Amino Acids (2010) 38:1361–1367
DOI 10.1007/s00726-009-0344-6

ORIGINAL ARTICLE

Tryptophan and iodothyronine transport interactions in HepG2 human hepatoma cells

James W. A. Ritchie · Peter Maving Taylor

Received: 10 June 2009 / Accepted: 27 August 2009 / Published online: 15 September 2009

Abstract This study identifies interactions between transport of the aromatic amino acid L-tryptophan (Trp) and thyroid hormones (TH) in HepG2 human hepatoma cells. The major portion of Trp uptake in HepG2 cells occurs via the NEM-sensitive amino acid transport System L2 (consistent with hepatic LAT3 expression), with a smaller aromatic-AA selective System T (MCT10) component. LAT3 and MCT10 mRNA were both detected in HepG2 cells. Uptake of TH does not involve System L2, but a significant portion of T_3 uptake is mediated by System T, alongside a taurocholate-sensitive organic anion transporter. T_4 uptake into HepG2 cells appears to be mediated principally by organic anion/monocarboxylate transporters, with smaller contributions by System T and receptor-mediated endocytosis. TH–Trp transport interactions in liver cells centre on System T which, due to a perivenous localisation alongside deiodinase 1, may impact on hepatic T_3 generation and release.

Keywords Thyroid hormone · Liver · Aromatic amino acid · Biomembrane transport

Introduction

The aromatic amino acids (L-tryptophan, L-phenylalanine, L-tyrosine) are important intermediary metabolites and precursors of neurotransmitters (tryptophan for serotonin; tyrosine for catecholamines) and hormones [tryptophan for melatonin; tyrosine for thyroid hormones (THs)] (Rose 1972 for review). Tryptophan also contributes to synthesis of the coenzymes NADH and NADPH (Rose 1972) and the tryptophan–kynurenine pathway has a significant immunoregulatory function (Kaper et al. 2007). Tryptophan and phenylalanine are essential constituents of the human diet. The liver is the main site of aromatic amino acid metabolism in the human body, oxidising any excess in the fed state and providing a systemic source from protein breakdown in the fasted state (Salter et al. 1986). The liver is the major extrathyroidal site of TH turnover and metabolism and an important source of circulating plasma T_3 (active form of TH) from thyroxine (T_4) deiodination (Gereben et al. 2008; Yen 2001 for review).

Intrahepatic metabolism of the aromatic amino acids and TH is regulated substantially by substrate transport across the plasma membranes of hepatocytes, rendering the transport step of key importance for processes including tryptophan catabolism (Salter et al. 1986), TH action in the liver and T_3 supply for the whole body (Gereben et al. 2008; Hennemann et al. 2001). THs retain a tyrosine-derived amino acid moiety within the iodothyronine molecular structure, allowing them to be accepted as substrates by transporters for aromatic amino acids which include System L (notably the SLC7A5/SLC3A2 heterodimer isoform LAT1; Ritchie et al. 2003; Ritchie and Taylor 2001) and System T (MCT10; SLC16A10; Friesema et al. 2008). THs are also recognised as substrates for a broad range of other transporter types including the monocarboxylate transporter MCT8, certain multi-specific organic anion/cation transporters as well as multi-drug resistance (MDR) pumps and fatty acid translocase (see e.g. Abe et al. 2002; Friesema et al. 2005 for review).

We have previously established a link between the transport of amino acids and TH in hepatocytes which is

J. W. A. Ritchie · P. M. Taylor (✉)
Division of Molecular Physiology,
College of Life Sciences, James Black Centre,
University of Dundee, Dundee DD1 5EH, UK
e-mail: p.m.taylor@dundee.ac.uk

modulated during altered thyroid status (Kemp and Taylor 1997). We are studying these interactions in greater detail using the human hepatoma cell line HepG2, which is a useful model system for the study of amino acid and TH metabolism in human liver (van Stralen et al. 1996; Goenner et al. 1992).

Materials and methods

Materials

[^{3}H]L-tryptophan, [^{125}I]L-triiodothyronine and [^{125}I]L-thyroxine were obtained from NEN DuPont (Hertfordshire, UK). Cell culture media were obtained from GibcoBRL Life Technologies (Paisley, UK) unless stated otherwise; other chemicals were obtained from either Sigma Chemicals (Poole, Dorset, UK) or BDH Merck Ltd (Poole, Dorset, UK).

Cell culture

HepG2 cells (ATCC® HB-8065) were maintained at 37°C, 5% CO_2 in a CO_2 incubator. Cells were seeded in flasks containing 20 ml of Dulbecco's modified Eagles medium (high glucose) plus 10% foetal calf serum and 1% antibiotic/antimycotic solution. At confluence, cells were resuspended by trypsinisation and reseeded into either 6- or 24-well plates (for radiotracer uptake measurements) or 21 cm^2 Petri dishes (for isolation of cell membranes) with the same culture medium. Confluent cell cultures were used for transport studies.

Radiotracer studies

All radiotracer uptake measurements were performed at 37°C as follows: prior to initiation of uptake, culture medium was aspirated and cells were washed twice in PBS (136 mM NaCl, 2.7 mM KCl, 7.7 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.3). Transport buffer (TB: 121 mM NaCl, 4.9 mM KCl, 2.5 mM $MgSO_4$, 20 mM Tris–HCl, 1 mM $CaCl_2$, pH 7.4), including radiotracer at the appropriate concentration as well as inhibitors, etc., as required, was then added for a timed period; NaCl was replaced with equimolar choline-Cl in TB as required. At the end of the experimental period, the TB was aspirated and cells were washed twice with ice cold PBS to terminate uptake. 1.25 ml of 0.2 M NaOH was immediately added to each well and left overnight to solubilise the cell monolayer, prior to assay for radioactivity (liquid scintillation counting) and protein (bicinchoninic acid method; Smith et al. 1985). In initial experiments, it was found that uptake of [^{3}H]-tryptophan into HepG2 cells was linear for 1 min (Fig. 1a) whereas uptakes of [^{125}I]-T_3 (Fig. 1b) and [^{125}I]-T_4 (not shown) were linear for at least 15 min. Subsequent [^{3}H]-Trp uptake experiments were conducted at 30 s, those using [^{125}I]-T_3 for 10 min and [^{125}I]-T_4 for 15 min, to enable accumulation of sufficient radioactivity whilst retaining first-order uptake kinetics. Extrapolation of these time frames for tracer uptake to time zero for the three compounds showed apparent instantaneous binding components equivalent to 22, 18 and 12% of the measured initial uptake velocities of tryptophan, T_3 and T_4, respectively. Preliminary studies using [^{14}C]-inulin as an extra-cellular marker established that extra-cellular radioactivity accounted for less than 2% of the total radiotracer uptake over these periods (data not shown), and correction for extra-cellular space was, therefore, not applied in subsequent experiments.

Kinetic parameters (K_m, V_{max}) of saturable transport processes were established by measuring the initial uptake of tracer substrate in the presence of different concentrations of unlabelled substrate (as indicated in text). All kinetic parameters were calculated from curves fitted using the least-squares method (SlideWrite Plus 5); the non-saturable component was subtracted from saturable component(s) as necessary.

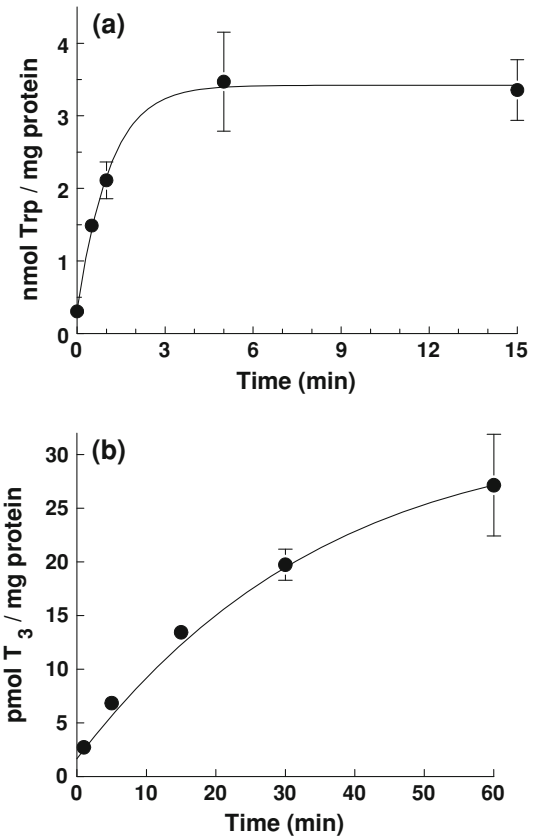

Fig. 1 Time course of uptake of **a** 50 μM Trp and **b** 50 nM T_3 into HepG2 cells. Radiotracer uptake was measured over the indicated time periods at 37°C. Results are expressed as total solute uptake ± SEM for three different experiments

Expression of thyroid hormone transporter genes in HepG2 cells

First-strand HepG2 cell cDNA (gifted by Dr C Sutherland, Biomedical Research Institute, University of Dundee) was stored at −20°C prior to use in PCR using gene-specific primers to test for specific mRNA expression. The primer sets used in the PCR program are detailed below:

LAT1	Forward	5′-tctccttgcccattgtcacc-3′
	Reverse	5′-atgactcccaggtggtagttcc-3′
LAT3	Forward	5′-ctcctgggctggggctccctgt-3′
	Reverse	5′-ttcccaaacatgttgggcag-3′
MCT10	Forward	5′-ctcatgtccagttcttttgtaag-3′
	Reverse	5′-agccgtccaactcctgaagtga-3′

For each PCR reaction, 2 µl of first-strand DNA, 1 µM each primer and standard 2× GoTaq Green PCR Master Mix (Promega) were used in a 20 µl total volume. The PCR programme used was 95°C, 3 min (94°C, 30 s; 55°C, 30 s; 72°C, 1 min) 40 cycles; 72°C, 2 min using a Thermo Scientific Hybaid Px2 thermal cycler.

Results

L-Tryptophan uptake into HepG2 cells

Tryptophan (50 µM) uptake into HepG2 cells was sensitive to inhibition by an excess (10 mM; Ritchie and Taylor 2001) of various amino acids and amino acid analogues (Fig. 2); L-Trp > Leu, BCH (System L specific), whereas Ala, Arg and Pro had no significant effect. Trp uptake was Na^+-independent since replacement of Na^+ with equimolar choline$^+$ in the uptake buffer had no significant effect on uptake of 50 µM Trp (not shown). Trp (1 µM) uptake into HepG2 cells was also significantly inhibited by iodothyronines (Fig. 3); L-T_3 > rT_3 > T_4, but not by the TH analogue TRIAC (all used in excess at 10 µM, close to the limit of aqueous solubility for TH). Uptake of 1 µM to 10 mM [^{3}H]-Trp into HepG2 cells included at least one saturable component (Fig. 4a), with an overall value for K_m of 570 µM and a V_{max} of 15.5 nmol/mg protein 30 s. The inhibition of Trp uptake by T_3 appeared to be a competitive effect, because a Dixon plot (Fig. 4b) of the effect of increasing concentrations of T_3 on Trp uptake reveals that the straight lines plotted at each concentration of Trp used intersected above the *x*-axis. A K_d value of 4.2 µM was estimated for T_3 inhibition of L-Trp uptake into HepG2 cells. The organic anion taurocholate (Taur; a model OATP substrate) at 500 µM (Inui et al. 2000) failed to inhibit 50 µM Trp uptake

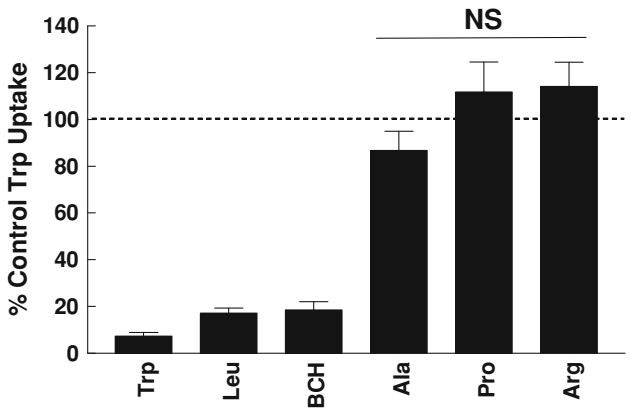

Fig. 2 Effect of amino acids on L-tryptophan uptake into HepG2 cells. Results are expressed as % remaining of control (50 µM) tryptophan uptake ± SEM for 3–12 separate experiments. Inhibitions were significantly different from zero ($p < 0.05$) except where NS indicated (non-significant). Concentrations of inhibitors used were all 10 mM

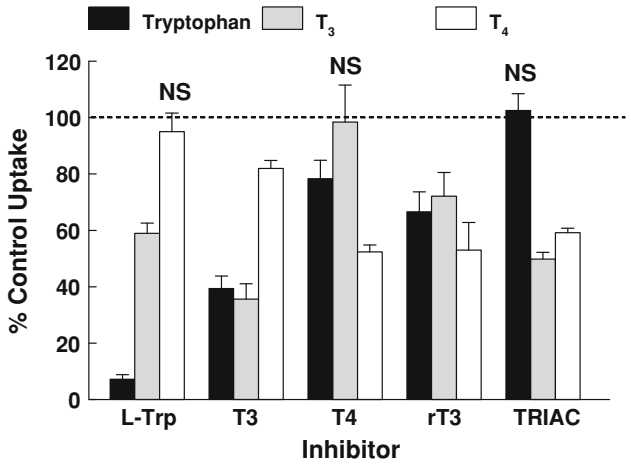

Fig. 3 Effect of iodothyronines on 1 µM tryptophan and 50 nM T_3/T_4 uptake into HepG2 cells. Results are expressed as % remaining of control uptake measured in the absence of putative inhibitor. Mean values ± SEM for 3–12 separate experiments are shown. Inhibitions were significantly different from zero ($p < 0.05$) except where NS indicated. Inhibitor concentrations were 10 µM for iodothyronines and 10 mM for L-Trp

(Table 1). Pre-incubation of HepG2 cells with the thiol-group reagent *N*-ethylmaleimide (2 mM, 30 min; Kemp and Taylor 1997) inhibited 50 µM Trp uptake by 60% (Table 1), although pre-incubation with the endocytosis inhibitor monodansylcadaverine (50 µM, 30 min; Horiuchi et al. 1982) had no significant effect (Table 1).

Iodothyronine uptake into HepG2 Cells

Both T_3 and T_4 uptakes into HepG2 cells showed major saturable components (see Fig. 3) which were Na^+-independent (no effect of Na^+ replacement with equimolar

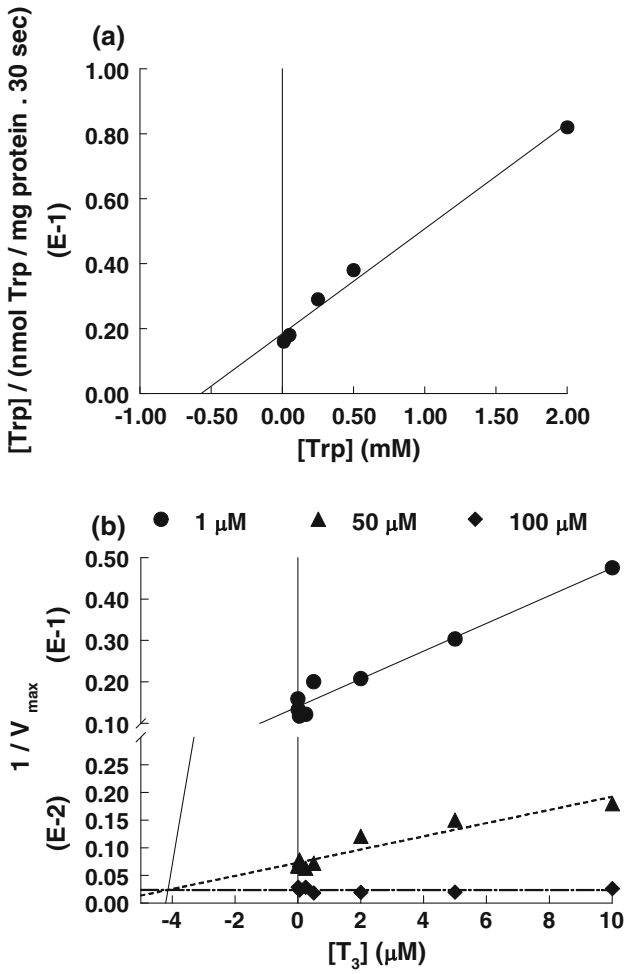

Fig. 4 **a** Hanes plot of tryptophan uptake into HepG2 cells; the saturable transport component has an estimated K_m of 570 μM and V_{max} of 15.5 nmol Trp/mg 30 s. **b** Dixon plot showing T3 inhibition of Trp uptake. The *lines* show inhibition at three different Trp concentrations (1, 50, 100 μM). All values shown are for $n = 3$ separate experiments

choline$^+$ salts in TB: data not shown). Iodothyronines inhibited 50 nM T_3 uptake to differing extents: T_3 > TRIAC (which lacks an α-amino group) > rT_3 > T_4 at 10 μM (Fig. 3). 50 nM T_3 uptake was sensitive to inhibition by an excess of aromatic amino acids (Trp, Phe at 10 mM each; Fig. 5a), whereas Arg, Glu, Leu, BCH and Ala (all at 10 mM) had no significant effect. Iodothyronines (T_4, rT_3 > TRIAC > T_3) also inhibited uptake of 50 nM T_4 into HepG2 cells (Fig. 3), although none of the amino acids tested (including Trp and Phe) had any significant effect (Fig. 5b). Taurocholate (500 μM; Inui et al. 2000) had a significant (~28%) inhibitory effect on both T_3 and T_4 uptakes (Table 1) and the inhibitory effects of Trp and taurocholate on T_3 uptake were additive. Pre-incubation with monodansylcadaverine significantly reduced T_4 uptake (by about 30%; Table 1) but had no effect on T_3 uptake, whereas pre-incubation with *N*-ethylmaleimide resulted in a marked (~95%) stimulation of T_3 uptake (Table 1).

Table 1 Effect of putative inhibitors on Trp and TH uptake into HepG2 cells

Inhibitor	Inhibition of uptake of identified substrate (%)		
	50 μM Trp	50 nM T3	50 nM T_4
2 mM NEM	61.8 ± 8.7*	−94.2 ± 8.8*	ND
50 μM MDC	19.1 ± 6.4	−12.8 ± 10.6	27.9 ± 0.4*
0.5 mM Taur	−25.8 ± 11.9	27.5 ± 5.5*	28.0 ± 1.4*
Taur/Trp (0.5/10 mM)		58.4 ± 2.5*	ND

Results are expressed as means + SEM for $n = 3$ to 12 different experiments

NEM *N*-ethylmaleimide (pre-incubation), *MDC* monodansylcadaverine (pre-incubation), *Taur* taurocholate, *ND* not determined

Degree of significance is expressed in relation to uptake values in the absence of any inhibitor, *$p < 0.05$

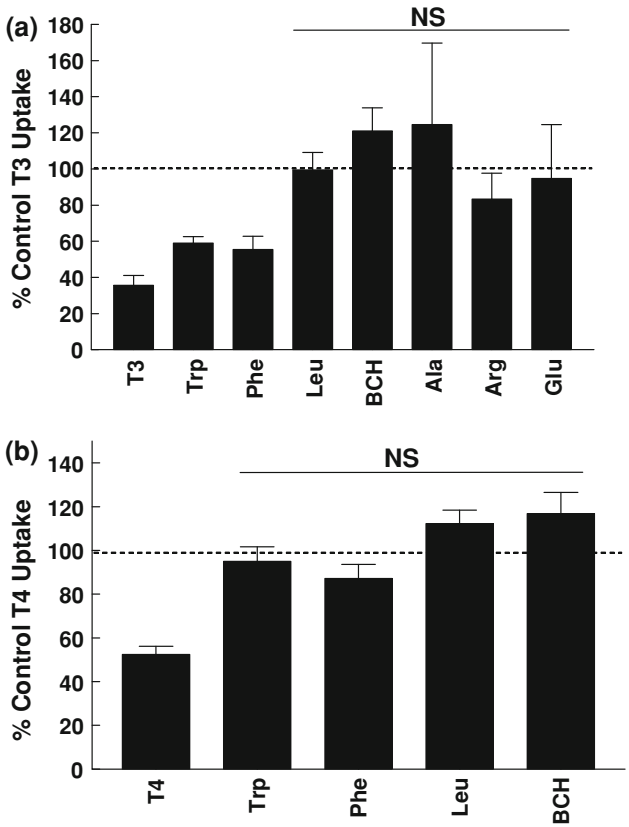

Fig. 5 Effect of amino acids on **a** T_3 and **b** T_4 uptake into HepG2 cells. Results are expressed as % remaining of 50 nM TH uptake ± SEM for 3–12 separate experiments. Inhibitions were significantly different from zero ($p < 0.05$), except where NS indicated. Self-inhibition by excess T_3/T_4 is shown to illustrate the extent of saturable TH uptake. Concentrations of inhibitors used were all 10 mM, except T_3/T_4 which were used at 10 μM

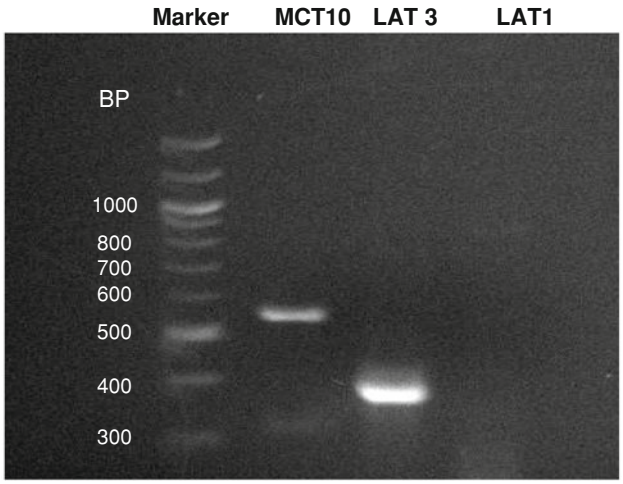

Fig. 6 Expression of LAT3 and MCT10 mRNA in HepG2 cells. PCR of HepG2 cell cDNA was performed using primer pairs for MCT10, LAT3 and LAT1. Amplicons corresponding to MCT10 (560 bp) and LAT3 (390 bp) but not LAT1 were detected. LAT1 was detected in human lymphocyte mRNA (not shown) as a positive control

Expression of thyroid hormone transporters in HepG2 cells

PCR of HepG2 cell cDNA revealed amplicons consistent with expression of mRNAs for LAT3 and MCT10, but not LAT1 (Fig. 6). Western blotting of HepG2 plasma membranes detected 4F2hc but not LAT1 System L amino acid transporter peptides (data not shown).

Discussion

Uptake of L-tryptophan into HepG2 cells is Na^+-independent and substantially inhibited by BCH, indicating that the major portion of Trp uptake occurs through amino acid transport System L, whilst the lack of any effect by Arg precludes involvement of Systems $b^{0,+}$ or y^+L. It is now clear that at least two distinct families of transporters contribute to System L transport activity. Neither of the two light chains of the heteromeric (SLC3/SLC7) System L transporters (LAT1 or LAT2) are normally expressed in the liver (Verrey et al. 2004) and we were unable to detect LAT1 expression (at least) in HepG2 cells. The System L transport of L-Trp demonstrated by HepG2 cells appears most likely to be mediated by the LAT3 transporter, which was first identified in human hepatocarcinoma cells (Babu et al. 2003) as a Na^+-independent and BCH-sensitive leucine transporter resembling hepatic System L2. LAT3, alongside LAT4, represents a new family of organic solute transporters (SLC43; Babu et al. 2003; Bodoy et al. 2005) which display System L-type transport but, unlike LAT1 and 2, do not require CD98/4F2hc for functional expression. *N*-Ethylmaleimide is a potent inhibitor of LAT3 (distinguishing it functionally from classical LAT1-mediated System L; Babu et al. 2003), hence the significant inhibitory effect of *N*-ethylmaleimide on Trp uptake into HepG2 cells is consistent with functional expression of LAT3-type System L2 and the detection of LAT3 mRNA expression in HepG2 cells. Neither BCH nor Arg had any significant effect on either T_3 or T_4 uptake into HepG2 cells, indicating that neither System L nor Systems y^+L/$b^{0,+}$, respectively, are involved with TH transport in this cell type. The System L permease LAT1 is a major effector of TH uptake into certain tissues (Taylor and Ritchie 2007 for review) but neither LAT3 nor LAT4 are reported to recognise T_3 as a substrate (Babu et al. 2003; Bodoy et al. 2005), providing an explanation as to why certain tissues (notably liver) express substantial System L transport activity but show no evident System L-type TH transport.

Interactions between transport of Trp (also Phe) and T_3 are likely to be through System T (Tryptophan preferring), which is functionally characterised as an Na^+-independent, *N*-ethylmaleimide-resistant transporter specific for aromatic amino acids which does not accept BCH as a substrate (Kemp and Taylor 1997; Blondeau et al. 1988). *N*-Ethylmaleimide and BCH were unable to completely abolish L-Trp uptake into HepG2 cells, indicating that there may be transport of L-Trp by the low-affinity System T in HepG2 cells secondary to that by System L. Uptake of T_3 into HepG2 cells appears to include at least two distinct components, based on the observation that inhibition by L-Trp and taurocholate on T_3 uptake together was largely additive. In HepG2 cells, it appears that T_3 is only capable of blocking the minor component of Trp transport through System T, but that System T contributes a much larger proportion of T_3 uptake itself. T_3 uptake in HepG2 cells is actually stimulated by *N*-ethylmaleimide, which may be due to direct effects on T_3 transport mechanisms or an indirect effect related to T_3 binding. A System T amino acid transporter (MCT10, also known as TAT1 or SLC16A10) has been characterised to transport aromatic amino acids and, most recently, iodothyronines (Friesema et al. 2008; Kim et al. 2001). MCT10 mRNA is expressed in HepG2 cells and the K_d value for the inhibition of L-Trp uptake by T_3 in this cell type (4.2 μM) is of the same order of magnitude as the T_3 concentration estimated to produce 50% inhibition of T_3 transport by human MCT10 overexpressed in COS1 cells (Friesema et al. 2008), consistent with our suggestion that System T is the site of TH–Trp interactions. MCT10 recognises substrates as anions, reflecting a structural similarity to H^+/monocarboxylate transporters of the SLC16 family, which includes the now well-established TH transporter MCT8 (Friesema et al. 2003). The exact mechanism by which TH and aromatic amino acids compete for transport by MCT10 remains

unresolved: the transporter mediates bidirectional facilitated diffusion of TH (Friesema et al. 2008) but appears to favour aromatic amino acid efflux (Friesema et al. 2008; Ramadan et al. 2006). MCT8 does not transport tryptophan or other amino acids although it accepts TRIAC alongside T_3, T_4 and rT_3 (Friesema et al. 2003). TRIAC had a substantial effect on the uptake of both T_3 and, especially, T_4 into HepG2 cells, probably reflecting a contribution of MCT8 (known to be expressed in HepG2 cells; Friesema et al. 2006) to their uptake.

The taurocholate-sensitive component of T_3 uptake into HepG2 cells is likely to be due at least partly to an organic anion transporter such as OATP1B1 (LST-1), the liver-specific bile acid transporter (Abe et al. 1999). THs are transported by multiple members of the organic anion transporting polypeptide (OATP) family, including OATP1A2, 1C1, 1B1 and 1B3 (Pizzagalli et al. 2002; van der Deure et al. 2008a, b), although these transporters do not interact with aromatic amino acids (Abe et al. 1998). The lack of any significant Na^+-dependent component of TH uptake in HepG2 cells precludes a substantial role for Na^+/taurocholate cotransporting polypeptide (Ntcp; SLC10A1) in iodothyronine uptake in this cell type (Friesema et al. 1999).

Amino acids do not significantly inhibit T_4 uptake into HepG2 cells under our experimental conditions, indicating that the mechanisms for T_4 uptake are largely distinct from those for Trp and also, to some extent, T_3. Taurocholate had a relatively large inhibitory effect on T_4 uptake into HepG2 cells, most likely due to competitive inhibition of uptake through an organic anion transporter (possibly OATP1B1) shared also by T_3 (Abe et al. 1999). The phenolic hydroxyl group of T_4 dissociates (becomes negatively charged) above pH 6.5, whereas that of T_3 only above pH 8.5 (Yen 2001); hence T_4 is (in relative terms) likely to be the better substrate for organic anion transporters. T_4 uptake into HepG2 cells also appears to include a small component (not shared by Trp or T_3) attributable to receptor-mediated endocytosis. Uptake of T_4 into trout liver (Riley and Eales 1994) has been shown to involve a binding/endocytosis mechanism distinct from that of T_3, lending support to this suggestion. MCT10 is able to transport T_4 (Friesema et al. 2008) and the absence of reciprocal inhibition between Trp and T_4 observed in our studies most likely reflects a relatively minor quantitative contribution of this transporter to total T_4 uptake. It should be noted that a small but significant proportion of total TH "uptake" associating with HepG2 cells appears to do so by non-saturable processes (this will include partitioning into the plasma membrane and passive diffusional uptake).

The HepG2 cell line is a useful model for studying the transport mechanisms of TH and aromatic amino acids in the liver. The TH and Trp uptake mechanisms described here for HepG2 cells are likely to be of importance for their intrahepatic metabolism and actions (e.g. genomic actions of TH binding to nuclear TH receptors; Gereben et al. 2008; Yen 2001). Indeed, we have shown previously that tryptophan suppresses T_3-dependent induction of TH-receptor–luciferase reporter gene expression in HepG2 cells (Taylor et al. 2003). It is possible that different transport mechanisms may be responsible for delivery of TH and Trp to different intrahepatic pools (e.g. nuclear-receptor bound and metabolic pools) and/or different hepatic compartments. For example, MCT10 is expressed selectively in the functionally distinct perivenous population of hepatocytes (Ramadan et al. 2006), alongside hepatic deiodinase D1 (Zandieh Doulabi et al. 2002): it is therefore tempting to speculate that MCT10 and D1 interact to facilitate extrathyroidal T_3 production from plasma T_4 and that this process may be influenced by nutritional circumstances altering Trp availability (e.g. high-protein diet, starvation).

Acknowledgments We are grateful to the UK Biotechnology and Biological Sciences Research Council and Tenovus (UK) for financial support, Fiona Mitchell and Lisa Roy for technical assistance and Dr Calum Sutherland for the gift of HepG2 cell cDNA.

References

Abe T et al (1998) Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate and comparison with oatp2. J Biol Chem 273:22395–22401

Abe T et al (1999) Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem 274:17159–17163

Abe T, Suzuki T, Unno M, Tokui T, Ito S (2002) Thyroid hormone transporters: recent advances. Trends Endocrinol Metab 13:215–220

Babu E et al (2003) Identification of a novel system L amino acid transporter structurally distinct from heterodimeric amino acid transporters. J Biol Chem 278:43838–43845

Blondeau JP, Osty J, Francon J (1988) Characterization of the thyroid hormone transport system of isolated hepatocytes. J Biol Chem 263:2685–2692

Bodoy S, Martin L, Zorzano A, Palacin M, Estevez R, Bertran J (2005) Identification of LAT4, a novel amino acid transporter with system L activity. J Biol Chem 280:12002–12011

Friesema EC et al (1999) Identification of thyroid hormone transporters. Biochem Biophys Res Commun 254:497–501

Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ (2003) Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 278:40128–40135

Friesema EC, Jansen J, Milici C, Visser TJ (2005) Thyroid hormone transporters. Vitam Horm 70:137–167

Friesema EC, Kuiper GG, Jansen J, Visser TJ, Kester MH (2006) Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism. Mol Endocrinol 20:2761–2772

Friesema ECH, Jansen J, Jachtenberg J-w, Visser WE, Kester MHA, Visser TJ (2008) Effective cellular uptake and efflux of thyroid

hormone by human monocarboxylate transporter 10. Mol Endocrinol 22:1357–1369

Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold A, Bianco AC (2008) Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev 29:898–938

Goenner S, Boutron A, Soni T, Lemonnier A, Moatti N (1992) Amino acid transport systems in the human hepatoma cell line Hep G2. Biochem Biophys Res Commun 189:472–479

Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ (2001) Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev 22:451–476

Horiuchi R, Cheng SY, Willingham M, Pastan I (1982) Inhibition of the nuclear entry of 3,3′,5′-triiodo-L-thyronine by monodansylcadaverine in GH3 cells. J Biol Chem 257:3139–3144

Inui KI, Masuda S, Saito H (2000) Cellular and molecular aspects of drug transport in the kidney. Kidney Int 58:944–958

Kaper T, Looger LL, Takanaga H, Platten M, Steinman L, Frommer WB (2007) Nanosensor detection of an immunoregulatory tryptophan influx/kynurenine efflux cycle. PLoS Biol 5:e257

Kemp HF, Taylor PM (1997) Interactions between thyroid hormone and tryptophan transport in rat liver are modulated by thyroid status. Am J Physiol 272:E809–E816

Kim DK, Kanai Y, Chairoungdua A, Matsuo H, Cha SH, Endou H (2001) Expression cloning of a Na^+-independent aromatic amino acid transporter with structural similarity to H^+/monocarboxylate transporters. J Biol Chem 276:17221–17228

Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ (2002) Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. Mol Endocrinol 16:2283–2296

Ramadan T, Camargo SM, Summa V, Hunziker P, Chesnov S, Pos KM, Verrey F (2006) Basolateral aromatic amino acid transporter TAT1 (Slc16a10) functions as an efflux pathway. J Cell Physiol 206:771–779

Riley WW Jr, Eales JG (1994) Characterization of 3,5,3′-triiodo-L-thyronine transport into hepatocytes isolated from juvenile rainbow trout (*Oncorhynchus mykiss*), and comparison with L-thyroxine transport. Gen Comp Endocrinol 95:301–309

Ritchie JW, Taylor PM (2001) Role of the System L permease LAT1 in amino acid and iodothyronine transport in placenta. Biochem J 356:719–725

Ritchie JW, Shi YB, Hayashi Y, Baird FE, Muchekehu RW, Christie GR, Taylor PM (2003) A role for thyroid hormone transporters in transcriptional regulation by thyroid hormone receptors. Mol Endocrinol 17:653–661

Rose DP (1972) Aspects of tryptophan metabolism in health and disease: a review. J Clin Pathol 25:17–25

Salter M, Knowles RG, Pogson CI (1986) Transport of the aromatic amino acids into isolated rat liver cells. Properties of uptake by two distinct systems. Biochem J 233:499–506

Smith PK et al (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85

Taylor PM, Ritchie JW (2007) Tissue uptake of thyroid hormone by amino acid transporters. Best Pract Res Clin Endocrinol Metab 21:237–251

Taylor PM, Baird FE, Christie GR, Hayashi Y, Ritchie JWA (2003) Membrane transporters as modulators of protein metabolism. Nova Acta Leopoldina 87(329):321–331

van der Deure WM, Hansen PS, Peeters RP, Kyvik KO, Friesema EC, Hegedus L, Visser TJ (2008a) Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. Endocrinology 149:5307–5314

van der Deure WM et al (2008b) Organic anion transporter 1B1: an important factor in hepatic thyroid hormone and estrogen transport and metabolism. Endocrinology 149:4695–4701

van Stralen PG, van der Hoek HJ, Docter R, de Jong M, Krenning EP, Everts ME, Hennemann G (1996) Uptake and metabolism of 3,5,3′-triiodothyronine and 3,3′,5′-triiodothyronine by human liver-derived cells: HepG2 cells as a model for thyroid hormone handling by human liver. J Clin Endocrinol Metab 81:244–248

Verrey F, Closs EI, Wagner CA, Palacin M, Endou H, Kanai Y (2004) CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch 447:532–542

Yen PM (2001) Physiological and molecular basis of thyroid hormone action. Physiol Rev 81:1097–1142

Zandieh Doulabi B, Platvoet-ter Schiphorst M, van Beeren HC, Labruyere WT, Lamers WH, Fliers E, Bakker O, Wiersinga WM (2002) TR{beta}1 protein is preferentially expressed in the pericentral zone of rat liver and exhibits marked diurnal variation. Endocrinology 143:979–984