM) in control and hTAUT cDNA-transfected cells were measured at various concentrations of Na^+ (10–140 mM) but at a constant Cl^- concentration (140 mM). The relationship between the uptake rate and the Na^+ concentration was found to be sigmoidal (Figure 2a), suggesting involvement of more than one Na^+ ion per transport of one taurine molecule. This sigmoidal relationship was evident for the taurine uptake catalysed by the endogenous taurine transporter of the HeLa cells as well as for that measured in HeLa cells transfected with hTAUT cDNA. To calculate the number of Na^+ ions involved per transport cycle, the experimental data were analysed according to the Hill-type equation:

$$v = \frac{V_{max.}[Na^+]^n}{K_{0.5}^n + [Na^+]^n}$$

where v is the uptake rate, $K_{0.5}$ is the Na^+ concentration necessary for half-maximal activation, $V_{max.}$ is the uptake rate maximally activatable by Na^+ and n is the Hill coefficient (i.e. the number of

Table 1 Na^+- and Cl^--dependence of taurine uptake and β-alanine uptake in HeLa cells transfected with either pBluescript SK(−) or hTAUT cDNA

Cells were transfected with either pBluescript SK(−) or hTAUT cDNA as described in the Materials and methods section. Uptake of [^{3}H]taurine (50 nM) and β-[^{3}H]alaline (20 nM) were measured over a 10 min incubation period. Three different uptake media were used: KRH buffer (NaCl), KRH buffer in which NaCl was replaced iso-osmotically with choline chloride (choline chloride), and a modified KRH buffer in which NaCl, KCl and $CaCl_2$ were replaced iso-osmotically with respective gluconate salts (sodium gluconate). Values in parentheses are percentage uptake compared with the control (100%) measured in NaCl-containing uptake medium.

	Uptake (pmol/10 min per 10^6 cells)			
	Taurine		β-Alanine	
Uptake medium	pBluescript SK(−)	hTAUT cDNA	pBluescript SK(−)	hTAUT cDNA
NaCl	2.10 ± 0.13 (100)	8.53 ± 0.48 (100)	0.41 ± 0.03 (100)	1.39 ± 0.06 (100)
Choline chloride	0.11 ± 0.01 (5)	0.11 ± 0.01 (1)	0.05 ± 0.01 (12)	0.05 ± 0.01 (4)
Sodium gluconate	0.27 ± 0.17 (13)	0.09 ± 0.01 (1)	0.04 ± 0.01 (10)	0.04 ± 0.01 (3)

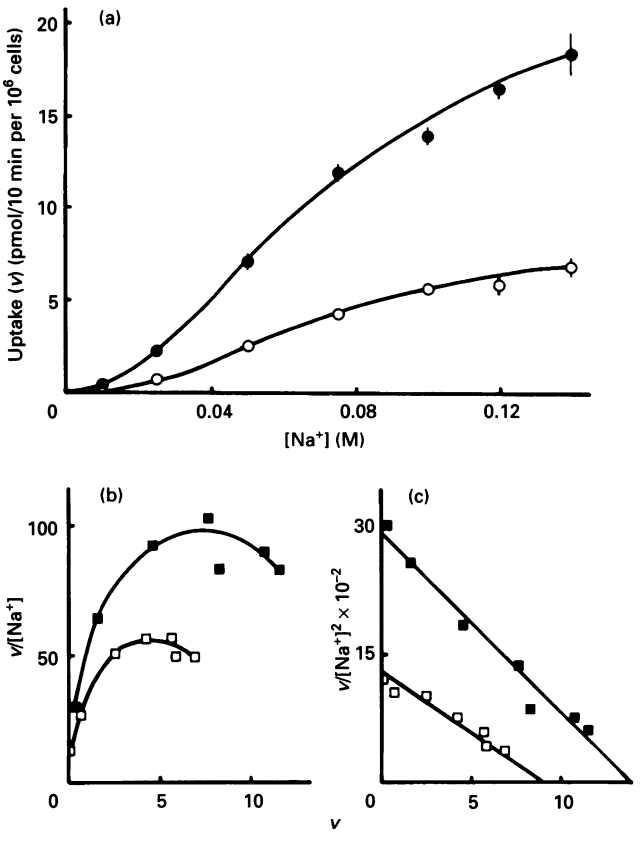

Figure 2 Dependence of taurine uptake on [Na^+] in HeLa cells transfected with pBluescript SK(−) vector alone or with hTAUT cDNA

Cells were transfected with either vector alone (○) or with vector carrying the hTAUT cDNA insert (●). Uptake of taurine (100 nM) was determined in these transfected cells over a 10 min incubation period in uptake media containing various concentrations of Na^+ (10–140 mM). Osmolality and concentration of Cl^- (140 mM) were maintained by appropriately substituting LiCl for NaCl. Uptake measured in the absence of Na^+ was subtracted from each uptake value to determine the Na^+-dependent component, which is plotted against Na^+ concentration (**a**). (**b**) and (**c**) Hill-type plots (v versus $v/[Na^+]^n$) with $n = 1$ and 2 respectively for the endogenous taurine transporter [(taurine uptake in HeLa cells transfected with vector alone (□)] and for the taurine transporter induced by hTAUT cDNA [taurine uptake in cells transfected with hTAUT cDNA minus that in cells transfected with vector lone (■)].

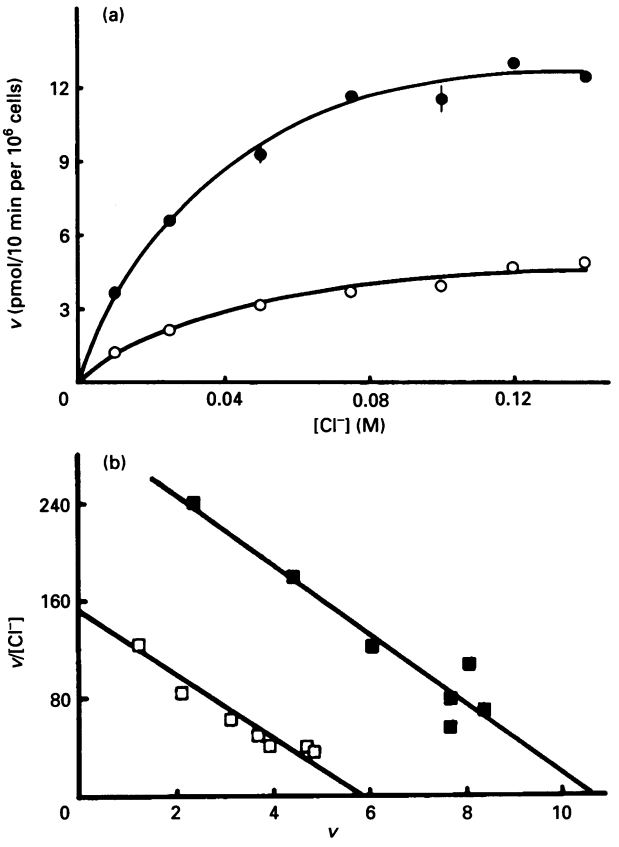

Figure 3 Dependence of taurine uptake on [Cl^-] in HeLa cells transfected with pBluescript SK(−) vector alone or with hTAUT cDNA

Cells were transfected with either vector alone (○) or with vector carrying the hTAUT cDNA insert (●). Uptake of taurine (100 nM) was determined in these transfected cells over a 10 min incubation period in uptake media containing various concentrations of Cl^- (10–140 mM). Osmolality and concentration of Na^+ (140 mM) were maintained by appropriately substituting sodium gluconate for NaCl. Uptake measured in the absence of Cl^- was subtracted from each uptake value to determine the Cl^--dependent component, which is plotted against Cl^- concentration (**a**). (**b**) Hill-type plot (v versus $v/[Cl^-]$ for the endogenous taurine transporter [taurine uptake in HeLa cells transfected with vector alone (□)] and for the taurine transporter induced by hTAUT cDNA [taurine uptake in cells transfected with hTAUT cDNA minus that in cells transfected with vector alone (■)].

Na^+ ions involved per transport cycle). This analysis was performed individually for the endogenous taurine transporter and for the taurine transporter encoded by the hTAUT cDNA, the activity of which was determined by subtracting the taurine uptake measured in control cells from that measured in hTAUT cDNA-transfected cells. For both transporters, the Hill-type plot was not linear when n was assigned a value of 1 (v versus $v/[Na^+]$; Figure 2b), but became linear ($r^2 > 0.99$) when n was assigned a value of 2 (v versus $v/[Na^+]^2$; Figure 2c). Thus the Na^+/taurine coupling ratio was 2:1 for the HeLa cell taurine transporter as well as for the cloned placental taurine transporter.

Similar experiments were carried out to determine the Cl^-/taurine coupling ratio. The uptake rates of taurine at a concentration of 100 nM were measured at various concentrations of Cl^- (10–140 mM) but at a fixed concentration of Na^+ (140 mM). In contrast with the activation by Na^+, the relationship between the uptake rate and the Cl^- concentration was hyperbolic for taurine uptake in control cells as well as in cells transfected with hTAUT cDNA (Figure 3a), suggesting involvement of one Cl^- ion per transport cycle in both cases. When the experimental data were analysed individually for the endogenous taurine transporter and for the taurine transporter induced by transfection with hTAUT cDNA (i.e. uptake in hTAUT cDNA-transfected cells minus uptake in control cells), the Hill-type plot was linear ($r^2 > 0.98$) when n was assigned a value of 1 (v versus $v/[Cl^-]$; Figure 3b). This suggests that the Cl^-/taurine coupling ratio was 1:1 for the HeLa cell taurine transporter as well as for the cloned placental taurine transporter.

Table 2 provides information on the substrate specificity of the endogenous taurine transporter and also of the hTAUT cDNA-induced taurine transporter. Uptake of [^{3}H]taurine in control as well as in cDNA-transfected cells was effectively blocked by unlabelled taurine, hypotaurine and β-alanine. GABA and α-alanine showed considerable inhibition but to a substantially smaller extent than the β-amino acids. Proline and leucine inhibited the uptake of [^{3}H]taurine only to a small extent. These data demonstrate that the taurine transporter induced by the hTAUT cDNA is specific for β-amino acids as has been shown in the case of the taurine transporter in human placental brush-

Table 2 Effects of unlabelled amino acids on [^{3}H]taurine uptake in HeLa cells transfected with either pBluescript SK(−) or hTAUT cDNA

Cells were transfected with either pBluescript SK(−) or hTAUT cDNA as described in the Materials and methods section. Uptake of [^{3}H]taurine (50 nM) was measured over a 10 min incubation period in the presence of NaCl (KRH buffer). The concentration of unlabelled amino acids was 250 μM. The values in parentheses are percentage uptake compared with the control (no unlabelled amino acid added).

Unlabelled amino acid	[^{3}H]Taurine uptake (pmol/10 min per 10^6 cells)			
	pBluescript SK(−)		hTAUT cDNA	
None	1.90±0.07	(100)	9.88±0.90	(100)
Taurine	0.09±0.01	(5)	0.33±0.01	(3)
Hypotaurine	0.08±0.01	(4)	0.29±0.01	(3)
β-Alanine	0.17±0.01	(9)	0.77±0.09	(8)
GABA	0.96±0.05	(51)	4.74±0.45	(48)
α-Alanine	1.14±0.12	(60)	6.52±0.24	(66)
Proline	1.39±0.16	(73)	7.24±0.16	(73)
Leucine	1.94±0.29	(102)	8.92±1.33	(90)

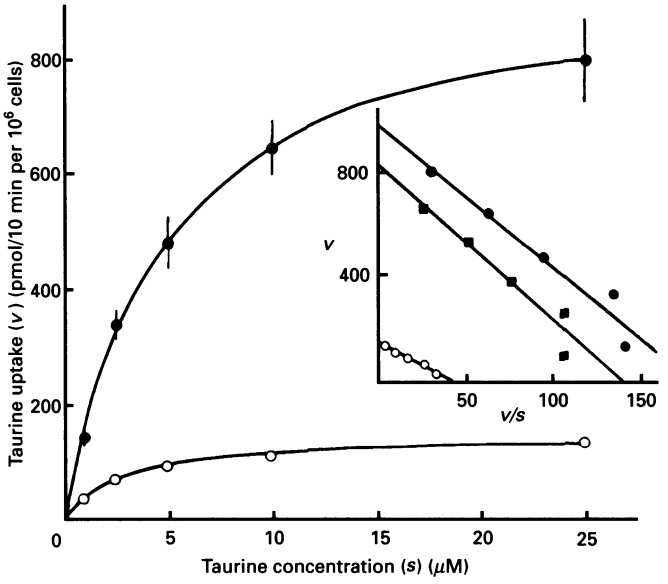

Figure 4 Kinetics of taurine uptake in HeLa cells transfected with pBluescript SK(−) vector alone or with hTAUT cDNA

Cells were transfected with either vector alone (○) or with vector carrying the hTAUT cDNA insert (●). Uptake of taurine was determined in these transfected cells in an uptake medium containing NaCl over a 10 min incubation period. The concentration of taurine was varied over the range 1–25 μM. At all concentrations, [^{3}H]taurine was kept constant at 0.1 μM. Non-saturable uptake was determined from the uptake of radiolabel measured in the presence of 1 mM unlabelled taurine and this value was subtracted from total uptake to calculate saturable mediated uptake. Inset: Eadie–Hofstee plot of the data for uptake in cells transfected with vector (○) or with hTAUT cDNA (●). At each concentration of taurine, uptake in vector-transfected cells was subtracted from uptake in hTAUT cDNA-transfected cells to determine the kinetic constants for the hTAUT cDNA-induced transport activity (■).

border membrane vesicles [6,9] and in JAR cells [12]. The endogenously expressed taurine transporter also showed similar substrate specificity.

The endogenous taurine transporter and the hTAUT cDNA-induced taurine transporter exhibit saturation kinetics (Figure 4). Eadie–Hofstee transformation of the experimental data (Figure 4, inset) showed that, for the endogenous taurine transporter, the apparent Michaelis–Menten constant (K_t) was 3.4±0.3 μM and the maximal velocity ($V_{max.}$) was 158±6 pmol/10 min per 10^6 cells. The corresponding kinetic constants for the taurine transport activity in hTAUT cDNA-transfected cells were 5.4±0.7 μM and 990±74 pmol/10 min per 10^6 cells. Kinetic constants were also determined for the cDNA-induced taurine transporter after subtraction of the taurine-transport activity measured in control cells from that measured in cDNA-transfected cells. The K_t was 5.9±1.0 μM and the $V_{max.}$ was 835±78 pmol/10 min per 10^6 cells. This K_t value is comparable with those reported for the human placental taurine transporter [6,9]. HeLa cells are also of human origin and therefore it is highly likely that the endogenous taurine transporter expressed in these cells is very similar to or identical with the transporter induced by the hTAUT cDNA. This is supported by the fact that the two transporters exhibit similar characteristics. Taken collectively, these results clearly show that the cloned cDNA (hTAUT) encodes a Na^+- and Cl^--coupled taurine transporter.

Primary structure of hTAUT

The cloned hTAUT cDNA (Figure 5) is 2864 bp long, with a coding region consisting of 1860 bp (corresponding to nucleotide positions 377–2236). This cDNA can encode a protein of 620 amino acids with an estimated M_r of 69853. The calculated isoelectric pH for hTAUT is 8.3. The 5′ non-coding region consists of 376 bp and the 3′ non-coding region 625 bp. Hydropathy analysis [33] showed that the protein possesses 12 putative transmembrane domains and a large hydrophilic loop between transmembrane domains 3 and 4. This loop contains three potential N-glycosylation sites. This membrane topology is similar to that of the other members of the Na^+- and Cl^--coupled transporter gene family [20–22]. When modelled in the same way as other members of the family, the N- and C-termini of the hTAUT are found to be intracellular and the N-glycosylation site-bearing loop extracellular. In addition to these three glycosylation sites on the loop between transmembrane domains 3 and 4, there is a fourth N-glycosylation site on the extracellular loop between transmembrane domains 11 and 12. The hTAUT amino acid sequence exhibits more than 80 % identity with the sequences of the canine, rat and mouse taurine transporters and is almost identical with the sequence for the taurine transporter from human FRTL-5 thyroid cells. The N-glycosylation site on the loop between the 11th and 12th transmembrane domains is unique to the human taurine transporter because the loop between the transmembrane domains 3 and 4 is the only segment bearing N-glycosylation sites in taurine transporters cloned from non-human species. hTAUT possesses five recognition sites for cyclic AMP-dependent protein phosphorylation and six recognition sites for protein kinase C-dependent phosphorylation [34]. Interestingly, studies from our laboratory have demonstrated that the taurine transporter expressed in a number of human cell lines is regulated by protein kinase C [12,35], therefore the presence of potential sites for phosphorylation by this enzyme in the hTAUT may be relevant to these findings.

Distribution of hTAUT mRNA in human tissues and cultured human cell lines

Northern-blot analysis of blotted human poly(A)$^+$ RNAs from several tissues probed with hTAUT cDNA under high-stringency conditions revealed that hybridizing mRNA species are present in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Figure 6). The size of the major transcript hybridizing

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                                                                G    1
   2  CCCAAAGAACTCAAACAAATTTACAAGAAACAAACAACCCCATCAACAAGTGGCCGAAGA   61
  62  TATGAACAGACACGTCTCAGAAGAAGGCATTTATGGAGACAACAGACACGCGAGGTCAGG  121
 122  AAGAAGCCGCTTATAAATTACCGCTTCCTTCGCGCCGCCGCCAACGCCGAGCCCCGAGGA  181
 182  CCGCAAGCCCAGAGGACAAGCTGCGCCAAGAGGGAGTGCGGACGTTCACCCAGCGGGTCA  241
 242  GAGAGCGAGCGGGCAGGCAGCCCCCGGCCGGCGGAACCCGGCACAGCCGAGCAGAGCGCG  301
 302  GGCGGCGCCGCAGCCACCCCAGATCCAGAACCAGAACCACAGCCCTTCTGAGGAGCTCCC  361
 362  AAACAAAGCAAGGAGATGGCCACCAAGGAGAAGCTGCAGTGTCTGAAAGATTTCCACAAG  421
                      M  A  T  K  E  K  L  Q  C  L  K  D  F  H  K
 422  GACATCCTGAAGCCCTCACCAGGGAAGAGCCCAGGCAAGCGGCCTGAGGACGAGGCTGAG  481
      D  I  L  K  P  S  P  G  K  S  P  G  K  R  P  E  D  E  A  E
 482  GGAAAACCTCCGCAGAGGGAGAAGTGGTCTAGCAAGATCGACTTTGTGCTCTCTGTGGCT  541
      G  K  P  P  Q  R  E  K  W  S  S  K  I  D  F  V  L  S  V  A
 542  GGCGGCTTCGTGGGCTTGGGCAACGTCTGGCGCTTCCCGTACCTCTGCTACAAGAATGGT  601
      G  G  F  V  G  L  G  N  V  W  R  F  P  Y  L  C  Y  K  N  G
 602  GGAGGTGCGTTTCTCATACCGTATTTTATTTCCTGTTTGGGAGCGGCCTGCCTGTGTTT  661
      G  G  A  F  L  I  P  Y  F  I  F  L  F  G  S  G  L  P  V  F
 662  TTCTTGGAGATCATCATAGGCCAGTACACCTCTGAAGGGGGCATCACCTGCTGGGAAAAG  721
      F  L  E  I  I  I  G  Q  Y  T  S  E  G  G  I  T  C  W  E  K
 722  ATCTGCCCCTTGTTCTCTGGTATCGGCTATGCCTCCGTTGTAATTGTGTCCCTCCTGAAT  781
      I  C  P  L  F  S  G  I  G  Y  A  S  V  V  I  V  S  L  L  N
 782  GTCTACTACATCGTCATCCTGGCCTGGGCCACATACTACCTGTTCCAGTCCTTCCAGAAG  841
      V  Y  Y  I  V  I  L  A  W  A  T  Y  Y  L  F  Q  S  F  Q  K
 842  GAGCTGCCCTGGGCACACTGCAACCACAGCTGGAACACACCTCACTGCATGGAGGACACC  901
      E  L  P  W  A  H  C  N  H  S  W  N  T  P  H  C  M  E  D  T
 902  ATGCGCAAGAACAAGAGTGTCTGGATCACCATCAGCTCCACCAACTTCACCTCCCCTGTC  961
      M  R  K  N  K  S  V  W  I  T  I  S  S  T  N  F  T  S  P  V
 962  ATCGAGTTCTGGGAGCGCAACGTGCTGAGCTTGTCCCCTGGAATCGACCACCCAGGCTCT 1021
      I  E  F  W  E  R  N  V  L  S  L  S  P  G  I  D  H  P  G  S
1022  CTGAAATGGGACCTCGCTCTCTGCCTTCTTTTAGTCTGGCTAGTGTGTTTCTTCTGCATC 1081
      L  K  W  D  L  A  L  C  L  L  L  V  W  L  V  C  F  F  C  I
1082  TGGAAGGGCGTCAGGTCCACTGGGAAGGTCGTCTACTTCACAGCCACTTTTCCATTCGCC 1141
      W  K  G  V  R  S  T  G  K  V  V  Y  F  T  A  T  F  P  F  A
1142  ATGCTCCTGGTGCTGCTGGTCCGAGGGCTGACGCTGCCGGGCGCGGGCGCAGGCATCAAG 1201
      M  L  L  V  L  L  V  R  G  L  T  L  P  G  A  G  A  G  I  K
1202  TTCTATCTGTATCCTGACATCACCCGCCTTGAGGACCCACAGGTGTGGATTGACGCTGGG 1261
      F  Y  L  Y  P  D  I  T  R  L  E  D  P  Q  V  W  I  D  A  G
1262  ACTCAGATATTCTTCTCTTATGCCATCTGCCTGGGGGCTATGACCTCGCTGGGGAGCTAC 1321
      T  Q  I  F  F  S  Y  A  I  C  L  G  A  M  T  S  L  G  S  Y
1322  AACAAGTACAAGTATAACTCGTACAGGGACTGTATGCTGCTGGGATGCCTGAACAGTGGT 1381
      N  K  Y  K  Y  N  S  Y  R  D  C  M  L  L  G  C  L  N  S  G
1382  ACCAGTTTTGTGTCTGGCTTCGCAATTTTTTCCATCCTGGGCTTCATGGCACAAGAGCAA 1441
      T  S  F  V  S  G  F  A  I  F  S  I  L  G  F  M  A  Q  E  Q
1442  GGGGTGGACATTGCTGATGTGGCTGAGTCAGGTCCTGGCCTGGCCTTCATTGCCTACCCA 1501
      G  V  D  I  A  D  V  A  E  S  G  P  G  L  A  F  I  A  Y  P
1502  AAAGCTGTGACAATGATGCCGCTGCCCACATTTTGGTCCATTCTTTTTTTTATTATGCTT 1561
      K  A  V  T  M  M  P  L  P  T  F  W  S  I  L  F  F  I  M  L
1562  CTCTTGCTTGGACTGGATAGCCAGTTTGTTGAAGTTGAAGGACAGATCACATCCTTGGTT 1621
      L  L  L  G  L  D  S  Q  F  V  E  V  E  G  Q  I  T  S  L  V
1622  GATCTTTACCCATCCTTCCTAAGGAAGGGTTATCGTCGGGAAATCTTCATCGCCTTCGTG 1681
      D  L  Y  P  S  F  L  R  K  G  Y  R  R  E  I  F  I  A  F  V
1682  TGTAGCATCAGCTACCTGCTGGGGCTGACGATGGTGACGGAGGGTGGCATGTATGTGTTT 1741
      C  S  I  S  Y  L  L  G  L  T  M  V  T  E  G  G  M  Y  V  F
1742  CAGCTCTTTGACTACTATGCAGCTAGCGGTGTATGCCTTTTGTGGGTTGCATTCTTTGAA 1801
      Q  L  F  D  Y  Y  A  A  S  G  V  C  L  L  W  V  A  F  F  E
1802  TGTTTTGTTATTGCCTGGATATATGGAGGTGATAACCTTTATGATGGTATTGAGGACATG 1861
      C  F  V  I  A  W  I  Y  G  G  D  N  L  Y  D  G  I  E  D  M
1862  ATTGGCTATCGGCCCGGGCCCTGGATGAAGTACAGCTGGGCTGTGATCACTCCAGTTCTC 1921
      I  G  Y  R  P  G  P  W  M  K  Y  S  W  A  V  I  T  P  V  L
1922  TGTGTTGGATGTTTCATCTTCTCGCTCGTCAAGTACGTACCCCTGACCTACAACAAAACA 1981
      C  V  G  C  F  I  F  S  L  V  K  Y  V  P  L  T  Y  N  K  T
1982  TACGTGTACCCCAACTGGGCCATTGGGCTGGGCTGGAGCCTGGCCCTTTCCTCCATGCTC 2041
      Y  V  Y  P  N  W  A  I  G  L  G  W  S  L  A  L  S  S  M  L
2042  TGCGTTCCCTTGGTCATCGTCATCCGCCTCTGCCAGACTGAGGGGCCGTTCCTTGTGAGA 2101
      C  V  P  L  V  I  V  I  R  L  C  Q  T  E  G  P  F  L  V  R
2102  GTCAAGTACCTGCTGACCCCAAGGGAACCCAACCGCTGGGCTGTGGAGCGCGAGGGAGCC 2161
      V  K  Y  L  L  T  P  R  E  P  N  R  W  A  V  E  R  E  G  A
2162  ACACCTTACAACTCTCGCACCGTCATGAACGGCGCTCTCGTGAAACCGACCCACATCATT 2221
      T  P  Y  N  S  R  T  V  M  N  G  A  L  V  K  P  T  H  I  I
2222  GTGGAGACCATGATGTGAGCTCTCTCGGGTCGACGGGGCCAACGGTTTCCTGCTGTTTAC 2281
      V  E  T  M  M  *
2282  TAACATTAGATTCTCATAGGACCAGGTTTACAGAGCTTTATATTTGCACTAGGATTTTTT 2341
2342  TTTTTTGTAATTGTCACAGAAAATGTAATTGTGGGTGTGTGTGCGTGCGTGTGTGTGTGT 2401
2402  GTGTGTATCGTGTGTGTGTGTTTTGTTTTGATTTGGGGGATATTTTGTACAAAAAGAAAA 2461
2462  CCCACGGGAAGATGTCCGTGGAGAGGCAGAGCTTTCATACTGAATTAGATGTATTTTATG 2521
2522  GGAATTTGGTAAATTTTTCTTTGTATTTTTTTTTTTTACATATAAGTATATATACACTTA 2581
2582  GAGATTGTCATATACTTTTACCACTTGAATTGATCTTCTTGCCAGCAATAGATCTCATTT 2641
2642  TCAAAAGCAATTCTTCGGTCTGTGTAGCTGGCAGAAAGTTCTGTCCAGTAAACGCAGGAT 2701
2702  GGAATTTTCCTGGGACTCTACACCCATCTTAAGGTGGTATACCTTCCAAATCCTGGTTCA 2761
2762  GATGGAAGAAATAGCAGGAGAGAGGACCCATTAGCTGGCAGACCCAGGGGGAAGAAAGGA 2821
2822  GGGCTGTGAGGAGATACCTCATTAAACTTGGCTTAGTGAAGAG                   2864
```

Figure 5 hTAUT cDNA and predicted primary amino acid sequence

to the hTAUT cDNA is about 6.9 kb. Other hybridizing transcripts (11.5, 8.5, 4.5, 2.3 and 1.1 kb in size) are also present in many of these tissues. The levels of the 6.9 kb transcript are highest in placenta and skeletal muscle, intermediate in heart,

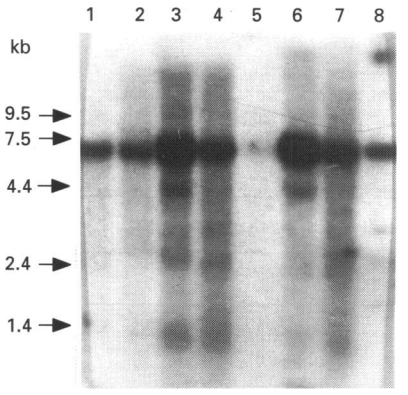

Figure 6 Northern-blot analysis of taurine-transporter mRNA transcripts in human tissues

A commercially available hybridization-ready blot containing poly(A)$^+$ RNA from different human tissues (Multiple Tissue Northern Blot; Clontech) was used to hybridize with the hTAUT cDNA probe. Each lane contained 2 μg of poly(A)$^+$ RNA. Lanes 1–8 represent heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas respectively. The sizes of hybridizing bands were determined using RNA standards run in parallel in an adjacent lane.

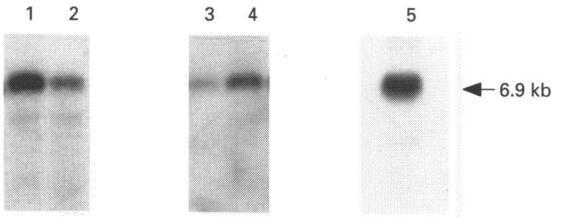

Figure 7 Northern-blot analysis of taurine-transporter mRNA transcripts in culture cell lines of human origin

Poly(A)$^+$ RNA (1 μg per lane), isolated from two placental cell lines, JAR (lane 1) and BeWo (lane 2), a cell line from retinal pigment epithelium HRPE (lane 3) and an intestinal carcinoma cell line HT-29 (lane 4), and total RNA (10 μg per lane) from a cervical carcinoma cell line, HeLa (lane 5) were separated on formaldehyde–agarose gels, blotted and hybridized with the hTAUT cDNA probe. The primary hybridizing transcript (6.9 kb in size) is indicated.

brain, lung, kidney and pancreas and lowest in liver. Interestingly, the relative levels of other hybridizing transcripts in comparison with the corresponding level of the principal 6.9 kb transcript differ significantly among the tissues, indicating a tissue-specific expression pattern.

We have also analysed poly(A)$^+$ RNA isolated from five different cultured cell lines of human origin for the presence of hTAUT transcripts (Figure 7). These cell lines were originally derived from human placental choriocarcinoma (JAR and BeWo), colon carcinoma (HT-29), cervical carcinoma (HeLa) and human retinal pigment epithelium (HRPE). All of these cell lines express Na^+- and Cl^--coupled taurine-transport activity [12,36,37, this study]. In accordance with these findings, the presence of the 6.9 kb major transcript as well as the other minor transcripts that hybridized to the hTAUT cDNA probe is evident in all these cells.

Chromosomal localization

Taurine has been implicated in a variety of biological functions in both the brain and peripheral tissues [38,39]. It is present in very high levels in several tissues and the ability of tissues to

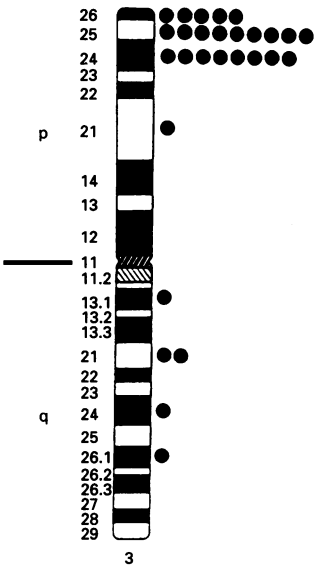

Figure 8 Chromosomal localization of the hTAUT gene

Position and relative abundance of silver grains on chromosome 3 as determined by *in situ* hybridization using the 2864 bp-long full-length hTAUT cDNA as a probe are indicated.

accumulate it is primarily determined by the activity of the taurine transporter. Changes in taurine levels have been observed in a number of diseases, including certain forms of epilepsy, Friedreich's ataxia, retinal blindness, abnormal neural development and cardiac myopathies. As an initial step to evaluating any possible relevance of changes in the taurine-transporter gene to any of the above-mentioned clinical conditions, we have mapped the chromosomal location of the cloned taurine transporter. Initially, we used the 2864 bp long full-length hTAUT cDNA as a probe for this purpose. Somatic-cell hybrid analysis with this probe revealed that DNA sequences homologous to the taurine transporter gene map to two different human chromosomes, 3 and 21. Southern-blot hybridization of the cDNA probe to *Eco*RI-digested DNA from 18 rodent–human somatic-cell hybrids detected three human-specific fragments of 17, 8.7 and 1.6 kb, two fragments of 12 and 9.1 kb in mouse DNA and three fragments of 21, 2.9 and 2.4 kb in Chinese hamster DNA. The 17 and 1.6 kb human fragments were concordant only with human chromosome 3 and the 8.7 kb fragment with human chromosome 21. Regional localization of these two sites was carried out by *in situ* hybridization using the ^{3}H-labelled full-length cDNA probe. Of 225 grains over 100 cells analysed, 22 (9.8 %) were located at chromosome 3 p24→p26 (Figure 8) and 17 (7.6 %) were located at chromosome 21 q11.2→q21 (results not shown). No other chromosomal site was labelled above background.

To determine which of these two chromosomal sites corresponds to the cloned taurine transporter, we repeated the somatic-cell hybrid analysis and *in situ* hybridization studies with another cDNA probe (612 bp long) derived from the 3′ non-coding region of the hTAUT cDNA. This probe detected a 3.8 kb *Eco*RI human-specific fragment and 2.8 and 3.6 kb hybridizing fragments in DNA from mouse and Chinese hamster respectively. The 3.8 kb human fragment was segregated with human chromosome 3. *In situ* hybridization with this probe confirmed the localization to the p24→p26 region of chromosome 3. These studies indicate that the gene for the cloned taurine transporter is located at chromosome 3 p24→p26.

The detection of a second chromosomal site (21 q11.2→q21) with the full-length hTAUT cDNA is interesting and suggests the possibility of the presence of another gene related to the taurine transporter. Although we cannot as yet rule out the presence of an inactive pseudogene, there is compelling biochemical evidence for the existence of two distinct taurine-transport systems. Certain strains of mice (C57BL/6J) exhibit hereditary taurinuria and this trait appears to be inherited in an autosomal recessive manner [40]. Measurement of taurine uptake in brush-border and basolateral membrane vesicles isolated from kidneys of these mice revealed that the taurine-uptake activity in the brush-border membrane is normal [41] but the taurine-uptake activity in the basolateral membrane is impaired [41,42]. These studies suggest that the brush-border and basolateral taurine transporters are under separate genetic control. A recent study using the polarized renal epithelial cell line LLC-PK$_1$ has clearly demonstrated the distinction in biochemical characteristics of the taurine transporters present in the apical (brush-border) and basolateral membranes [43]. Therefore it is very likely that the polarized placental syncytiotrophoblast also expresses two different taurine transporters, one in the maternal-facing brush-border membrane and the other in the fetal-facing basal membrane. In LLC-PK$_1$ cells, it has been shown [43] that the apical taurine transporter exhibits a Na^+/taurine coupling ratio of 2:1, whereas the basolateral taurine transporter exhibits a Na^+/taurine coupling ratio of 1:1. Thus the Na^+/taurine stoichiometry may serve to distinguish between these two taurine transporters. Taurine transporters have been cloned from various sources, but stoichiometric analysis has not been performed with any of them. Ours is the first report on the Na^+/Cl^-/taurine stoichiometry for a cloned taurine transporter. The cloned placental taurine transporter exhibits a Na^+/Cl^-/taurine stoichiometry of 2:1:1. The Na^+/taurine coupling ratio for the cloned placental taurine transporter is similar to the coupling ratio for the taurine transporter in the placental brush-border membrane [7,11] and for the taurine transporter in the apical membrane of the LLC-PK$_1$ cell line [43]. Therefore it appears that the cloned placental taurine transporter represents the brush-border-membrane taurine transporter.

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