Role of the System L permease LAT1 in amino acid and iodothyronine transport in placenta

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The feto-placental unit relies on a maternal supply of indispensable amino acids and iodothyronines for early development and normal growth. We examined the role of the System L transporter in placental uptake of these substances, using the human placental choriocarcinoma cell line BeWo as a model experimental system. BeWo cells express both heavy (4F2hc) and light (LAT1, LAT2) chains of the System L holotransporter. Saturable transport of both L-[^{3}H]tryptophan and [^{125}I]tri-iodo-L-thyronine in BeWo cells includes components sensitive to inhibition by the System-L-specific substrate 2-endoamino-bicycloheptane-2-carboxylic acid; kinetic properties of these components indicate that the 4F2hc-LAT1 transporter isoform is likely to predominate for the carriage of both substances at physiologically relevant concentrations. Both 4F2hc and LAT1 proteins are also expressed in human placental membranes and LAT1 at least is localized largely to the syncytiotrophoblast layer of the term human placenta. The 4F2hc-LAT1 transporter might therefore serve a vital role in supplying the developing fetus and the placenta with both thyroid hormones and indispensable amino acids from the maternal circulation.

Key words: BeWo cells, membrane transport, System L, tri-iodothyronine, tryptophan.

INTRODUCTION

The placenta utilizes indispensable amino acids from the maternal supply as well as delivering them to the fetus [1,2]; for example, the branched-chain amino acids are oxidized within the placenta as fuel (reviewed in [3]) and tryptophan is involved in the placental regulation of feto-maternal immune relations [4]. The main transporter of indispensable neutral amino acids into the placenta (and choriocarcinoma cell lines) has been functionally identified as System L [1,5–7]. Recent research has identified members of a new family of amino acid permeases (e.g. LAT1, LAT2) that form heteromeric System L 'transporter units' with 4F2hc (CD98) glycoprotein in the cell membrane (reviewed in [8,9]). Both LAT1 and LAT2 isoforms of the System L permease are reported to be expressed in placental tissue [10–13].

We have recently [14] shown that thyroid hormones (THs) are also substrates for System-L-type amino acid transport mediated by the *Xenopus* LAT1 homologue IU12 [15]. THs are essential for normal fetal development and it is well recognized that maternal–fetal transfer supplies the fetus with THs, particularly in early gestation before the onset of fetal thyroid function [16,17]. The exact mechanisms by which THs cross the placenta are not fully understood.

The human placental choriocarcinoma cell line BeWo is recognized to be a useful experimental system for studying the mechanisms and regulation of nutrient transport in the placenta [18,19]. Given the potential importance of amino acid and TH transport by System L to normal placental function, we have examined the expression of LAT1 and LAT2 transporter isoforms in BeWo cells and their respective contributions to the uptake of L-tryptophan and 3,3′,5-tri-iodo-L-thyronine (T_3). We have also shown the localization of LAT1 protein in the term human placenta.

MATERIALS AND METHODS

Reagents

Chemicals were obtained from Sigma (Poole, Dorset, U.K.) and radiotracers were purchased from NEN (Hounslow, Middx., U.K.). Tissue culture disposables and media were obtained from Life Technologies, BRL (Paisley, Renfrewshire, Scotland, U.K.).

Cell culture

The BeWo cell line (b30 clone; a gift from Dr H. McArdle, Rowett Research Institute, Aberdeen, Scotland, U.K.) was maintained in continuous culture in 90% (v/v) Ham's F-12 Nutrient Mixture/10% (v/v) foetal calf serum at 37 °C in an atmosphere of air/CO_2 (19:1). BeWo cells were cultured on 175 cm^2 flasks and subcultured on 10 cm dishes or on 6-well or 24-well plates as required. The culture media were changed every second day and on the day before experimentation.

Human placental tissue

The use of human placental tissue was approved by the Tayside Committee on Medical Research Ethics. All placental material was obtained at routine full-term caesarean section from normal pregnancies.

Tracer uptake experiments

All experiments were performed on cells that had just reached confluence. Basic experimental medium contained (in mM): 121 NaCl, 4.9 KCl, 2.5 $MgSO_4$, 20 Tris/HCl and 1 $CaCl_2$ at pH 7.4; where indicated, NaCl was replaced with equimolar LiCl or choline chloride. Preliminary experiments showed that the time course of uptake of L-[^{3}H]tryptophan (50 μM) was linear for up to 45 s and that of [^{125}I]T_3 (50 nM) was linear for up to 5 min

Abbreviations used: 4F2hc (CD98), heavy chain of System L holotransporter; BCH, 2-endoamino-bicycloheptane-2-carboxylic acid; DS, donkey serum; hLAT, human LAT; LAT, L-type amino acid transporter light chain; MeAiB, methylamino-isobutyric acid; OATP, organic-anion-transporting polypeptide; rT_3, ('reverse') 3,3′,5′-tri-iodothyronine; RT–PCR, reverse-transcriptase-mediated PCR; T_3, 3,3′,5-tri-iodo-L-thyronine; T_4, L-thyroxine; TH, thyroid hormone.

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(results not shown) at 37 °C; subsequent uptake experiments using the two tracers were performed at 30 s and 5 min respectively at 37 °C. Uptake was stopped by rapidly washing cells with ice-cold PBS, followed by the addition of 0.2 M NaOH to solubilize the cells for liquid-scintillation counting and total protein determination by the bicinchoninic acid method [20]. Preliminary experiments with [^{14}C]inulin as an extracellular marker demonstrated that over 95% of cell-associated tracer was intracellular.

Reverse-transcriptase-mediated PCR (RT–PCR)

Oligonucleotide primers were synthesized by MWG Biotech AG (Ebersberg, Germany). Human LAT1 (hLAT1) specific primers were matched to bp 372–395 (hLAT1 forward, 5′-CTACGCGGAGCTCGGCACCACCAT-3′) and bp 1232–1209 (hLAT1 reverse, 5′-AAGGCGTAGAGCAGCGTCATCACA-3′) of the coding sequence (GenBank accession number AF104032). Human LAT2 (hLAT2) specific oligonucleotide primers were matched to bp 896–915 (hLAT2 forward, 5′-TTTCCAGGAACCTGACATCG-3′) and bp 1076–1095 (hLAT2 reverse, 5′-ACATTGCAGTGACATAAGCG-3′) of the coding sequence [21]. Total RNA was isolated from BeWo cells by using the RNeasy method (Qiagen). RT–PCR was performed with the Access RT–PCR System (Promega) in accordance with the manufacturer's protocol with the following PCR amplification conditions: 94 °C for 30 s, 55 °C for 1 min, 60 °C for 1 min, repeated for 40 cycles. This was followed by a single additional extension step at 68 °C for 7 min. RT–PCR products were resolved on a 2% (w/v) agarose/Tris/acetate/EDTA gel containing 0.5 μg/ml ethidium bromide, and revealed under UV illumination.

Western blotting

Crude BeWo plasma membranes were prepared by subcellular fractionation with a method previously described for L6 myotubes [22]. In brief, confluent cells were scraped into Hepes-buffered saline and centrifuged at 700 ***g*** for 10 min (4 °C); the pellet was resuspended in sucrose buffer [250 mM sucrose/20 mM Hepes/2 mM EGTA/3 mM NaN_3 (pH 7.4)], followed by two rounds of 20 strokes in a Dounce homogenizer. The supernatant was spun at 31000 ***g*** for 1 h (4 °C) and the resulting pellet of crude membranes was suspended in an appropriate volume of sucrose buffer containing protease inhibitors [200 μM PMSF, 1 μM leupeptin, 1 μM pepstatin and 10 μM *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane ('E-64')]. Total protein content was then measured with the bicinchoninic acid method. Crude cotyledon membranes from normal human term placenta were obtained from Emma Stanley (Department of Molecular Pathology, University of Dundee, Dundee, Scotland, U.K.).

Crude membrane proteins were resolved by SDS/PAGE [in the presence or absence of 5% (v/v) 2-mercaptoethanol as a reducing agent] and electroblotted to nitrocellulose membranes. LAT1 protein was detected by enhanced chemiluminescence (ECL®; Amersham, Little Chalfont, Bucks., U.K.) with affinity-purified rabbit polyclonal antibody (Genosys, Cambridge, U.K.) (1:250 dilution) to a peptide (CQKLMQVVPQET; single-letter amino acid codes) mapped to the C-terminal sequence of hLAT1. Horseradish-peroxidase-labelled mouse anti-rabbit secondary antibody (Scottish Antibody Production Unit) was then used for visualization. 4F2hc protein was detected with anti-(human CD98) goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) (1:250 dilution) followed by horseradish-peroxidase-labelled donkey anti-sheep secondary antibody for visualization (Scottish Antibody Production Unit).

Confocal laser scanning microscopy

Confluent BeWo cells were fixed on their culture dish for 5 min at −20 °C in 90% (v/v) methanol/10% (v/v) Mes buffer [100 mM Mes/1 mM EGTA/1 mM $MgSO_4$ (pH 6.9)], then extracted with 1% (v/v) Nonidet P40 in PBS for 10 min at room temperature. Antibodies against 4F2hc and hLAT1 were added at 1:100 dilution in DMEM (Dulbecco's modified Eagle's medium) for 1 h at room temperature. Cells were washed with PBS and secondary antibodies were added (1:2000 donkey anti-sheep conjugated to ELEXA and 1:20 pig anti-rabbit conjugated to FITC) for 1 h at room temperature. Coverslips were mounted over cells in Citifluor (Agar Scientific, Stansted, Essex, U.K.) and sealed with nail varnish. Cells were then viewed with a Bio-Rad MRC-600 confocal laser-scanning microscope.

Immunohistology of term human placenta sections

Human placenta sections were prepared by Emma Stanley with the following procedure. Pieces (1 cm^3) of freshly dissected placental cotyledon were immediately fixed in 10% (v/v) buffered formalin for 3 days, then dehydrated with alcohol before being embedded in paraffin wax. The embedded material was cut into 3 μm microtome sections, then fixed on glass slides overnight at 47 °C.

The pre-prepared placenta sections were de-waxed, placed in methanol containing 1% (v/v) H_2O_2 for 30 min with gentle shaking, then washed with Tris-buffered saline (TBS). Each section was then incubated with donkey serum (DS; Scottish Antibody Production Unit) diluted 1:5 in PBS for 5 min to block non-specific binding sites on the sections. The primary antibody, anti-hLAT1, was applied diluted 1:25 in DS for 30 min (controls were incubated in DS without primary antibodies). Slides were washed in TBS, treated with DS as before and the secondary antibodies (anti-rabbit IgG) applied for 30 min at a dilution of 1:25 in DS. Slides were again washed in TBS, re-blocked with DS as before and the tertiary antibodies [anti-(rabbit PAP)] applied for 30 min at a dilution of 1:100 in DS followed by a wash with TBS. Sections were then incubated in 0.5 mg/ml 3,3′-diaminobenzidine containing 1% (v/v) H_2O_2 for 7 min to reveal staining and then washed under running water. The sections were then counterstained with haematoxylin for 10 s, then washed and dehydrated in solvents. The slides were allowed to dry in air; cover slips were then mounted on top of the sections. Section images were produced on slide film with an MC-100 camera mounted on an Axioscope microscope.

Data analysis and handling

Differences between mean values were assessed by using Student's unpaired t test, with significance assigned at $P < 0.05$. All experiments were performed in triplicate and results are expressed for at least $n = 3$ different BeWo cell preparations.

RESULTS

Characterization of tryptophan uptake into BeWo cells

Tryptophan uptake into BeWo cells was Na^+-independent, because replacing NaCl with equimolar choline chloride in the uptake buffer had no significant effect on the uptake of 50 μM [^{3}H]Trp tracer (results not shown). [^{3}H]Trp uptake was sensitive to inhibition by an excess of various amino acids and amino acid analogues: L-Trp, Leu, 2-endoamino-bicycloheptane-2-carboxylic acid (BCH) > D-Trp, Gln > Ala, all at 10 mM inhibited 50 μM Trp uptake (Figure 1, upper panel). The System-

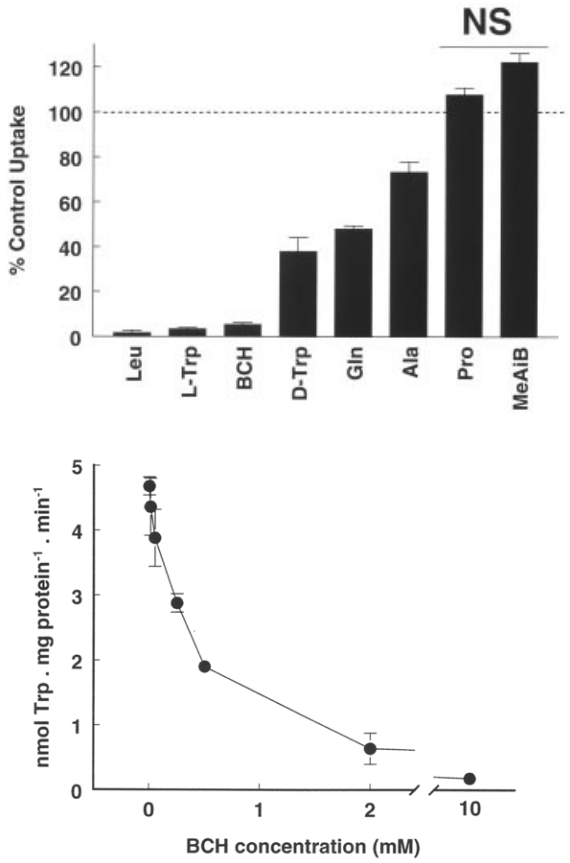

Figure 1 Effect of amino acids on tryptophan uptake into BeWo cells

Upper panel: results are expressed as percentages of control 50 μM tryptophan uptake (means $\pm$ S.E.M. for $n > 3$ experiments). All inhibitions were significantly different from zero ($P < 0.05$) except those indicated NS (non-significant). The concentrations of inhibitors used were all 10 mM. Lower panel: concentration dependence of the effect of BCH on Trp uptake. A K_i of 0.38 mM was estimated as the concentration of BCH producing half-maximal inhibition of Trp transport. The curve was fitted to the experimental data by using commercial software (SlideWrite Plus 5 for Windows; Advanced Graphics Software). Uptakes are expressed as nmol of Trp/min per mg of protein (means $\pm$ S.E.M. for $n = 3$ separate experiments) for 50 μM [^{3}H]Trp tracer in NaCl buffer.

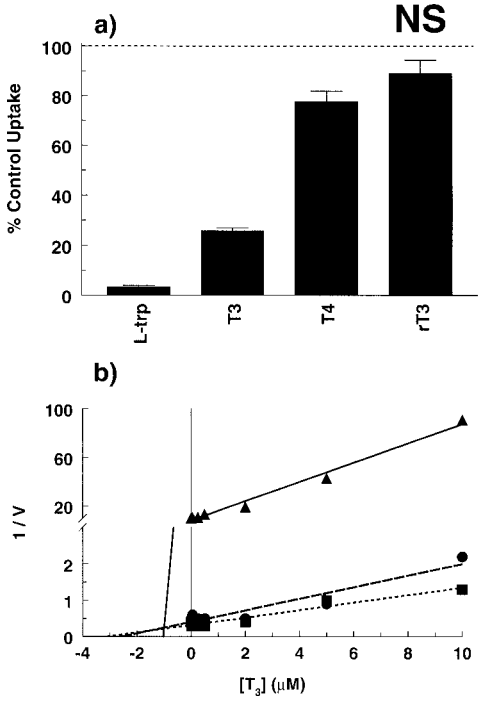

Figure 2 Effect of iodothyronines on tryptophan uptake into BeWo cells

(**a**) Results are expressed as percentages of control (50 μM tryptophan) uptake remaining in the presence of inhibitor (means $\pm$ S.E.M. for $n > 3$ separate experiments). All inhibitions were significantly different from zero ($P < 0.05$) except that indicated NS (non-significant). Concentrations of iodothyronines used were all 10 μM and unlabelled L-Trp was at 10 mM. (**b**) Dixon plot of T_3 inhibition of Trp uptake (measured over 30 s). Results shown are for $n = 3$ separate experiments at each concentration of L-Trp: ▲, 1 μM; ●, 50 μM; ■, 100 μM.

L-specific substrate BCH inhibited Trp uptake with a K_i of 0.38 mM (Figure 1, lower panel). The imino acid proline and the System-A-specific substrate methylamino-isobutyric acid (MeAiB) at 10 mM had no significant effect on 50 μM Trp uptake (Figure 1, upper panel). These results show that the greater part of L-Trp uptake into BeWo cells occurs by amino acid transport System L. Ala is a substrate of the LAT2 but not of the LAT1 permease isoform [11,13,23], so it seems from our results that 4F2hc-LAT1 is the predominant System L transporter in BeWo cells at physiologically relevant Trp concentrations (plasma [Trp] is approx. 50 μM). Total Trp uptake measured over a range of Trp concentrations (0.01, 0.05, 0.25, 0.5, 2 and 5 mM) showed overall values for K_m of 90 μM and V_{max} of 8.4 nmol/min per mg of protein.

Transport of T_3 by the System L amino acid transporter

We next investigated the possibility that iodothyronines were substrates for System L in BeWo cells. Uptake of the System L substrate Trp was substantially inhibited by T_3 but to a much smaller extent by L-thyroxine (T_4) and 3,3′,5′-tri-iodothyronine (rT_3) (Figure 2a). The inhibition of Trp uptake by T_3 seems to be competitive because a Dixon plot (Figure 2b) showing effects of increasing concentrations of T_3 inhibitor on Trp uptake reveals that the straight lines plotted at three different concentrations of Trp intersect above the x-axis. A K_i of 1.2 μM was estimated for the inhibition by T_3 of L-Trp uptake into BeWo cells.

T_3 uptake into BeWo cells was saturable; the iodothyronines T_3, rT_3 and, to a much smaller extent, T_4 (Figure 3, upper panel) at 10 μM blocked 50 nM T_3 uptake. The uptake of 50 nM T_3 into BeWo cells was also sensitive to inhibition by an excess of the amino acids L-Trp, D-Trp $\gg$ Leu, BCH at 10 mM each (Figure 3, upper panel), although neither Ala nor MeAiB (a System-A-specific analogue) had any significant effect on T_3 uptake. The inhibition by L-Trp, D-Trp and Phe was greater than that of 10 μM T_3 but the addition of T_3 and L-Trp together did not produce an additive effect (results not shown). Up to 40% of 50 nM T_3 uptake therefore seems to occur via non-saturable mechanisms, presumably including simple diffusion. Saturable T_3 uptake included a Na^+-dependent component: replacement of NaCl with equimolar choline chloride in the uptake buffer decreased the transport of 50 nM T_3 by 35 $\pm$ 5% (mean $\pm$ S.E.M.; $n = 11$), although replacement of Na^+ with Li^+ in the uptake buffer had no effect on the transport of T_3 into BeWo cells (results not shown). The Na^+-independent component of tracer T_3 uptake was inhibited by BCH to the same extent as self-inhibition by unlabelled T_3 (Figure 3, lower panel). This component (presumably System L) had values for K_m of 1.6 μM and V_{max} of 20 pmol/min per mg of protein, as estimated

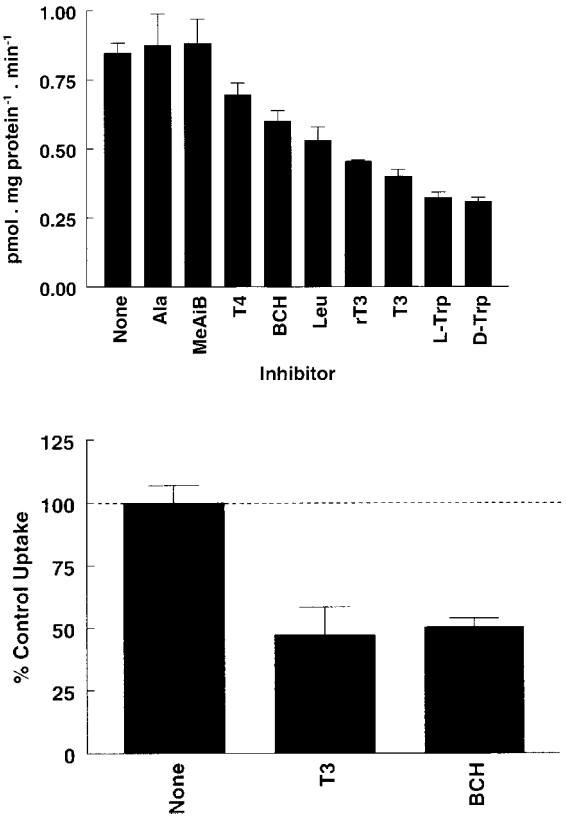

Figure 3 Effect of putative inhibitors on the uptake of T_3 into BeWo cells

Upper panel: uptakes are expressed as pmol of T_3/min per mg of protein (means ± S.E.M. for $n > 3$ separate experiments) for 50 nM [^{125}I]T_3 tracer in NaCl buffer. Concentrations of inhibitors were 10 mM for amino acids and 10 μM for iodothyronines. All inhibitions were significantly different from zero ($P < 0.05$) except for MeAiB and Ala. Lower panel: effect of inhibitors (percentages of control uptake; means ± S.E.M. for $n = 3$ experiments) on 50 nM T_3 uptake in Na^+-free (choline chloride) buffer. All inhibitions were significantly different from zero ($P < 0.05$).

from [^{125}I]T_3 uptakes into BeWo cells at 0.05, 0.25, 0.5, 2, 5 and 10 μM T_3 in choline chloride buffer (results not shown). Organic anion transporters [Na^+-dependent taurocholate co-transporting polypeptide ('Ntcp'), organic-anion-transporting polypeptide (OATP)] are also known to accept THs as substrates but the model OATP substrate taurocholate (500 μM) had no effect on the uptake of either 50 nM T_3 or 50 μM Trp into BeWo cells.

RT–PCR of total BeWo RNA

Expression of the System L permeases hLAT1 and hLAT2 was identified in BeWo cells from total RNA extract as the production of single RT–PCR products of the expected size (861 and 200 bp respectively) (Figure 4). In subsequent studies we focused on LAT1 because of its greater importance in mediating T_3 uptake into BeWo cells.

Identification and localization of System L transporter subunits in BeWo cells

The proteins required for L-type amino acid transport, namely the heavy (4F2hc) and light (LAT1) chains, were detected in crude membranes of BeWo cells by Western blotting (Figures 5a and 5b respectively). Preincubation of the anti-LAT1 antibody with the LAT1 peptide completely blocked the appearance

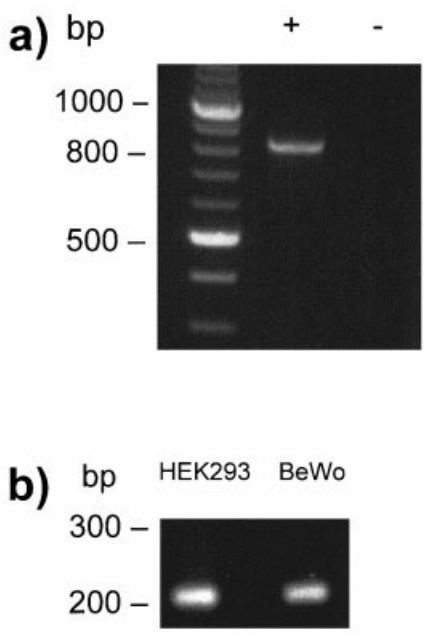

Figure 4 RT–PCR of total BeWo RNA with hLAT1 (a) and hLAT2 (b) primers

The amount of RNA used was 1 μg. (**a**) Reactions performed with (+) and without (−) reverse transcriptase are shown in adjacent lanes. Omission of the reverse transcriptase enzyme from the reaction mixture, as a negative control, resulted in a loss of product. Molecular size markers are shown in the left lane. (**b**) RNA from HEK293 cells was used as a positive control for LAT2.

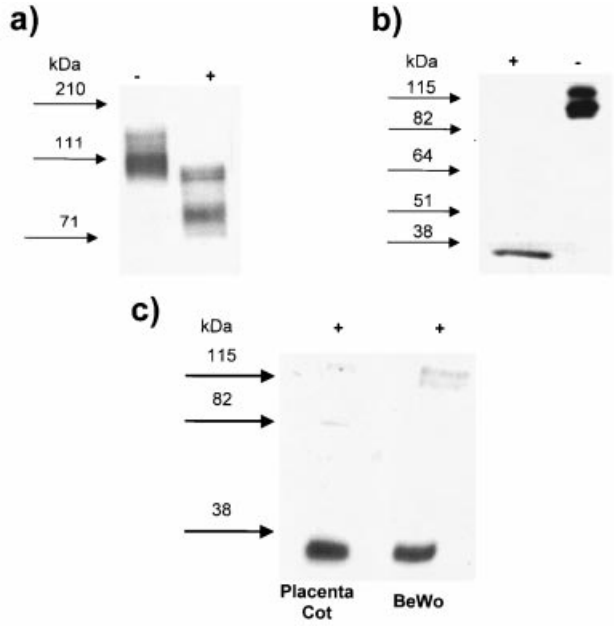

Figure 5 Expression of System L transporter subunits in BeWo plasma membranes and placental cotyledons

(**a**, **b**) Western blots of BeWo plasma membranes (40 μg of protein) probed with anti-(human 4F2hc) (**a**) and anti-LAT1 (**b**). Samples prepared under reducing (+) and non-reducing (−) conditions (with and without 2-mercaptoethanol respectively) are shown. (**c**) Western blot of human crude placental cotyledon membranes (Cot) (50 μg of protein) probed with anti-LAT1, with BeWo plasma membranes (25 μg of protein) as a positive control.

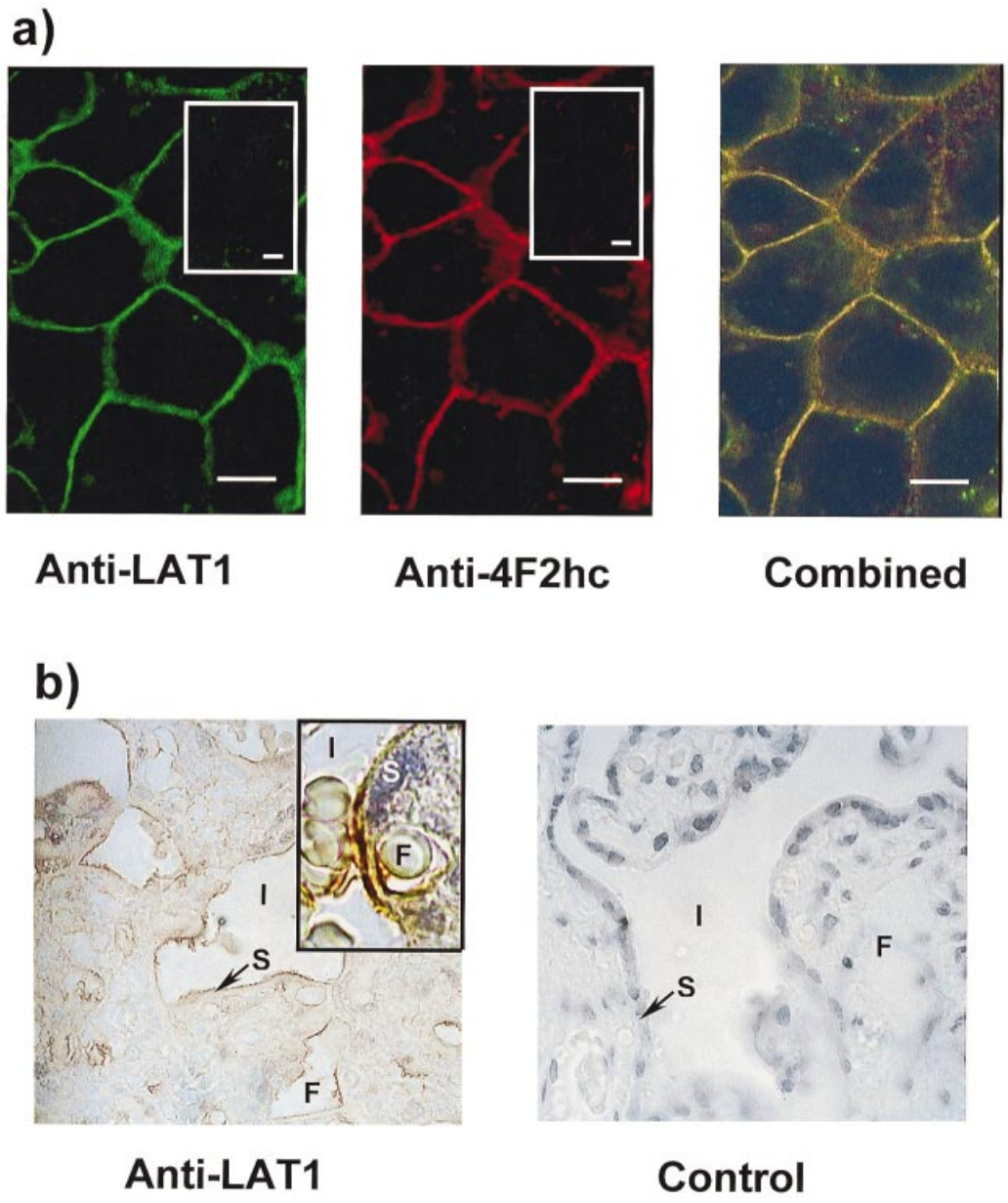

Figure 6 Subcellular localization of 4F2hc and LAT1 in BeWo cells (a) and immunohistology of term human placental sections (b)

(**a**) Confocal, immunofluorescence microscopy of BeWo cells using anti-(human CD98) (anti-4F2hc) and anti-LAT1. Z-sections taken through the centres of cells. Insets: control images produced with cells incubated in secondary antibodies only. Scale bars, 1.5 μm. (**b**) Images produced from sections of a single placenta with an oil-immersion lens. Abbreviations: S, syncytiotrophoblast layer; I, inter-villus space; F, fetal blood capillary. Control slices were incubated without anti-hLAT1; incubation conditions were otherwise the same. Magnifications: large panels, $\times 360$; inset to left panel, $\times 990$. The inset shows that LAT1 (intense brown staining) is localized predominantly but not exclusively at the apical surface of syncytiotrophoblastic cells. Erythrocytes are visible in the inter-villus space and fetal blood capillary; in addition, nuclei (purple staining) are visible within the syncytiotrophoblast. Similar results were obtained from a second placenta.

of banding in Western blots (results not shown). Treatment of BeWo membranes with the reducing agent 2-mercaptoethanol (to break disulphide bridges between subunit peptides of the holotransporter) caused a marked band-shift of both LAT1 and 4F2hc on Western blots. Under reducing conditions, 4F2hc appeared as a broad band with two distinct peaks at approx. 75 and 100 kDa (probably reflecting different glycosylation states of the peptide), whereas LAT1 appeared as a single band at 35 kDa. In contrast, both peptides were identified in a broad band (centred on 115 kDa) under non-reducing conditions, consistent with the formation of a heterodimeric holotransporter [8,24].

Confocal laser microscopy of intact BeWo cells also revealed that 4F2hc and LAT1 are found localized together mainly in the plasma membrane (Figure 6a), which is consistent with their role as a cell-surface amino acid transporter complex.

Expression and localization of 4F2hc and LAT1 in term human placenta

Western blotting of human crude placental cotyledon membranes, under reducing conditions confirmed the presence of LAT1 (Figure 5c). LAT1 was seen as a single band under

reducing conditions at approx. 35 kDa in both cotyledon membranes and BeWo crude membranes (Figure 5c). This suggests that LAT1 exists in placental cotyledons, which is consistent with previous reports of its mRNA distribution [12].

Immunohistology of term placental sections with the use of anti-LAT1 antibody show that LAT1 protein is distributed mainly along the syncytiotrophoblast layer on the maternal-blood-facing side (Figure 6b), although some staining is also apparent on the fetal side.

DISCUSSION

The present study provides both functional and molecular characterization of a System-L-type amino acid transporter in BeWo cells. Our functional results show that uptake of the aromatic amino acid Trp into BeWo cells is both Na^+-independent and inhibited by BCH, two characteristic features of classical System L transport [8,10]. Transport of both branched-chain amino acids and aromatic amino acids in human placental membrane vesicles [5,6] and villous explants from human placenta [21,25] are also reported to occur almost exclusively by System L. Indeed the K_i (0.44 mM) for the inhibition of Trp transport by BCH in villous explants [25] is very similar to that reported here for BeWo cells. T_3 is a competitive inhibitor of Trp uptake in BeWo cells, with a K_i (1.2 μM) of the same order of magnitude as the estimated K_m for Na^+-independent T_3 transport (1.6 μM). These results are consistent with the idea that T_3 and L-Trp compete for a common transport mechanism in BeWo cells, presumably System L because this seems to be the sole mode of Trp uptake. System L activity (i.e. uptake inhibited by BCH) represents approx. 30 % of the total T_3 uptake in our BeWo cells and also seems to contribute significantly to the transport of iodothyronines (including both T_3 and T_4) in the JAR human choriocarcinoma cell line [26]. The K_m values for T_3 and Trp transport by System L in BeWo cells (1.6 and 90 μM respectively) are similar to those that we have reported previously for 4F2hc-LAT1 holotransporters expressed in *Xenopus* oocytes [14]. The relatively low K_m for T_3 transport (approx. 1/55 that for Trp) should help T_3 to compete effectively with amino acids for transport by System L under physiological conditions. RT–PCR studies of BeWo mRNA reveal the expression of both LAT1 and LAT2 permease subunits in these cells. The lack of substantial inhibition by Ala of either Trp or T_3 transport indicates that LAT1 rather than LAT2 is the predominant permease isoform operating in BeWo cells, at least under our experimental conditions (Ala also fails to inhibit iodothyronine transport in JAR cells [26]). We find no evidence for significant transport of Trp by System T (a BCH-resistant transport mechanism selective for aromatic amino acids and iodothyronines [3]) in BeWo cells.

A role for 4F2hc-LAT1 holotransporters in the normal function of the placenta *in vivo* is indicated by the identification of both peptide subunits in cell membranes prepared from human term placental cotyledons. This preparation includes membranes from trophoblastic and endothelial cells, although from our immunohistological studies it seems that LAT1 is localized mainly to the syncytiotrophoblast layer of the villi in term placenta. The transport of metabolites from mother to fetus is facilitated by the polarized distribution of transporters in the syncytiotrophoblast [1,6,27] but the exact subcellular localization of LAT1 and LAT2 in placental tissue remains to be elucidated. The brush border (maternal-facing) membranes display System-L-like transport of a type consistent with major expression of LAT1 protein at that surface [12], where it would contribute to the extraction of amino acids and iodothyronines from the maternal blood. A System-L-type mechanism has also been identified at the microvillous border of differentiated BeWo cells cultured on microbeads [7]. LAT2 protein is localized predominantly to the basolateral membrane in intestinal and renal epithelium [23] and might therefore be predicted to be expressed at fetal-facing membranes in the placenta. Both LAT1 and LAT2 operate as exchangers, so net maternal–fetal transfer of indispensable amino acids by these permeases should overall be accompanied by the reciprocal transfer of other (ideally dispensable) LAT substrates such as glutamine [3].

The uptake of T_3 in BeWo cells includes multiple transport components. The observation that the aromatic amino acids D-Trp and L-Trp had inhibitory effects larger than T_3 itself (Figure 3, upper panel) leads us to suggest that 10 μM T_3 (a concentration approaching the limit of its solubility) was insufficient to saturate fully all T_3 carriers present in BeWo cells. System L transport does not account for all the saturable T_3 uptake present in BeWo cells and it seems unlikely that the iodothyronine-transporting System T has any significant activity in this cell type (or indeed in JAR cells [28]). Members of the OATP family have been shown to transport THs in other tissues [29,30]. The lack of an inhibitory effect of the prototypical OATP substrate, taurocholate, on either Trp or T_3 uptake into BeWo cells indicates that OATPs are not involved with the transport of either solute in this cell type. The mechanisms for T_3 uptake into JAR choriocarcinoma cells include a discrete transport component inhibited by aromatic drugs including nitrendipine and diazepam [26]. Detailed functional and molecular information on this mechanism are as yet unavailable, although it is possible that the mechanism accounts for the remaining (apparently Na^+-dependent) component of T_3 transport noted for BeWo cells in the present study. The overall importance of amino acid transport systems in effecting TH movement across plasma membranes remains to be fully established. Nevertheless our results indicate that the 4F2hc-LAT1 System L holotransporter might serve an important role in supplying the placenta and developing fetus with THs as well as indispensable amino acids.

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