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# Thyroid Hormone Transport by the Heterodimeric Human System L Amino Acid Transporter

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**Transport of thyroid hormone across the cell membrane is required for thyroid hormone action and metabolism. We have investigated the possible transport of iodothyronines by the human system L amino acid transporter, a protein consisting of the human 4F2 heavy chain and the human LAT1 light chain. *Xenopus* oocytes were injected with the cRNAs coding for human 4F2 heavy chain and/or human LAT1 light chain, and after 2 d were incubated at 25 C with 0.01–10 μM [$^{125}$I]$T_4$, [$^{125}$I]$T_3$, [$^{125}$I]$rT_3$, or [$^{125}$I]3,3′-diiodothyronine or with 10–100 μM [$^3$H]arginine, [$^3$H]leucine, [$^3$H]phenylalanine, [$^3$H]tyrosine, or [$^3$H]tryptophan. Injection of human 4F2 heavy chain cRNA alone stimulated the uptake of leucine and arginine due to dimerization of human 4F2 heavy chain with an endogenous *Xenopus* light chain, but did not affect the uptake of other ligands. Injection of human LAT1 light chain cRNA alone did not stimulate the uptake of any ligand. Coinjection of cRNAs for human 4F2 heavy chain and human LAT1 light chain stimulated the uptake of phenylalanine > tyrosine > leucine > tryptophan (100 μM) and of 3,3′-diiodothyronine > $rT_3 \sim T_3 > T_4$ (10 nM), which in all cases was $Na^+$ independent. Saturation analysis provided apparent Michaelis constant ($K_m$) values of 7.9 μM for $T_4$, 0.8 μM for $T_3$, 12.5 μM for $rT_3$, 7.9 μM for 3,3′-diiodothyronine, 46 μM for leucine, and 19 μM for tryptophan. Uptake of leucine, tyrosine, and tryptophan (10 μM) was inhibited by the different iodothyronines (10 μM), in particular $T_3$. *Vice versa*, uptake of 0.1 μM $T_3$ was almost completely blocked by coincubation with 100 μM leucine, tryptophan, tyrosine, or phenylalanine.**

**Our results demonstrate stereospecific $Na^+$-independent transport of iodothyronines by the human heterodimeric system L amino acid transporter. (*Endocrinology* 142: 4339–4348, 2001)**

THYROID HORMONE IS essential for the development of different organs, in particular the brain, and for the metabolic control of virtually all tissues throughout life (1–3). Its major effects include stimulation of oxygen consumption and thermogenesis; acceleration of carbohydrate, protein, lipid, and bone mineral turnover; and increased contractility of skeletal muscles and heart (2, 3). Most actions of thyroid hormone are initiated by binding of the active form of $T_3$ to nuclear receptors, which are associated with regulatory elements in the promoter region of target genes (3, 4). Binding of $T_3$ to its receptor induces the release of corepressors and the recruitment of coactivators, usually resulting in the stimulation of gene transcription (4).

Although $T_3$ is the receptor-active form of thyroid hormone, its precursor $T_4$ is the predominant product secreted by the follicular cells of the thyroid gland (5, 6). Although some $T_3$ is also secreted, most $T_3$ is produced by enzymatic outer ring deiodination of $T_4$ in peripheral tissues (5, 6). Both $T_4$ and $T_3$ are inactivated by inner ring deiodination to the metabolites $rT_3$ and 3,3′-diiodothyronine (3,3′-$T_2$), respectively (5, 6). The three deiodinases (D1–D3) involved in these conversions are homologous selenoproteins with different catalytic profiles, tissue distributions, and physiological functions (7, 8). D1 in liver and kidney appears important for systemic $T_3$ production, D2 in tissues such as brain and pituitary for local $T_3$ production, and D3 in brain and other tissues for $T_4$ and $T_3$ degradation. All three deiodinases are transmembrane proteins with their active site exposed to the cytoplasm (5–8).

The metabolism and action of thyroid hormone are intracellular events requiring the uptake of extracellular hormone through the plasma membrane. Although iodothyronines are lipid-soluble compounds, they cannot readily cross the lipid bilayer of the cell membrane by simple diffusion. This is because the polar zwitter-ionic alanine side-chain prevents passage of the iodothyronine molecule through the hydrophobic inner part of the cell membrane constituted of the aliphatic fatty acid chains. Evidence accumulated over the last two decades indicates that uptake of thyroid hormone in different tissues is mediated by transporters (6, 9–12). Work in our laboratory has demonstrated the presence of multiple iodothyronine transporters in rat and human liver cells (6, 11, 12). Two energy- and $Na^+$-dependent transporters appear of particular importance for hepatic uptake of $T_4$ and $rT_3$, and of $T_3$, respectively, showing nanomolar affinities for their ligands, but different dependencies on cellular ATP levels (6, 11, 12). However, iodothyronine transporters in other tissues show different characteristics. Isolated rat pituitary cells also show carrier-mediated uptake of different iodothyronines,

Abbreviations: Arg, Arginine; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; BSP, bromosulfophthalein; D1, D2, D3, deiodinases 1, 2, and 3; 4F2hc, 4F2 heavy chain; *h*4F2hc, human 4F2 heavy chain; *h*LAT1, human LAT1 light chain; Leu, leucine; MIT, 3-(mono)iodotyrosine; Phe, phenylalanine; 3,3′-$T_2$, 3,3′-diiodothyronine; TC, taurocholate; Triac, 3,3′,5-triiodothyroacetic acid; Trp, tryptophan; Tyr, tyrosine; $V_{max}$, maximum uptake rate.

but this appears to be mediated by a single transporter (13). In contrast, cultured neonatal rat cardiomyocytes show specific uptake of $T_3$, but not of $T_4$ (14). A variety of cells, including rat pituitary cells (13), GH-producing tumor cells (15), erythrocytes (16, 17), cardiomyocytes (14), and astrocytes (18); mouse neuroblastoma (19) and thymocytes (20); and human choriocarcinoma cells (21, 22), show competition between uptake of iodothyronines and neutral amino acids such as leucine (Leu) and tryptophan (Trp). This may not be surprising, because iodothyronines are iodinated amino acid derivatives built from two tyrosine (Tyr) molecules. Based on the above observations it has been suggested that thyroid hormone may be taken up in different tissues at least in part through system L or system T amino acid transporters (15–19).

In particular through the pioneering work of Christensen (23, 24), different classes of amino acid transporters have been distinguished on the basis of their preference for certain types of amino acids (*e.g.* neutral, acidic, or basic), their specificity for natural or artificial prototypic ligands, as well as their mechanism of transport (*e.g.* $Na^+$ dependent or $Na^+$ independent). A rapidly increasing number of amino acid transporters has been characterized in recent years, including the 4F2-related heterodimeric transporters (for reviews, see Refs. 25–27). The 4F2 or CD98 cell surface antigen has been known for some time to be expressed in many tissues, especially on activated lymphocytes and tumor cells, but only recently has it been identified as a family of amino acid transporters (25–28). These heterodimeric transporters each consist of a common 4F2 heavy chain (4F2hc) and a member of a family of homologous light chains, 7 of which have now been cloned (25–41). 4F2hc is a glycosylated protein with a single transmembrane domain, whereas the light chains are not glycosylated and have 12 putative transmembrane domains; they are linked through a disulfide bond (25–28). One of the 7 light chains mentioned above appears to dimerize preferentially with another heavy chain, termed rBAT (for related to basic amino acid transport), which is homologous to 4F2hc, suggesting the existence of a superfamily of heterodimeric amino acid transporters consisting of multiple heavy and light chains (25–28, 38–40).

In combination with 4F2hc, two 4F2 light chains mediate the $Na^+$-independent transport of large neutral (branched chain and aromatic) amino acids such as Leu, Tyr, Trp, and phenylalanine (Phe). This is typical for the system L amino acid transporter, hence the names LAT1 and LAT2 for these light chains (25–27, 29–33). Two other light chains forming heterodimers with 4F2hc mediate the $Na^+$-dependent uptake of neutral amino acids such as Leu as well as the $Na^+$-independent uptake of basic amino acids such as arginine (Arg). This is characteristic of the system $y^+L$ amino acid transporter, which is why these light chains are named $y^+$LAT1 and $y^+$LAT2 (25–27, 34–36). We have tested the possible involvement of these heterodimeric 4F2 transporters in the transport of thyroid hormone by studying the uptake of the iodothyronines $T_4$, $T_3$, $rT_3$, and 3,3′-$T_2$ by *Xenopus laevis* oocytes injected with cRNA coding for human 4F2hc (*h*4F2hc) alone or in combination with cRNA coding for human LAT1 (*h*LAT1), mouse LAT2 (*m*LAT2), *h*$y^+$LAT1, or *h*$y^+$LAT2. Whereas the system $y^+L$ transporters did not mediate the uptake of iodothyronines, effective thyroid hormone transport was observed with the system L transporters, in particular the *h*4F2hc/*h*LAT1 heterodimer, which is the subject of this report.

## Materials and Methods

### *Materials*

Nonradioactive L-iodothyronines and 3,3′,5-triiodothyroacetic acid (Triac) were obtained from Henning Berlin GmbH & Co. (Berlin, Germany). 3′,5′-[$^{125}$I]$T_4$, 3′-[$^{125}$I]$T_3$, and carrier-free $Na^{125}I$ were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). 3′,5′-[$^{125}$I]$rT_3$ and 3,3′-[$^{125}$I]$T_2$ were prepared by radioiodination of 3,3′-$T_2$ and 3-monoiodothyronine, respectively, using the chloramine-T method, followed by purification on Sephadex LH-20 (Amersham Pharmacia Biotech). [$^{125}$I]$T_4$ and [$^{125}$I]$rT_3$ were also purified on Sephadex LH-20 immediately before use (42). D-$T_3$, Phe, Tyr, and 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) were purchased from Sigma (St. Louis, MO), Arg and Leu were obtained from Merck & Co., Inc. (Darmstadt, Germany), and Trp and bromosulfophthalein (BSP) were purchased from Fluka (Buchs, Switzerland). $^3$H-Labeled Arg, Leu, Phe, Tyr, and Trp were purchased from Amersham Pharmacia Biotech. All other chemicals were of reagent grade.

### *RNA preparation*

The plasmids containing cDNA coding for *h*4F2hc and *h*LAT1, pSPORT1-*h*4F2hc (43) and pcDNA1-E16 (29), were linearized with *Hin*dIII and *Eco*RV (Roche, Mannheim, Germany), respectively, and transcribed using the Ampliscribe High Yield T7 RNA transcription kit (Epicentre, Madison, WI). The cRNAs were capped with the m7G (5′)ppp (5′)G cap analog (Epicentre) and stored in sterile water at −80 C.

### *Oocyte isolation and cRNA injection*

Oocytes were prepared as described previously (44). After isolation, oocytes were sorted on morphological criteria and defolliculated manually. Healthy-looking stage V–VI oocytes were kept at 18 C in modified Barth's solution containing 20 IU/ml penicillin and 20 μg/ml streptomycin (44). The next day, oocytes were injected with 2.3 ng *h*4F2hc cRNA and/or 2.3 ng *h*LAT1 cRNA in 23 nl water using the Nanoject system (Drummond Scientific, Broomall, PA). Uninjected oocytes were used as controls, as similar results were obtained using water-injected oocytes. Injected and uninjected oocytes were kept for 2 d at 18 C in modified Barth's solution.

### *Uptake*

Uptake assays were performed as reported previously (44). Groups of 8–10 oocytes were incubated for 2–60 min at 25 C with 0.01–10 μM [$^{125}$I]$T_4$, [$^{125}$I]$T_3$, [$^{125}$I]$rT_3$, or [$^{125}$I]3,3′-$T_2$, or with 10–100 μM [$^3$H]Arg, [$^3$H]Leu, [$^3$H]Phe, [$^3$H]Tyr, or [$^3$H]Trp in 0.1 ml incubation medium [100 mM NaCl or choline chloride (ChCl), 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM Tris, pH 7.5]. After incubation, oocytes were washed four times with 2.5 ml ice-cold $Na^+$-containing incubation medium containing 0.1% BSA. Oocytes were transferred to new tubes and counted individually.

### *Efflux*

Oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1 were incubated in groups of 8–10 for 30 min at 25 C with 10 μM [$^3$H]Leu or 0.1 μM [$^{125}$I]$T_3$ or [$^{125}$I]$T_2$ in 0.1 ml $Ch^+$-containing incubation medium. One group of oocytes was processed to determine the total uptake of each ligand as described above. Efflux of internalized ligand from other groups of oocytes was analyzed as follows. After removal of the medium, oocytes were rapidly washed with 0.5 ml $Ch^+$-containing incubation medium at 25 C and incubated for successive 2-min periods at 25 C with 0.5 ml of the same medium without or with 10 mM unlabeled Leu. After each interval, medium was rapidly replaced by fresh medium and counted for radioactivity. Radioactivity still associated with the oocytes

at the end of the 20-min total efflux period was counted as well. Efflux was quantified by expressing the cumulative release of radioactivity as a percentage of that present in the oocytes at the start of the efflux period.

### *Statistics*

Data are presented as the mean ± SEM. Differences were tested for statistical significance by *t* test. Kinetic parameters were determined by fitting the plot of uptake rate (v) *vs.* ligand concentration (S) to the Michaelis-Menten equation: $v = V_{max}/(1 + K_m/S)$, where $V_{max}$ is the maximum uptake rate, and $K_m$ is the Michaelis constant.

## Results

Initial experiments were carried out to reproduce the induction of amino acid transport in *Xenopus* oocytes after injection of cRNAs coding for 4F2-related proteins as reported by others (25–41). Uninjected and water-injected oocytes showed negligible uptake of the basic amino acid Arg and different neutral amino acids, *i.e.* the aliphatic amino acid Leu and the aromatic amino acids Phe, Tyr, and Trp. Figure 1A shows the effects of injection of 2.3 ng *h*4F2hc cRNA alone or together with 2.3 ng *h*LAT1 cRNA on the uptake of Leu, Phe, Tyr, Trp, and Arg. Incubations were performed 2 d after cRNA injection at a ligand concentration of 50 μM using incubation medium containing $Na^+$ or choline ($Ch^+$). Injection of oocytes with cRNA coding for *h*LAT1 alone did not stimulate transport of the different amino acids (data not shown). Injection of *h*4F2hc cRNA alone did not effect transport of Phe, Tyr, or Trp, but induced $Na^+$-dependent transport of Leu and $Na^+$-independent transport of Arg. This is characteristic for the induction of a $y^+$L-type transporter, which has been documented in different studies and is explained by the dimerization of exogenous *h*4F2hc with an endogenous $y^+$LAT-type light chain expressed in native oocytes (25–30). This assumption is supported by observations that injection of cRNA coding for *h*$y^+$LAT1 or *h*$y^+$LAT2 in addition to *h*4F2hc cRNA further markedly increased $Na^+$-dependent transport of Leu and $Na^+$-independent transport of Arg compared with those in oocytes injected with *h*4F2hc cRNA alone (data not shown). Coinjection of oocytes with *h*4F2hc cRNA and *h*LAT1 cRNA did not increase Arg transport above that observed after injection of *h*4F2hc cRNA alone. Compared with oocytes injected with *h*4F2hc cRNA alone, oocytes injected in addition with *h*LAT1 cRNA showed a further marked increase in Leu uptake, which, however, became almost completely $Na^+$ independent. Coinjection of cRNA coding for *h*4F2hc and *h*LAT1 also resulted in a large induction of the transport of Phe and Tyr and a much smaller increase in the uptake of Trp, which in all cases was $Na^+$ independent. This is in agreement with previous reports, and characteristic for the induction of an L-type amino acid transporter. Marked stimulation of the $Na^+$-independent uptake of 100 μM Leu was also observed after coinjection of *h*4F2hc and *m*LAT2 cRNA (data not shown).

Figure 1B shows the uptake of 10 μM Leu, Tyr, and Trp in $Ch^+$-containing medium by oocytes coinjected with *h*4F2hc and *h*LAT1 cRNA as a function of incubation time. Data were corrected for transport in uninjected oocytes, which was significant only for Trp because of the relatively small induction of Trp transport by *h*4F2hc plus *h*LAT1 cRNA injection. Trp uptake was linear with time of incubation for at least 60 min. Transport of Leu and Tyr was much faster than Trp uptake and was linear with time for only approximately 15 min.

Figure 2A shows the uptake of $T_4$, $T_3$, $rT_3$, or 3,3′-$T_2$ by uninjected oocytes and oocytes injected with cRNA coding for *h*4F2hc and *h*LAT1 after incubation for 1 h with 10 nM iodothyronine in medium with or without $Na^+$. As shown previously, significant uptake of iodothyronines was observed in uninjected oocytes, which is a major drawback of this expression system for the cloning of thyroid hormone transporters. Iodothyronine uptake by native oocytes decreased in the order 3,3′-$T_2 \sim T_3 > T_4 > rT_3$ and was somewhat lower in incubation medium containing $Ch^+$ instead of $Na^+$. Injection of oocytes with *h*4F2hc cRNA alone or with *h*LAT1 cRNA alone did not increase the uptake of any iodothyronine (data not shown). The lack of effect of injection with *h*4F2hc cRNA alone suggests that the $y^+$L-type transporter generated by dimerization of the exogenous heavy chain with an endogenous light chain does not mediate transport of iodothyronines. This is supported by findings that coinjection of *h*4F2hc cRNA and cRNA coding for *h*$y^+$LAT1 or *h*$y^+$LAT2 did not stimulate iodothyronine transport in oocytes (data not shown). However, injection of oocytes with both cRNA coding for *h*4F2hc and *h*LAT1 resulted in significant increases in net iodothyronine uptake, which decreased in the order 3,3′-$T_2 > rT_3 \sim T_3 > T_4$, and in all cases was $Na^+$ independent. Smaller increments in iodothyronine uptake were noted after coinjection of *h*4F2hc cRNA and *m*LAT2 cRNA (data not shown).

Figure 2B shows the uptake of 0.1 μM $T_3$ and 3,3′-$T_2$ induced by injection of oocytes with both *h*4F2hc and *h*LAT1 cRNA as a function of time of incubation in $Ch^+$-containing medium. Transport of $T_3$ was linear with time for at least 60 min. Transport of 3,3′-$T_2$ was much faster than $T_3$ uptake and was linear with time for about 45 min.

The saturation kinetics of iodothyronine uptake by the heterodimeric *h*4F2hc/*h*LAT1 transporter were studied by incubation of oocytes injected with cRNA for both subunits during 1 h with 0.1–10 μM ligand in $Ch^+$-containing medium. Iodothyronine uptake through the oocytes' endogenous transporter(s) was determined in parallel incubations with uninjected oocytes. The results are presented in Fig. 3, showing that iodothyronine uptake was saturable in both uninjected and cRNA-injected oocytes. Michaelis-Menten analysis of the results obtained with uninjected oocytes provided apparent $K_m$ values of 2–14 μM for the different iodothyronines. Iodothyronine transport mediated by the *h*4F2hc/*h*LAT1 transporter was determined by subtraction of the uptake rates in uninjected oocytes from those observed in oocytes injected with the cRNAs for both subunits. Michaelis-Menten analysis of the corrected data provided apparent $K_m$ values of 7.9 μM for $T_4$, 0.8 μM for $T_3$, 12.5 μM for $rT_3$, and 7.9 μM for 3,3′-$T_2$. $V_{max}$ values were 2.6, 1.1, 11.3, and 28 pmol/oocyte·h for $T_4$, $T_3$, $rT_3$, and 3,3′-$T_2$, respectively. The fold stimulation of iodothyronine uptake induced by injection of oocytes with cRNA for *h*4F2hc and *h*LAT1 varied with increasing ligand concentration (0.1–10 μM) from 2.1–2.7 for $T_4$, from 2.2–1.9 for $T_3$, from 4.4–7.6 for $rT_3$, and from 3.2–13.7 for 3,3′-$T_2$. The kinetics of transport of Leu and Trp by the

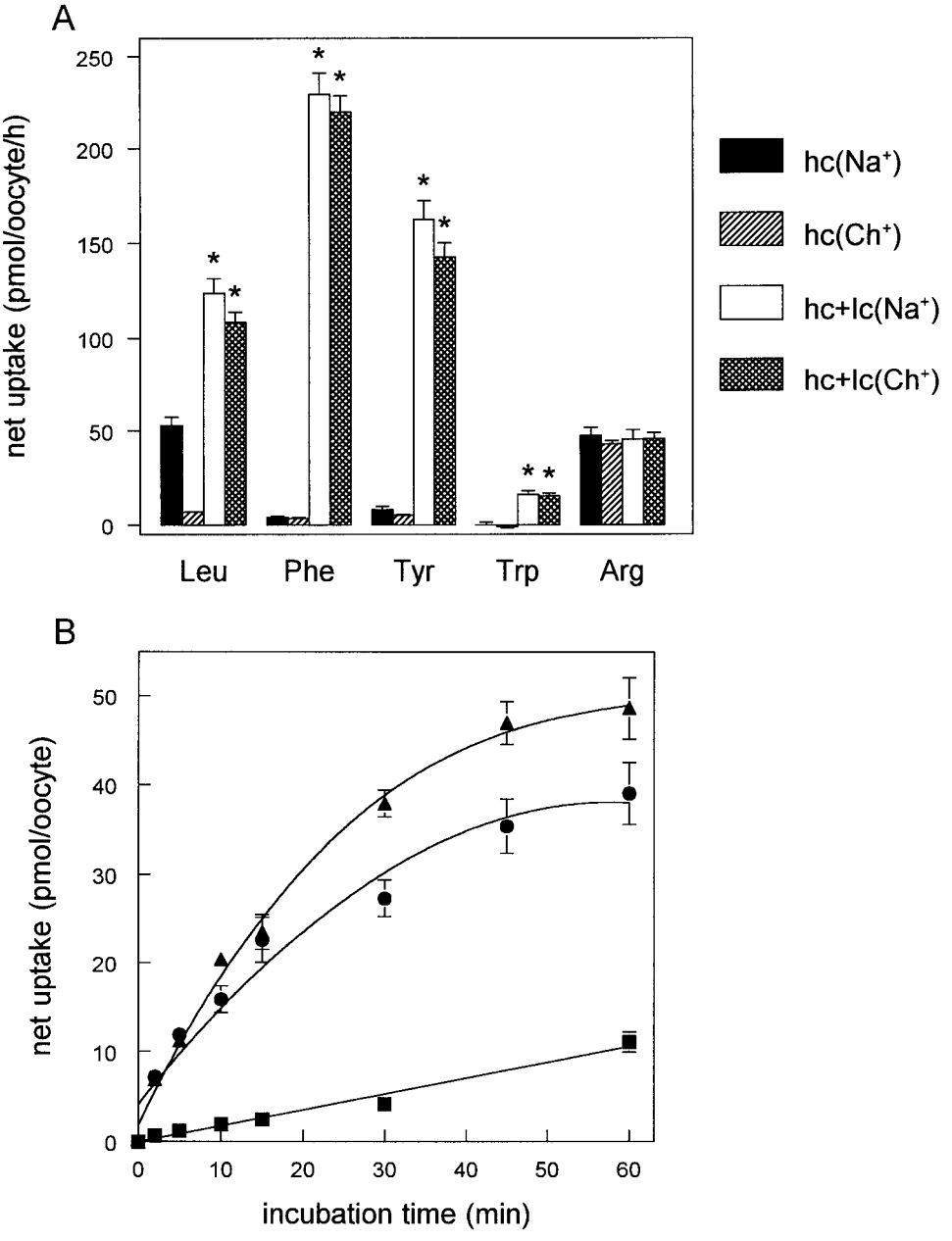


FIG. 1. A, Uptake of amino acids by *Xenopus* oocytes injected with cRNA coding for *h*4F2hc alone (hc) or in combination with cRNA coding for *h*LAT1 (hc+lc). Oocytes were incubated for 60 min at 25 C with 50 $\mu$M $^3$H-labeled Leu, Phe, Tyr, Trp, or Arg in incubation medium containing $Na^+$ or $Ch^+$. Data were corrected for minor uptake observed in uninjected oocytes. B, Time course of uptake of amino acids by oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1. Oocytes were incubated for 2–60 min with 10 $\mu$M $^3$H-labeled Leu (●), Tyr (▲), or Trp (■) in $Ch^+$-containing medium. Data were corrected for minor uptake observed in uninjected oocytes. Data are the mean ± SEM of 8–10 oocytes. *, $P < 0.001$ *vs.* corresponding oocytes injected with *h*4F2hc cRNA alone.

*h*4F2hc/*h*LAT1 transporter were analyzed similarly using ligand concentrations of 1–100 $\mu$M, yielding $K_m$ values of 46 $\mu$M for Leu and 19 $\mu$M for Trp (data not shown). These data are in good agreement with previous reports (29–32).

Competition between iodothyronine and amino acid transport by the heterodimeric *h*4F2hc/*h*LAT1 transporter was studied by testing the effects of 10 $\mu$M unlabeled iodothyronine on the uptake of 10 $\mu$M labeled Leu, Tyr, or Trp or the effects of 100 $\mu$M unlabeled amino acid on the uptake of 0.1 $\mu$M labeled $T_3$. Figure 4A demonstrates that uptake of 10 $\mu$M Leu, Tyr, and Trp by *h*4F2hc/*h*LAT1 was inhibited by the different iodothyronines. The degree of competition was greatest with $T_3$, in agreement with its low apparent $K_m$ value. *Vice versa*, uptake of 0.1 $\mu$M $T_3$ by the *h*4F2hc/*h*LAT1 transporter was almost completely inhibited by 100 $\mu$M Leu, Trp, Tyr, or Phe, whereas iodothyronine uptake by the endogenous transporter(s) was not affected (Fig. 4B).

The specificity of iodothyronine and amino acid transport by the *h*4F2/*h*LAT1 transporter was further investigated by testing the effects of the $T_3$ analogs D-$T_3$ and Triac, the organic anions BSP and taurocholate (TC), and the prototypic L-type ligand BCH on uptake of L-$T_3$ and L-Leu by the heterodimeric amino acid transporter (Fig. 5). Uptake was studied in the absence of $Na^+$ using 0.1 $\mu$M [$^{125}$I]$T_3$ or 10 $\mu$M [$^3$H]Leu as ligands and oocytes injected with cRNA for *h*4F2hc and *h*LAT1 and was corrected for uptake in oocytes injected with *h*4F2hc cRNA only. In general, Leu uptake was somewhat less sensitive to the different competitors than $T_3$ uptake, perhaps because Leu uptake was tested at a relatively high ligand concentration. Neither [$^{125}$I]$T_3$ nor [$^3$H]Leu uptake

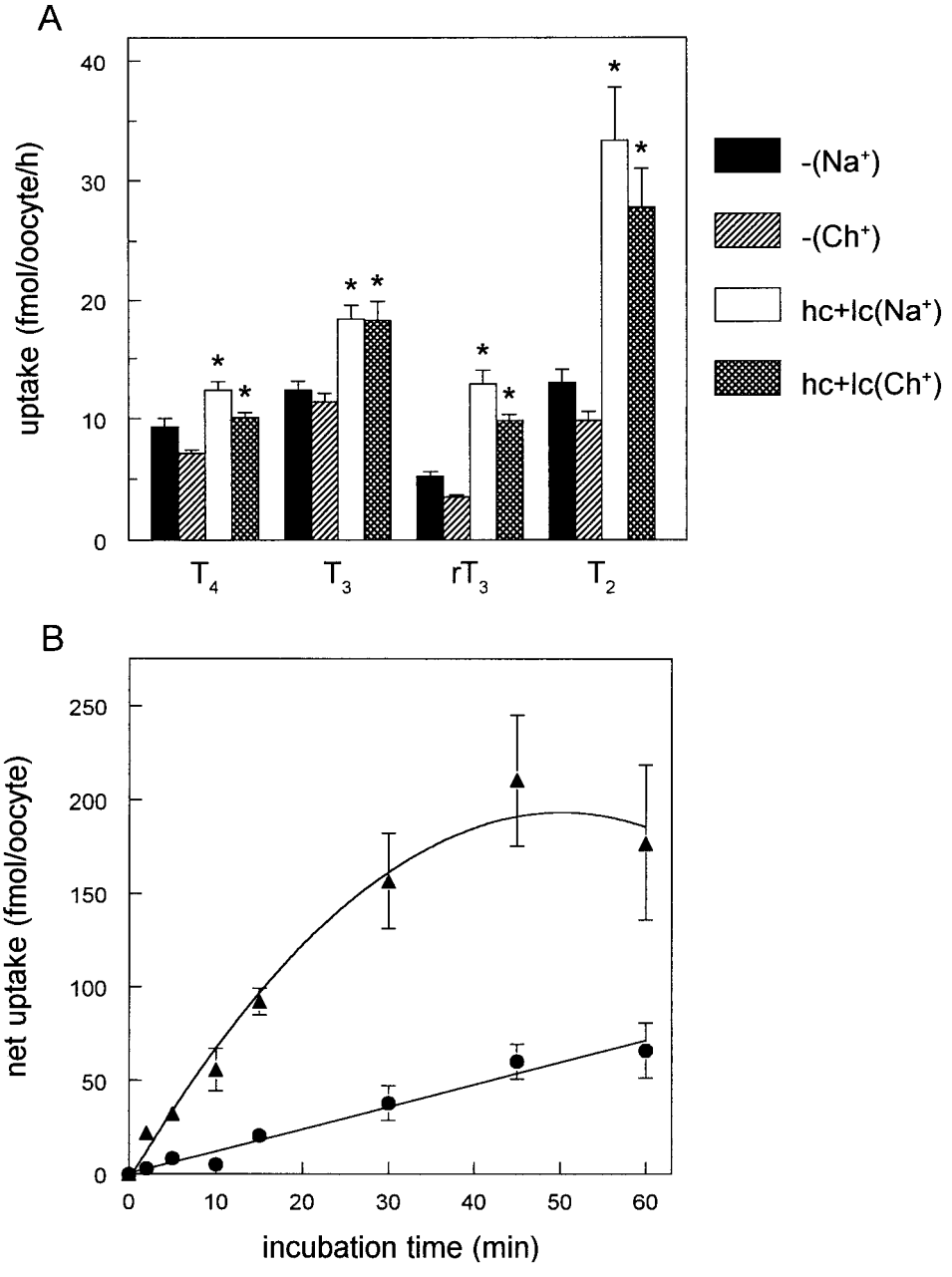


FIG. 2. A, Uptake of iodothyronines by uninjected *Xenopus* oocytes (−) or oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1 (hc+lc). Oocytes were incubated for 60 min at 25 C with 10 nM $^{125}$I-labeled $T_4$, $T_3$, $rT_3$, or 3,3′-$T_2$ in incubation medium containing $Na^+$ or $Ch^+$. B, Time course of uptake of iodothyronines by oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1. Oocytes were incubated for 2–60 min with 0.1 μM $^{125}$I-labeled $T_3$ (●) or 3,3′-$T_2$ (▲) in $Ch^+$-containing medium. Data were corrected for uptake observed in uninjected oocytes. Data are the mean ± SEM of 8–10 oocytes. *, $P < 0.01$ *vs.* corresponding uninjected oocytes.

was significantly inhibited by 10 μM D-$T_3$ or Triac, in contrast to the potent inhibition by 10 μM L-$T_3$. Tested at 100 μM, BCH produced at least the same marked inhibition of [$^3$H]Leu and [$^{125}$I]$T_3$ uptake as Leu itself. The prototypic organic anion transporter ligand BSP and the bile acid TC had little effect on uptake of either ligand (Fig. 5).

It has been demonstrated that the *h*4F2hc/*h*LAT1 transporter mediates the exchange of intra- and extracellular amino acids (29, 30). In agreement with these reports, efflux of labeled Leu taken up by *h*4F2hc/*h*LAT1-expressing oocytes was greatly stimulated by the addition of 10 mM unlabeled Leu to the efflux medium (Fig. 6A). In contrast, 10 mM extracellular Leu only slightly stimulated the efflux of 3,3′-$T_2$ internalized by *h*4F2hc/*h*LAT1-expressing oocytes (Fig. 6B), whereas it did not affect the efflux of $T_3$ taken up by such oocytes (data not shown).

## Discussion

In agreement with previous reports from our laboratory, uninjected or water-injected oocytes show significant transport of iodothyronines (44–46). Uptake of iodothyronines by native oocytes is partially $Na^+$ dependent and is saturated at increasing ligand concentrations, with apparent $K_m$ values of 2–14 μM, suggesting the involvement of one or more unidentified transporters. This endogenous iodothyronine transport is not inhibited by the addition of large concentrations of different amino acids, which suggests that iodothyronines are not taken up by amino acid transporters na-

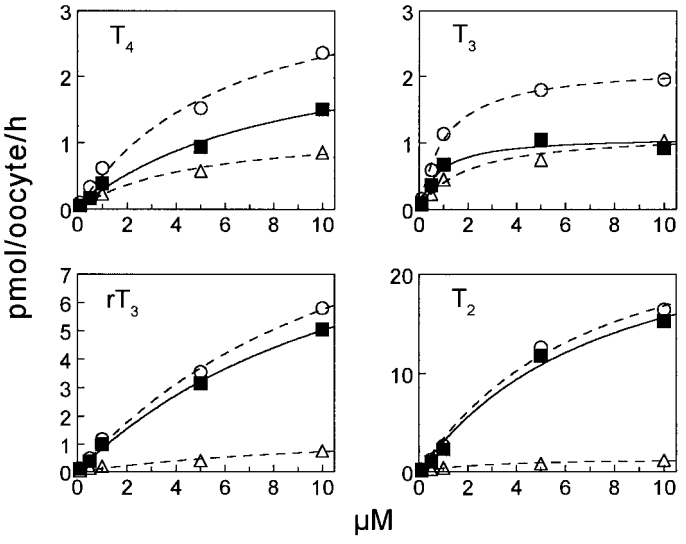


FIG. 3. Ligand concentration-dependent uptake of iodothyronines by uninjected *Xenopus* oocytes (△) or oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1 (○). Oocytes were incubated for 60 min with 0.1–10 $\mu$M $^{125}$I-labeled $T_4$, $T_3$, $rT_3$, or 3,3′-$T_2$ in $Ch^+$-containing medium. Uptake induced by expression of *h*4F2hc/*h*LAT1 was calculated by subtraction of uptake in uninjected oocytes from that observed in injected oocytes (■). Curve-fitting was performed using the Michaelis-Menten equation $v = V_{max}/(1+K_m/S)$. Data are the means of 8–10 oocytes.

tive to the oocytes. This is supported by observations that uptake of various amino acids by uninjected oocytes is negligible. However, injection of oocytes with cRNA coding for *h*4F2hc results in a marked induction of $Na^+$-dependent uptake of the neutral amino acid Leu and $Na^+$-independent uptake of the basic amino acid Arg. This is in agreement with previous publications from other laboratories and is explained by the formation of a functional $y^+$L-type transporter by dimerization of the exogenous *h*4F2 heavy chain with an endogenous $y^+$LAT-type light chain (25–41). Uptake of iodothyronines is not stimulated by the injection of *h*4F2hc cRNA alone, indicating that iodothyronines are not transported by $y^+$L-type transporters. This idea is supported by our findings that coexpression of *h*4F2hc and either *h*$y^+$LAT1 or *h*$y^+$LAT2 does not induce iodothyronine transport, although the $Na^+$-dependent transport of Leu as well as the $Na^+$-independent uptake of Arg are further markedly increased.

Injection of oocytes with *h*LAT1 cRNA alone does not induce the transport of the various amino acids and iodothyronines, indicating that expression of a 4F2-like heavy chain by native oocytes is negligible. However, if oocytes are coinjected with cRNA for both *h*4F2hc and *h*LAT1, uptake of the large, neutral amino acids Leu, Phe, Tyr, and Trp, but not that of the basic amino acid Arg, is markedly stimulated above that seen after injection of *h*4F2hc cRNA alone. The *h*LAT1-induced increment in amino acid transport is completely independent of $Na^+$, which confirms the characteristics of the L-type amino acid transporter (23–27). Also, transport of the different iodothyronines is markedly stimulated by coexpression of *h*4F2hc and *h*LAT1, and for all iodothyronines the induced transport is completely $Na^+$ independent. Tested at low ligand concentrations, the rate of iodothyronine uptake by the *h*4F2hc/*h*LAT1 transporter decreases in the order 3,3′-$T_2$ > $rT_3$ ~ $T_3$ > $T_4$. This does not appear to be a simple reflection of the affinity of the different iodothyronines for the *h*4F2hc/*h*LAT1 transporter, as the apparent $K_m$ value is much lower for $T_3$ than for $T_4$, $rT_3$, and 3,3′-$T_2$. The apparent $K_m$ of 0.8 $\mu$M for $T_3$ is the lowest value reported for a ligand of the *h*4F2hc/*h*LAT1 transporter (25–27, 29, 30). Among the different iodothyronines, by far the highest $V_{max}$ value is observed for 3,3′-$T_2$.

As iodothyronines and large neutral amino acids are all ligands for the *h*4F2hc/*h*LAT1 transporter, it is not surprising that they inhibit each other's transport. Leu uptake is more strongly inhibited by L-$T_3$ than by L-$T_4$, L-$rT_3$, 3,3′-L-$T_2$, D-$T_3$, and Triac, in keeping with the low $K_m$ value for $T_3$ and the stereospecificity of this L-type amino acid transporter (24–30). This is reminiscent of the competition between iodothyronine and amino acid uptake in different cell systems reported previously (13–22). Thus, uptake of $T_4$ and $T_3$ by NB41A3 mouse neuroblastoma cells is stereospecific, saturable ($K_m$: $T_3$, 3 nM; $T_4$, 6 nM), and inhibited by high concentrations of Leu and Phe, but not by $\alpha$-aminoisobutyric acid, a system A transporter-specific ligand (19). Similar characteristics of iodothyronine uptake were observed in the Hs683 human glioma cell line (47). Somewhat different results were reported for $T_3$ uptake by cultured rat astrocytes, which express both high affinity (L1) and low affinity (L2) system L transporters (18). Apparent $K_m$ values for uptake of Leu and Trp by the L1 transporter amount to 8–9 $\mu$M. $T_3$ uptake by these cells is $Na^+$ independent and saturable, with an apparent $K_m$ of 2–3 $\mu$M. L1-mediated uptake of Leu and Trp is competitively inhibited by $T_3$, and $T_3$ uptake is competitively inhibited by Trp, with corresponding $K_m$ and $K_i$ values. However, $T_3$ uptake is not inhibited by up to 30 mM Leu (18). It is also interesting to mention the characterization of saturable and stereospecific iodothyronine transport in GH4C1 rat pituitary tumor cells (15), showing high affinity for $T_3$ ($K_m$, 0.4 $\mu$M) and $T_4$, low affinity for $rT_3$ and thyronine, and strong inhibition by Leu, Phe, Tyr, Trp, and the L-type transporter-specific ligand BCH. GH4C1 cells also show high affinity transport of Leu ($K_m$, 17 $\mu$M), which is potently inhibited by $T_3$ ($IC_{50}$, 2 $\mu$M), further supporting the involvement of an L-type transporter in $T_3$ (and $T_4$) uptake (15). Also in cultured rat anterior pituitary cells, uptake of $T_4$ and $T_3$ is mediated by a common transporter and inhibited by the aromatic amino acids Phe, Tyr, and Trp (13).

Blondeau and co-workers (16, 17) demonstrated that $T_3$ transport by rat erythrocytes is $Na^+$ independent, saturable ($K_m$, 0.14 $\mu$M), and specific (L-$T_3$ ≫ D-$T_3$ > $T_4$ > $rT_3$ > thyronine). $T_3$ uptake is competitively inhibited by the aromatic amino acids Trp, Phe, and Tyr, but not by D-Trp or Leu. They also showed low affinity uptake of Trp by rat erythrocytes, with an apparent $K_m$ of 558 $\mu$M. Trp uptake is competitively inhibited by Phe, Tyr, and iodothyronine analogs, with $K_i$ values identical to those for inhibition of $T_3$ transport. These results suggest the involvement of a T-type, aromatic amino acid-specific transporter in the uptake of both Trp and $T_3$ (16, 17). Interestingly, $T_3$ uptake is markedly *trans*-stimulated by intracellular Trp, although Trp uptake is *trans*-inhibited by intracellular $T_3$ (16, 17). Both $T_3$ and Trp uptake are inhibited by the thiol-blocking reagent *N*-ethylmaleimide. A 45-kDa protein was identified by photo-

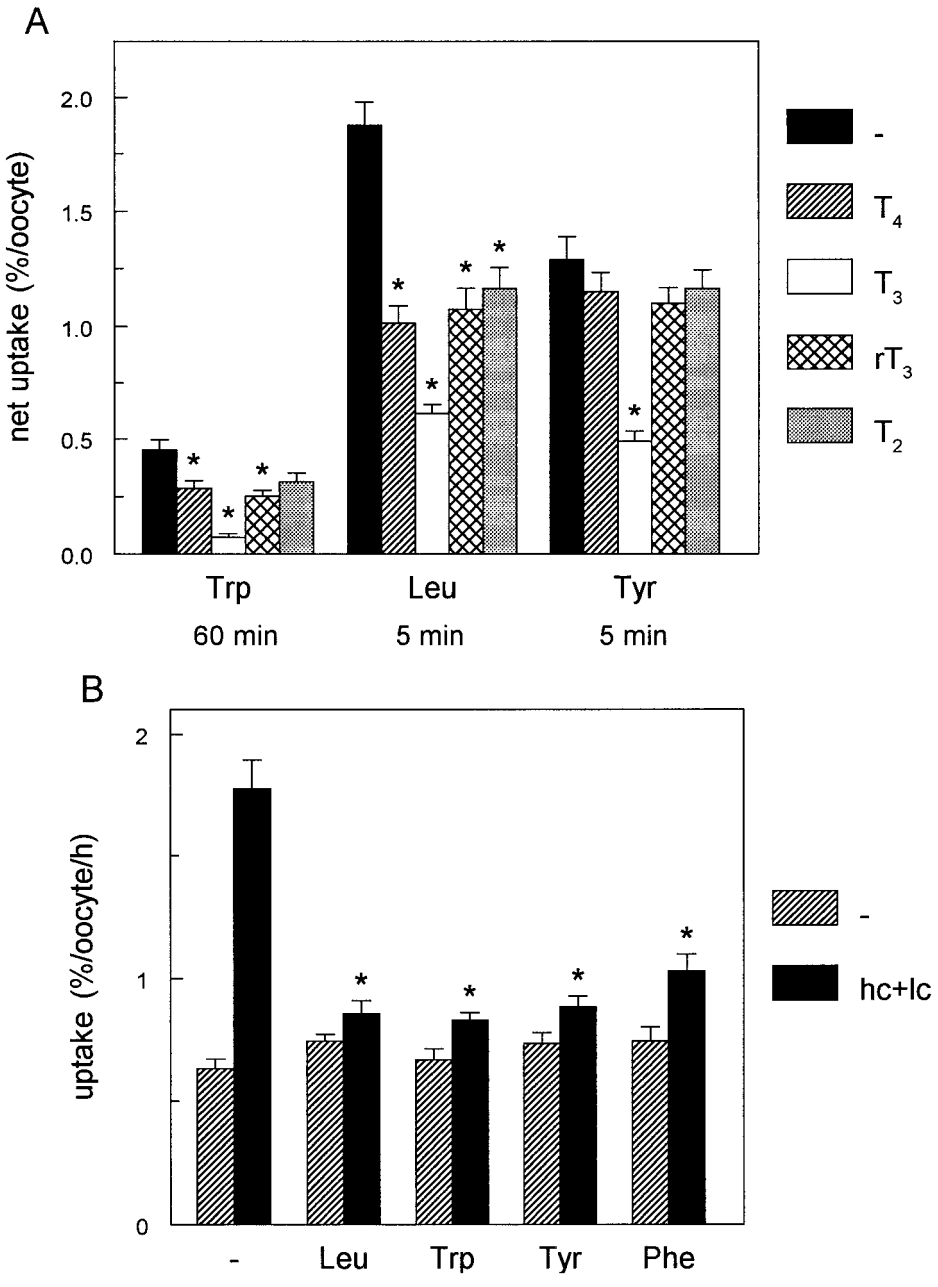


FIG. 4. A, Effects of iodothyronines on the uptake of amino acids by oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1. Oocytes were incubated for 5 min (Leu, Tyr) or 60 min (Trp) at 25 C with 10 $\mu$M $^3$H-labeled Leu, Tyr, or Trp in the absence or presence of 10 $\mu$M $T_4$, $T_3$, $rT_3$, or 3,3′-$T_2$ in $Ch^+$-containing medium. Data were corrected for minor amino acid uptake in uninjected oocytes. B, Effects of amino acids on the uptake of $T_3$ by uninjected *Xenopus* oocytes (−) or oocytes injected with cRNAs coding for *h*4F2hc and *h*LAT1 (hc+lc). Oocytes were incubated for 60 min at 25 C with 0.1 $\mu$M [$^{125}$I]$T_3$ in the absence or presence of 100 $\mu$M Leu, Trp, Tyr, or Phe in $Ch^+$-containing medium. Data are the mean ± SEM of 8–10 oocytes. *, $P < 0.01$ *vs.* incubation without competitor.

affinity labeling with [$^{125}$I]$T_3$ that may be a subunit of the T-type transporter, but this was not further characterized (48).

Competition between iodothyronine and aromatic amino acid (*e.g.* Trp) transport has also been demonstrated in other cells, *e.g.* JAR human choriocarcinoma cells (21, 22) and neonatal rat cardiomyocytes (14), but it has not been established whether iodothyronine uptake in these cells is indeed mediated by amino acid transporters. Of special interest are observations of countertransport of Tyr derivatives by the system h transporter located in thyroidal lysosomal membranes (49, 50). Loading of lysosomes with Tyr or 3-(mono)iodotyrosine (MIT) greatly stimulates the influx of Tyr, MIT, 3,5-diiodotyrosine, Phe, and Leu. Potent competition by $T_4$ and $T_3$ suggests that iodothyronines are also countertransported against Tyr derivatives. The apparent $K_m$ value for MIT is 1.5 $\mu$M, and Tyr, 3,5-diiodotyrosine, $T_4$, and $T_3$ show similar high affinities. This exchange mechanism probably plays an important role in thyroid hormone biosynthesis, as the iodotyrosines released by lysosomal hydrolysis of Tg must be transported to the cytoplasm for deiodination, and the iodothyronines to the cell membrane for secretion (6).

The apparent $K_m$ values of $T_4$, $T_3$, Leu, and Trp in the $10^{-6}$–$10^{-5}$ M range for the *h*4F2hc/*h*LAT1 transporter expressed in oocytes most closely resemble those reported for their uptake by rat GH4C1 pituitary tumor cells (15), supporting the involvement of an L-type transporter. They are also in reasonable agreement with the apparent $K_m$ values for $T_4$, $T_3$, Leu, and Trp uptake by cultured rat astrocytes (18) and rat erythrocytes (16, 17), but the complete lack of effect of

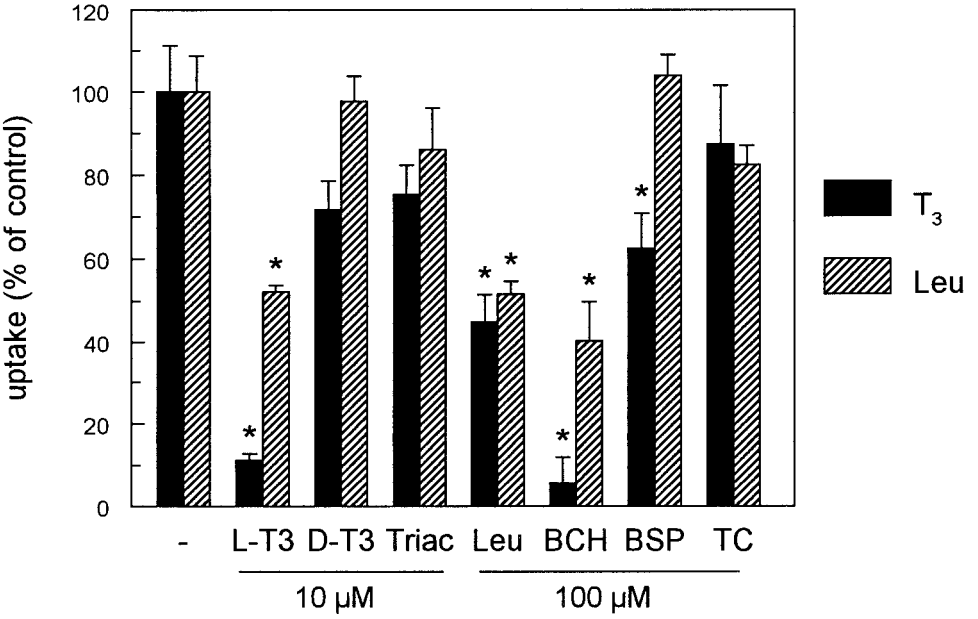


FIG. 5. Effects of various compounds on the uptake of $T_3$ and Leu by the *h*4F2hc/*h*LAT1 transporter. Oocytes were injected with *h*4F2hc cRNA alone or together with *h*LAT1 cRNA, and after 2 d were incubated for 60 min with 0.1 $\mu$M [$^{125}$I]$T_3$ or for 5 min with 10 $\mu$M [$^3$H]Leu in $Ch^+$ medium without or with 10 $\mu$M L-$T_3$, D-$T_3$, or Triac or 100 $\mu$M Leu, BCH, BSP, or TC. Uptake by *h*4F2hc cRNA-injected oocytes was subtracted from uptake by *h*4F2hc and *h*LAT1 cRNA-injected oocytes. Net uptake in the presence of competitor was expressed as a percentage of control net uptake in the absence of competitor. Data are the mean ± SEM of 8–10 oocytes. *, $P < 0.01$ *vs.* control.

$>10^{-2}$ M Leu on iodothyronine uptake by these cells suggests that an aromatic amino acid-specific (T-type) transporter is involved. Also, the much lower $K_m$ values reported for $T_4$ and $T_3$ uptake by the NB41A3 mouse neuroblastoma cells appear to implicate another (sub)type of amino acid transporter than 4F2hc/LAT1.

The L-type amino acid transporter mediates not only influx, but also efflux, of amino acids. Our results show that the release of intracellular Leu is stimulated by exchange with extracellular Leu in agreement with previous reports (29, 30). Extracellular Leu only induces a small increase in the release of 3,3-$T_2$ from the oocytes and no release of internalized $T_3$, which may be explained by strong binding of iodothyronines to intracellular sites in oocytes.

The above-mentioned properties of the thyroidal lysosomal system h transporter, mediating the exchange of amino acids such as Leu, Tyr, iodotyrosines, and iodothyronines, suggest that it may actually be an L-type amino acid transporter. This is also supported by evidence that the high uptake of radioiodine-labeled MIT and 3-iodo-$\alpha$-methyltyrosine by different tumors is mediated by an L-type transporter (51–53). This principle is used in nuclear medicine for the scintigraphic visualization of such tumors. If the *h*4F2hc/*h*LAT1 and/or *h*4F2hc/*h*LAT2 transporters are indeed responsible for tumor uptake of the radioactive Tyr derivatives, then the availability of cell systems overexpressing these transporters would greatly facilitate the development of improved tumor-seeking radiopharmaceuticals.

Obviously, all cells require amino acid transporters, but, in contrast to the ubiquitous expression of the 4F2 heavy chain, the LAT1 and, in particular, LAT2 light chains show restricted tissue distributions; neither of them is expressed in liver (25–33). This suggests the existence of other light chains as yet to be identified, which are involved in the uptake of aromatic amino acids in tissues that do not express LAT1 or LAT2. Presumably, one of these constitutes with 4F2hc a T-type transporter specific for aromatic amino acids, including iodothyronines (16–18). Perhaps, additional light chains exist that associate specifically with the homologous rBAT heavy chain (28), generating transporters that also accept iodothyronines. However, cellular uptake of iodothyronines is not only mediated by amino acid transporters. We and others have demonstrated recently that iodothyronines are also transported into liver by $Na^+$-dependent and $Na^+$independent organic anion transporters, although various members of the $Na^+$-independent organic anion transporter family are also expressed in other tissues, in particular kidney and brain (46, 54–58). Typical ligands for these organic anion transporters, BSP and TC, have no effect on iodothyronine uptake by the *h*4F2hc/*h*LAT1 transporter, in contrast to the potent inhibition by the L-type ligand BCH. However, the major, $Na^+$-dependent hepatic transporters for $T_4$ and $T_3$ remain to be identified. Iodothyronine uptake by uninjected oocytes is saturable (apparent $K_m$, 2–14 $\mu$M), suggesting that it is carrier mediated. The lack of effect of high concentrations of different amino acids on iodothyronine uptake by native oocytes argues against the involvement of an amino acid transporter. The type of endogenous iodothyronine transporter(s) in *Xenopus* oocytes remains to be determined.

In summary, we have demonstrated that *h*4F2hc/*h*LAT1 and, albeit less effectively, also *h*4F2hc/*m*LAT2 are capable of transporting iodothyronines, in agreement with previous suggestions that thyroid hormone is taken up in different tissues via L-type amino acid transporters. Our findings are in agreement with a recent report published after completion of our study, showing iodothyronine transport by the heterodimeric transporter composed of *h*4F2hc and the IU12 light chain from *Xenopus*, which is homologous to *h*LAT1 (59). One of the questions that remains to be clarified is the extent to which cellular uptake of iodothyronines through 4F2-related transporters is stimulated by countertransport of different intracellular amino acids. Of course, these transporters may also mediate cellular efflux of iodothyronines.

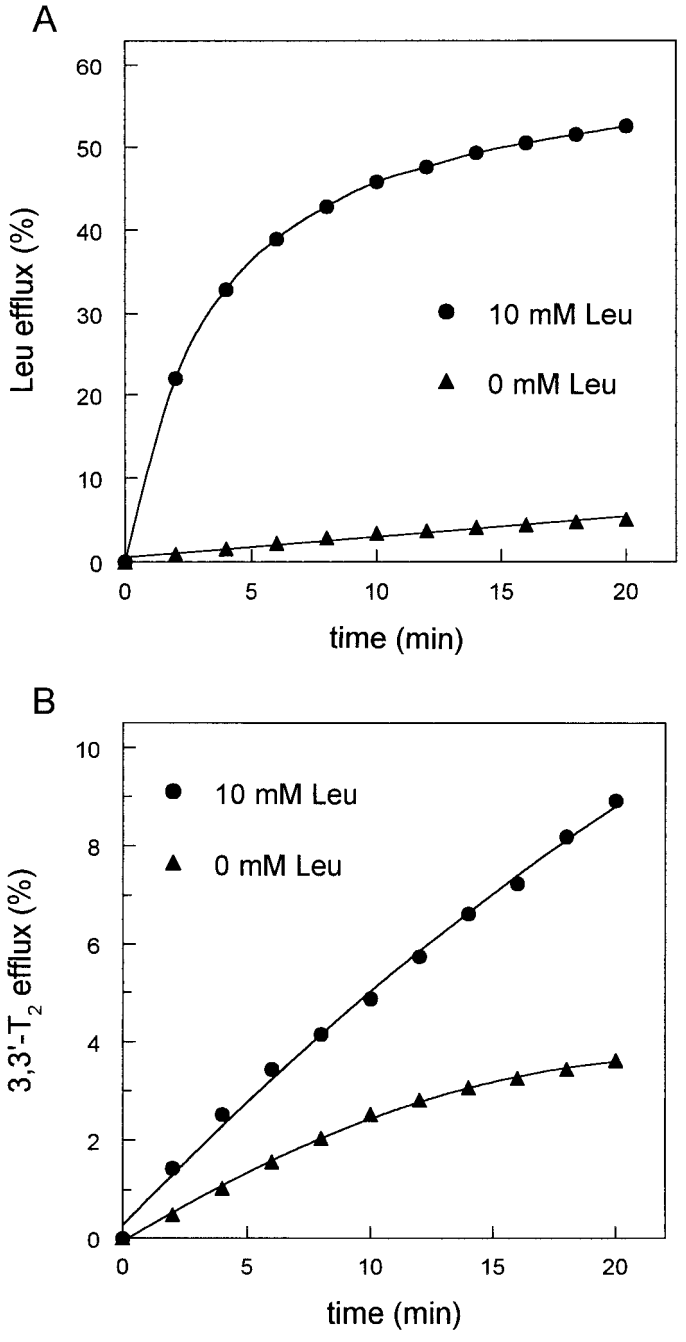


FIG. 6. Effects of extracellular unlabeled Leu on the efflux of labeled Leu (A) and 3,3′-$T_2$ (B) internalized by *Xenopus* oocytes injected with cRNA coding for *h*4F2hc and *h*LAT1. Oocytes were preincubated for 30 min with 10 $\mu$M [$^3$H]Leu (A) or 0.1 $\mu$M [$^{125}$I]$T_2$ (B) in $Ch^+$-containing medium. After washing, efflux of internalized radioactive ligand was determined by incubation of oocytes during successive 2-min periods at 25 C with the same medium without (▲) or with 10 mM nonradioactive Leu (●). Data are the mean ± SEM of 8–10 oocytes.

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