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Involvement of the L-Type Amino Acid Transporter Lat2 in the Transport of 3,3′-Diiodothyronine across the Plasma Membrane

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Key Words
Thyroid hormones · Transporters · L-type amino acid transporter 2 · Molecular uptake · 3,3′-Diiodothyronine · T_3 · Oocytes

Abstract
Thyroid hormones are transported across cell membranes by transmembrane transporter proteins, for example by members of the monocarboxylate transporter (MCT) and the L-type amino acid transporter (LAT) families. LATs consist of a light chain (e.g. LAT2) and a heavy chain (CD98), which is essential for their cell surface expression and functionality. The specificity of Lat2 for thyroid hormones and their metabolites and its role in their transport was not fully clear. This fact motivated us to establish a cell system to elucidate the uptake of thyroid hormones and their metabolites by mouse Lat2. The coinjection of cRNA coding for Lat2 and CD98 into *Xenopus laevis* oocytes resulted in a markedly increased level of 3,3′-diiodo-L-thyronine (3,3′-T_2) and to some extent also enhanced T_3 transport. To gain insight into properties of thyroid hormones and their metabolites transported by Lat2, we inhibited 3,3′-T_2 uptake by various iodothyronine derivatives. T_1 and T_2 derivatives as well as 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid strongly competed with 3,3′-T_2 uptake. In addition, we performed T_2 uptake measurements with the thyroid hormone-specific transporter MCT8. For both Lat2 and MCT8, K_m values in a low micromolar range were calculated. We demonstrated that oocytes are a suitable system for thyroid hormone transport studies mediated by Lat2. Our data indicates that Lat2 compared to other thyroid hormone transporters prefers 3,3′-T_2 as the substrate. Thus, Lat2 might contribute to the availability of thyroid hormone by importing and/or exporting 3,3′-T_2, which is generated either by T_3 inactivation or by rapid deiodinase 1-mediated rT_3 degradation.

Introduction

Thyroid hormones are essential for processes such as the development of the human brain, growth and metabolism [1–3]. For their intracellular availability thyroid hormones are transported across cell membranes by a variety of thyroid hormone transmembrane transporter proteins. Several transporters with high affinity for thyroid hormones but with different tissue distributions and ligand affinities have been identified. They include members of the monocarboxylate transporter (MCT), organic anion-transporting polypeptide (OATP) and L-type amino acid transporter (LAT) families [4, 5].

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LAT2 expression studies in the human and mouse brain showed no expression of LAT2 in developing neurons of the human, while expression is clearly present in mouse neurons at all stages of development [6]. In murine neurons and astrocytes Lat2 is coexpressed with Mct8 and Lat1. To study the role of Lat2 in the transport of thyroid hormones, *Lat2* knock-out mice were analyzed by Braun et al. [7]. The animals display a significantly reduced T_3 uptake in primary astrocytes, which confirms the participation of Lat2 in the transport of thyroid hormones in brain cells. Together with others we have suggested that coexpression of Lat2 in neurons complements the inactivation of *Mct8* in mice [6, 7].

The characterization of *Lat2*-deficient mice showed normal growth and development. Movement coordination is slightly impaired indicating light neurobehavioral alterations, although cerebellar development and structure remained inconspicuous [1, 8]. Circulating thyroid hormones, thyroid-stimulating hormone and thyroid hormone-responsive genes remained unchanged, possibly because of functional compensation by Mct8 [8]. The role of Lat2 during the early postnatal cerebral cortex development is indicated by the combined Mct8/Lat2 mouse [9].

Lat2 was first identified as a sodium-independent transporter protein of large and neutral amino acids, such as alanine, phenylalanine and leucine, and requires association with CD98 (4F2 heavy chain, 4f2hc) for its cell surface expression. The so-called light chain, Lat2, consists of 12 transmembrane helices (TMHs), whereas the heavy chain, CD98, shows only 1 transmembrane helix with a large extracellular C-terminal domain. Both chains are proposed to be linked via a disulphide bridge [10–12]. Lat2 is not only expressed in the brain, but also in the kidney, intestine and liver [7, 8]. As already mentioned, studies indicate the involvement of Lat2 in the transport of thyroid hormones [7, 13], but its role in the transport of these hormones, such as T_3 or the metabolite 3,3′-T_2, including its thyroid hormone substrate specificity, is still unclear. So far, transport studies by LAT2 have mainly been performed in oocytes from *Xenopus laevis* [10, 11, 13]. Using this cell system and the known LAT inhibitor 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) we observed a preferential uptake of 3,3′-T_2 by Lat2 and its strong competition by thyroid hormone metabolites, such as 3-T_1 and other T_2 isomers. In comparison to the uptake of 3,3′-T_2, only limited T_3 uptake was detectable. Our results indicate that Lat2 is involved in the transport of thyroid hormones across cell membranes and participates in the regulation of intracellular availability or cellular release of these hormones.

Materials and Methods

Materials

Iodo-L-thyronines (3,3′-T_2, T_3, rT_3, T_4), L-amino acids (Leu, IIe, Met, His) and BCH were obtained from Sigma (St. Louis, Mo., USA). All iodothyronines were dissolved in DMSO. [^{125}I]rT_3, [^{125}I]T_4 and [^{14}C]Leu were purchased from Perkin Elmer (Boston, Mass., USA). [^{125}I]3,3′-T_2 and [^{125}I]T_3 were purchased from Hartmann Analytic GmbH (Braunschweig, Germany). All other chemicals were of reagent grade.

Plasmids

Murine Lat2 and CD98 were amplified from kidney cDNA and cloned into the expression vector pcDNA3 (Invitrogen, Carlsbad, Calif., USA) [6]. The C-terminal FLAG-tag was introduced into Lat2-pcDNA3 by oligonucleotide-based cloning using PpuMI and XbaI. An additional BamHI restriction site for CD98-pcDNA3 was carried out by site-directed mutagenesis with the QuikChange Kit (Stratagene, Heidelberg, Germany). The C-terminal His tag was introduced into CD98-pcDNA3 by oligonucleotide-based cloning using BamHI and XbaI.

Both constructs were cloned into the pTLB expression vector [14], which was kindly provided by T.J. Jentsch (Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany). For stable transfection of MDCK1 cells CD98-His was cloned into pcDNA3.1Hygro$^{(+)}$ (Invitrogen) using HindIII and XbaI. Clones of human MCT8 in pcDNA3 and empty pcDNA3 were used for stable transfection of MDCK1 cells as previously described [15].

Transfection of MDCK1 Cells

Cells at 50% confluency were transfected with Lat2-FLAG-pcDNA3 or CD98-His-pcDNA3.1Hygro$^{(+)}$ and exposed to selective pressure with 400 μg/ml of G418 or 50 μg/ml of hygromycin for 2 weeks, respectively. Derived clones were used for stable co-transfection. The expression was ascertained by SDS-PAGE and immunoblotting with FLAG or CD98 antibody.

cRNA Preparation and Injection into Isolated X. laevis Oocytes

Plasmids containing CD98-His or Lat2-FLAG in pTLB were cut by MluI. Linearized plasmids were transcribed using the mMESSAGE mMACHINE® SP6 RNA Transcription Kit (Life Technologies GmbH, Darmstadt, Germany) following the manufacturer's protocol.

Oocytes were kindly provided and isolated by the group of T.J. Jentsch or prepared by EcoCyte Bioscience (Castrop-Rauxel, Germany). Stage V–VI oocytes (35) were sorted on morphological criteria and kept at 18°C in ND96 containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES and 50 mg/ml gentamycin (pH 7.5). Oocytes were injected with 23 ng CD98-His and/or 23 ng Lat2-FLAG cRNA or 46 nl of RNase-free water.

Uptake Assays

After 2 days of expression oocytes were washed with washing buffer (100 mM NaCl or 100 mM ChCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM Tris, 0.1% BSA, pH 7.5). For the thyroid hormone uptake assay oocytes were incubated in 600 μl of uptake buffer (washing buffer containing 100 nM L-iodothyronine and 0.1 nM of the corresponding ^{125}I-labeled iodothyronine). Further oocytes were washed six times with ice-cold wash-

ing buffer. Radioactivity was determined by a gamma counter (Wizard; PerkinElmer, Waltham, Mass., USA).

Two days before the experiments with stably transfected MDCK1 cells (MCT8, pcDNA3), 100,000 cells per well were seeded into 24-well plates. For time course assays, cells were washed once with PBS and incubated with uptake buffer (125 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM HEPES, 0.1% BSA, 5.6 mM glucose, pH 7.4) supplemented with 10 nM 3,3′-T_2.

For competitive inhibition studies, cells were exposed to 100 nM 3,3′-T_2 (Lat2) or 10 nM 3,3′-T_2 (MCT8) containing uptake buffer with 0.1 nM of the corresponding radioactive ^{125}I-labeled thyroid hormone. Incubation for 60 or 2 min, respectively, was performed in the presence or absence of test compounds (10 μM iodothyronines, 1 mM or 100 μM BCH, 1 mM amino acid).

For L-Leu uptake, oocytes and MDCK1 cells were incubated with uptake buffer without BSA for 10 min, and washed with ice-cold buffer or PBS, respectively. The radioactivity of cells was measured using a beta counter (Wallac, Perkin Elmer). L-Leu was dissolved in uptake buffer with the following concentrations: 500 nm [^{14}C]L-Leu plus 10 μM nonlabeled L-Leu for MCT8 or 50 μM L-Leu for Lat2/CD98 transport studies, respectively. Each experiment was performed at least twice with two different batches of oocytes and cRNAs or in triplicates for MDCK1 cells, respectively.

Transport Kinetics

Saturation of 3,3′-T_2 uptake in Lat2/CD98 coinjected oocytes was analyzed after 60 min of incubation in uptake buffer without BSA containing 3,3′-T_2 at final concentrations of 0.1–25 μM containing [^{125}I]3,3′-T_2 as tracer. Transport kinetics of 3,3′-T_2 uptake by MCT8-transfected MDCK1 cells were measured for 2 min in uptake buffer without BSA. 3,3′-T_2 concentrations ranged from 0.5 to 12 μM containing [^{125}I]3,3′-T_2 as tracer.

Western Blot

Stably transfected MDCK1 cells or oocytes (2 days after Lat2/CD98 injection) were lysed in homogenization buffer (250 mM saccharose, 20 mM HEPES, 1 mM EDTA, pH 7.4) and treated with ultrasound. For oocytes the buffer was supplemented with 1 mM DTT.

Equal amounts of cell lysates were separated on SDS gels, transferred onto nitrocellulose membranes and probed with antibodies for Lat2 (a kind gift from Ulrich Schweizer, Rheinische Friedrich-Wilhelms-Universität Bonn, and Eva K. Wirth, Charité-Universitätsmedizin Berlin), FLAG (Cell Signaling Technology; Danvers, Mass., USA) or CD98 (Santa Cruz Biotechnology, Dallas, Tex., USA). The experiments were performed at least twice.

Statistics

Data are presented as means ± SD or means ± SEM after the subtraction of noninjected or empty-vector transfected cells, respectively. The kinetic parameters were determined by fitting the plot of uptake rate (v) versus 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. Calculations were performed using GraphPad Prism 4.0.

Results

Substrate Specificity

Initial experiments were carried out to reproduce the previously described induction of amino acid transport in cRNA microinjected *X. laevis* oocytes [10, 11] and stably transfected MDCK1 cells. The expression of Lat2 and CD98 in both cell systems was confirmed by Western blotting using antibodies against Lat2, FLAG or CD98 (online suppl. fig. S2A–D; for all online suppl. material, see www.karger.com/doi/10.1159/000381542).

Uninjected oocytes showed negligible uptake of the neutral amino acid L-Leu. Online supplementary figure S1 shows uptake of L-Leu in Lat2/CD98 coinjected oocytes or in Lat2/CD98 stably transfected MDCK1 cells. Coexpression of Lat2/CD98 resulted in a large induction (about 16-fold) in L-Leu uptake in oocytes, while a lower increase in L-Leu uptake was determined in Lat2/CD98-expressing MDCK1 cells (online suppl. fig. S1). Based on these functional results we used the oocyte system for further Lat2 transport studies.

Figure 1a shows the uptake of 100 nM iodothyronines (T_4, rT_3, T_3, 3,3′-T_2) by noninjected oocytes and oocytes injected with cRNAs coding for Lat2 and CD98 after 60 min of incubation in Na^+-free uptake buffer. The coexpression of both chains induces the uptake of 3,3′-T_2 by a factor of 4 and only to a lower extent the uptake of T_3. The lower induction of T_3 uptake seems to be the result of a higher background uptake. Thyroid hormones T_4 and rT_3 were not transported into oocytes by Lat2. The specific 3,3′-T_2 uptake over time was calculated after the subtraction of uptake in noninjected oocytes as background (fig. 1b, c).

Functional properties of 3,3′-T_2 transport by Lat2 were examined by the single expression of Lat2 or CD98 and coexpression of Lat2/CD98 in *X. laevis* oocytes (fig. 2a). As shown in figure 2a, the expression of Lat2 alone is not sufficient for 3,3′-T_2 transport. The coexpression of Lat2 and CD98 resulted in a large 3,3′-T_2 uptake, indicating that CD98 is indispensable for the functional expression of Lat2.

To clarify a sodium dependence of 3,3′-T_2 uptake by Lat2, uptake assays were performed in Na^+- or Ch^+-containing uptake buffer. After subtraction of the background, we could characterize 3,3′-T_2 uptake as sodium independent (fig. 2b, c).

Transport Kinetics

Saturation kinetics of 3,3′-T_2 uptake by Lat2 were studied by incubation of noninjected oocytes and oocytes

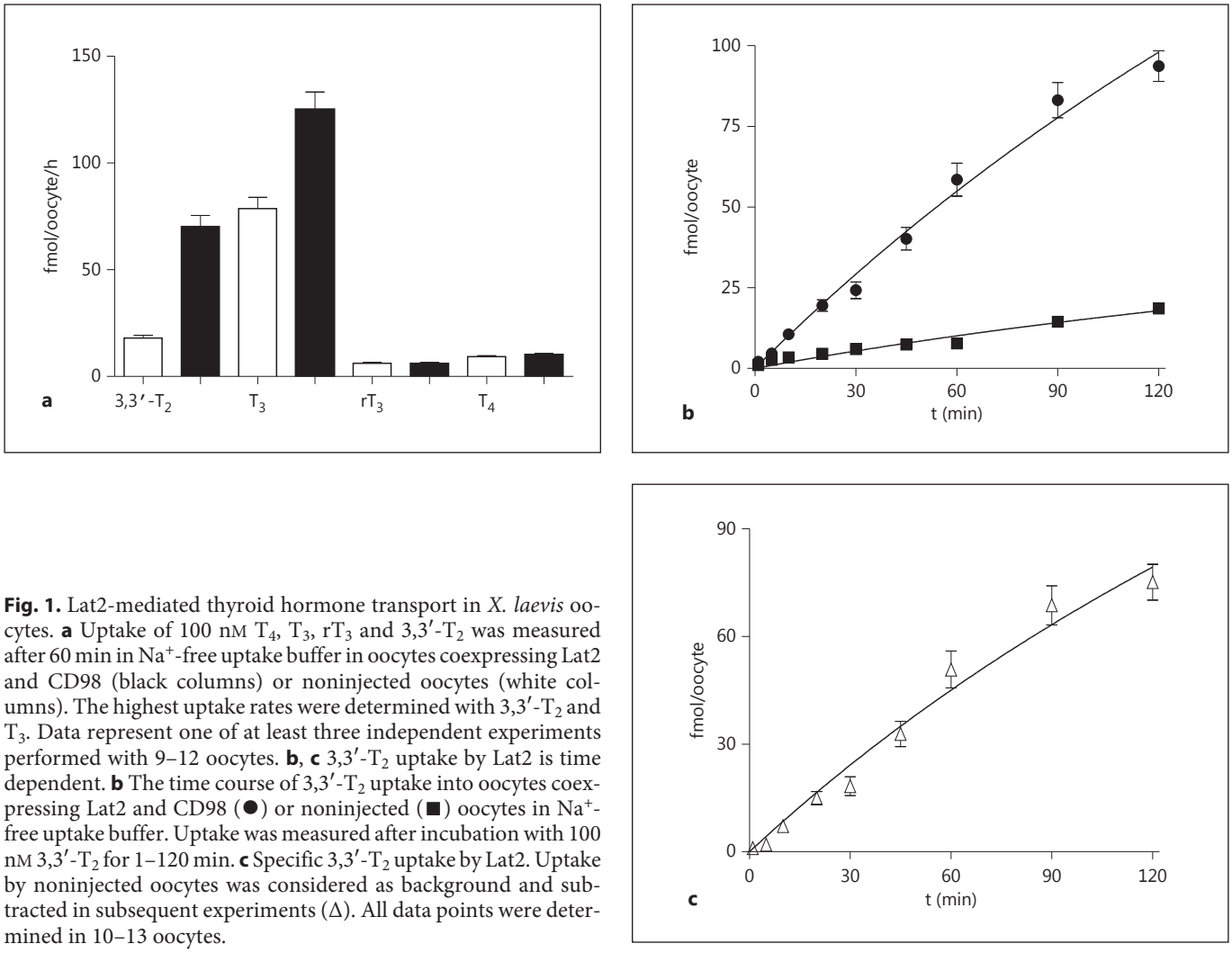


Fig. 1. Lat2-mediated thyroid hormone transport in *X. laevis* oocytes. **a** Uptake of 100 nM T_4, T_3, rT_3 and 3,3′-T_2 was measured after 60 min in Na^+-free uptake buffer in oocytes coexpressing Lat2 and CD98 (black columns) or noninjected oocytes (white columns). The highest uptake rates were determined with 3,3′-T_2 and T_3. Data represent one of at least three independent experiments performed with 9–12 oocytes. **b**, **c** 3,3′-T_2 uptake by Lat2 is time dependent. **b** The time course of 3,3′-T_2 uptake into oocytes coexpressing Lat2 and CD98 (●) or noninjected (■) oocytes in Na^+-free uptake buffer. Uptake was measured after incubation with 100 nM 3,3′-T_2 for 1–120 min. **c** Specific 3,3′-T_2 uptake by Lat2. Uptake by noninjected oocytes was considered as background and subtracted in subsequent experiments (Δ). All data points were determined in 10–13 oocytes.

injected with Lat2 and CD98 cRNAs for 60 min with 0.1–25 μM concentrations of the substrate in Ch^+-containing buffer. The results are presented in figure 3a and b. 3,3′-T_2 transport by Lat2/CD98 was determined by subtraction of the uptake rates in noninjected oocytes from those observed in oocytes injected with the cRNAs for both subunits.

Competition Studies on Thyroid Hormone Uptake

Competitive transport experiments by Lat2 were studied in the absence of Na^+ using 100 nM 3,3′-T_2 as tracer. Oocytes were coinjected with CD98 and Lat2 and corrected for uptake in noninjected oocytes. Uptake without an inhibitor was set to 100%.

The specificity of iodothyronine uptake by Lat2/CD98 was investigated using the known Lat inhibitor BCH, amino acids and several iodothyronines as agents potentially interfering with 3,3′-T_2 uptake (fig. 4). A potent inhibition of about 80% on 3,3′-T_2 uptake was determined for BCH at a concentration of 1 mM. Tested at 100 μM, BCH produced at least the same marked inhibition of 3,3′-T_2 uptake as 3,3′-T_2 itself (fig. 4a).

Competition between iodothyronine and amino acid transport by Lat2 was studied by testing the effects of 1 mM of unlabeled amino acid on the uptake of 3,3′-T_2. Figure 4b illustrates the inhibition by L-Leu, L-Ile, L-Met and L-His. Uptake of 3,3′-T_2 was reduced between 80 and 95% depending on the amino acid tested, whereas the strongest inhibition was measured with L-Ile and L-Leu. In addition, the effect of several iodothyronines (10 μM) was investigated on 3,3′-T_2 uptake. As shown in figure 4c, 3,3′-T_2 uptake by Lat2 was inhibited by iodothyronines

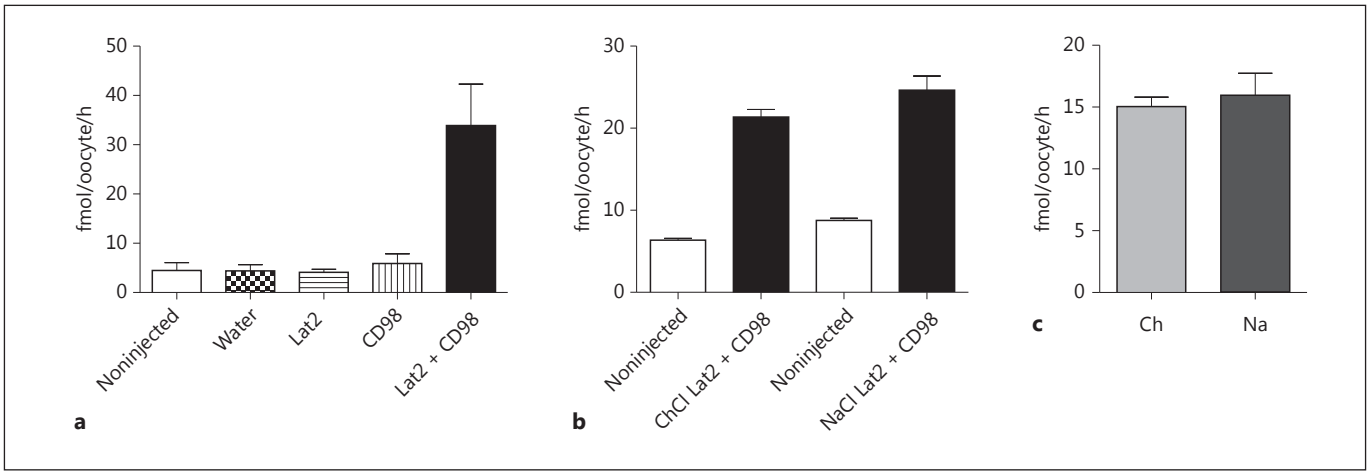


Fig. 2. Functional expression of Lat2 in *X. laevis* oocytes. **a** Effect of coexpression of Lat2 and CD98 on 3,3′-T_2 uptake. The uptake of 100 nM 3,3′-T_2 was measured after 60 min in Na^+-free uptake solution on noninjected oocytes or oocytes injected with water, Lat2 or CD98 cRNA, or both, 2 days after injection. The coexpression of Lat2 and CD98 resulted in a marked uptake of 3,3′-T_2. **b**, **c** Ion dependence of 3,3′-T_2 transport in oocytes that express Lat2 and CD98. **b** Noninjected or Lat2 and CD98 coexpressing oocytes were incubated for 60 min with 100 nM 3,3′-T_2 in NaCl or ChCl containing buffer. **c** 3,3′-T_2 uptake by noninjected oocytes was considered as background and subtracted. Uptake of 3,3′-T_2 measured in Na^+ uptake solution (Na) was not different from uptake medium containing choline$^+$ (Ch). Data represent one of at least two independent uptake experiments performed with 10–14 oocytes.

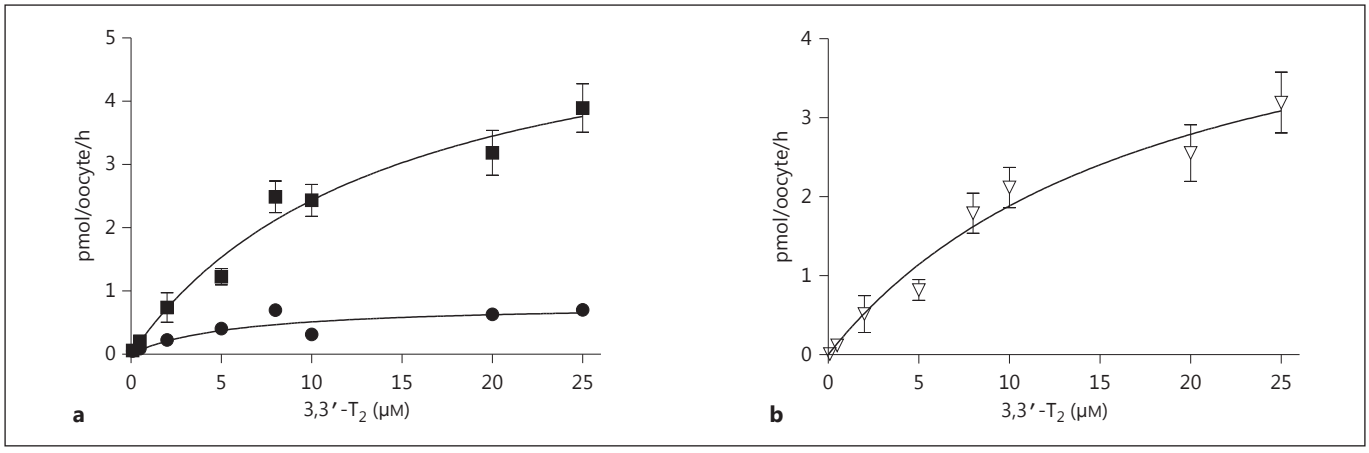


Fig. 3. Concentration dependence of Lat2-mediated 3,3′-T_2 uptake. **a** Two days after injection, 3,3′-T_2 uptake was measured at different substrate concentrations (0.1–25 μM) in Na^+-free uptake buffer in noninjected (●) or Lat2 and CD98 coexpressing (■) oocytes after 60 min. **b** The transport activity of noninjected oocytes was subtracted from that of cRNA-injected oocytes (▽). A Michaelis-Menten transport mechanism was assumed for calculations (GraphPad 4.0). Data correspond to a representative experiment with 8–10 oocytes per group. The kinetic parameters were V_{max} = 5.4 pmol/60 min and K_m = 18.6 μM.

without iodine substituents or containing up to three iodine atoms. In contrast, 3,3′-T_2 uptake by MCT8 was only strongly inhibited by iodothyronines carrying at least one iodine atom per aromatic ring [15] (fig. 5e).

MCT8 Studies

3,3′-T_2 transport studies by MCT8 were performed in MDCK1 cells as previously described [15]. Figure 5 shows the time course of 3,3′-T_2 uptake in pcDNA3 and MCT8 stably transfected cells. Expression of MCT8 induced a 6-fold increase in the initial uptake of 3,3′-T_2 compared

with the control, and the 3,3′-T_2 transport into MCT8-expressing cells was only linear for the first 4 min. Therefore, all further transport experiments were performed at 2-min incubations.

To determine the K_m value of 3,3′-T_2 for MCT8, stably transfected MDCK1 cells were incubated with substrate concentrations ranging from 0.5 to 12 μM for 2 min. After the subtraction of pcDNA3-transfected MDCK1 as background from MCT8-expressing cells, Michaelis-Menten analysis provided apparent K_m values of 18.6 μM in oocytes and 2.6 μM in MDCK1 cells for 3,3′-T_2 for Lat2 or MCT8, respectively.

Discussion

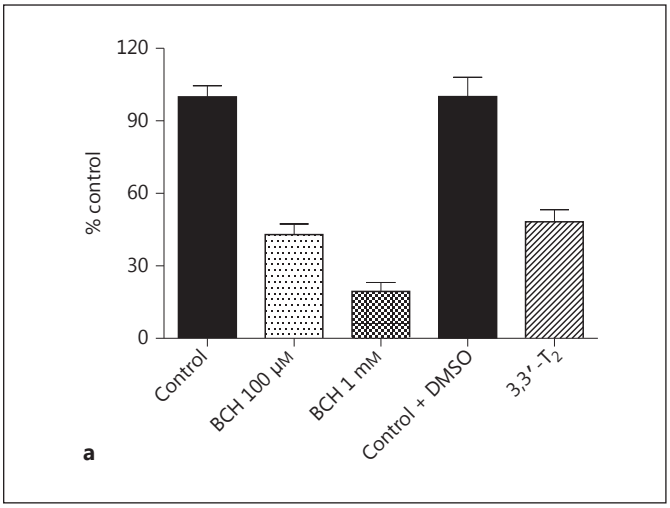
In this study, we used *X. laevis* oocytes to establish a cell system to investigate the transport of thyroid hormones and their metabolites by Lat2. Oocytes represent a well-described system that is widely used in transport studies. They have been used to characterize the thyroid hormone transporters MCT8 and LAT1 in vitro [13, 16]. The endogenous expression of transport proteins in this system is low, leading to only minor levels of background transport activity [17].

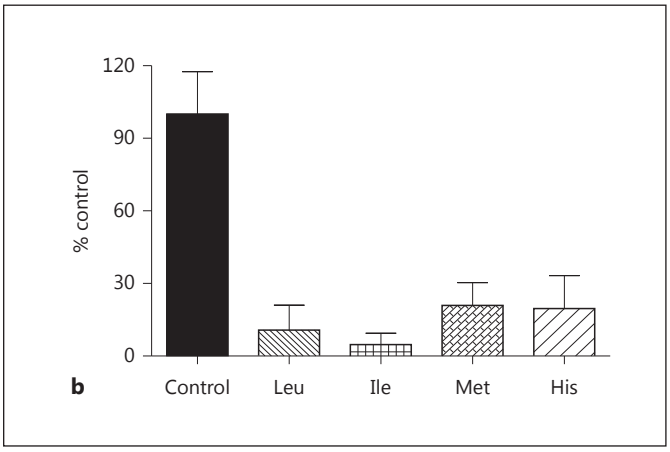
To show the functionality of Lat2 expression, we analyzed the transport of the known substrate L-Leu. For this purpose, we injected oocytes with cRNAs coding for Lat2 and CD98 and stably transfected MDCK1, which we used for thyroid hormone uptake studies by MCT8 [15].

Although both systems showed marked uptake of the substrate, the endogenous leucine uptake in oocytes was much lower than in MDCK1 cells. The presence of endogenous amino acid transporters other than Lat2 in this cell line is plausible. MDCK1 cells are isolated from the kidney, where amino acids need to be reabsorbed.

The highest uptake rate by Lat2 in oocytes was determined for 3,3′-T_2 (about 4-fold). However, transport of T_3 is only mildly induced by the coexpression of CD98 and Lat2 (about 1.6-fold). By testing different iodothyronine concentrations (10–100 nM) we could not enhance the uptake of T_3, which remained low, while rT_3 and T_4 are not transported by Lat2. These results support and extend similar studies with human LAT1 and human LAT2 [13, 18, 19].

Previously it was demonstrated by others that LAT1 and, albeit less effectively, also Lat2 is capable of transporting iodothyronines. In contrast to Lat2, LAT1 markedly induced the uptake of rT_3 when coexpressed with





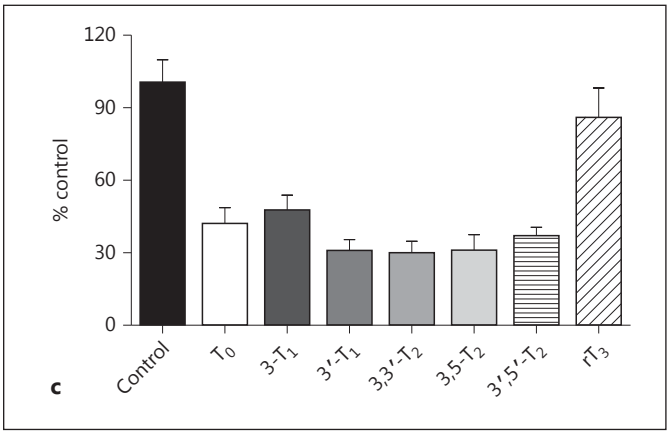


Fig. 4. Inhibition of 3,3′-T_2 uptake by Lat2. *X. laevis* oocytes were coinjected with Lat2 and CD98 cRNAs and the uptake of 3,3′-T_2 was measured after an incubation time of 60 min in Na^+-free uptake buffer. 3,3′-T_2 uptake of noninjected oocytes was considered as background and subtracted. Uptake without inhibitor was set to 100%. Inhibition on 3,3′-T_2 uptake by BCH (100 μM and 1 mM) and 3,3′-T_2 (10 μM; **a**), 1 mM amino acids (**b**) and 10 μM iodothyronine derivatives (**c**).

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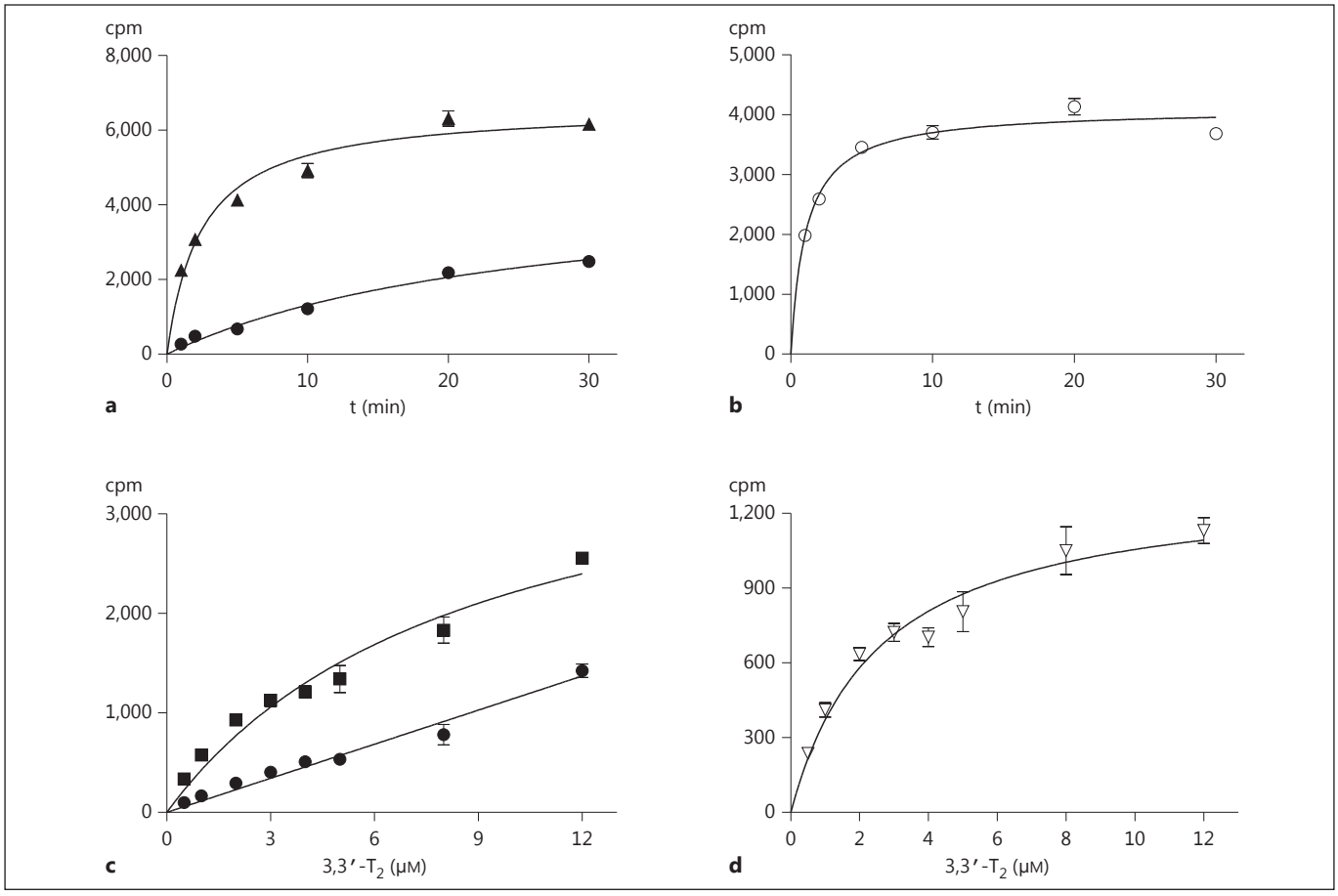
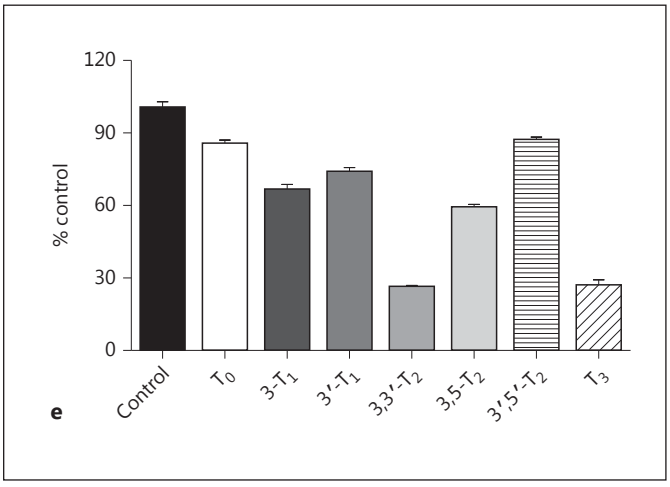


Fig. 5. 3,3′-T_2 uptake in MCT8 (▲) or pcDNA3 (●) stably transfected MDCK1 cells. **a** Time course experiments were measured after incubation of 10 nM 3,3′-T_2 for 1–30 min in Na^+-free uptake buffer. **b** Uptake by pcDNA3-transfected cells was considered as background and subtracted (○). **c**, **d** The concentration dependence of MCT8-mediated 3,3′-T_2 uptake. **c** 3,3′-T_2 uptake by MCT8 was measured at different substrate concentrations (0.5–12 μM) in Na^+-free uptake buffer without BSA in MCT8- (■) and pcDNA3- (●) transfected MDCK1 cells after 2 min. **d** The transport activity of pcDNA3-transfected cells was subtracted from that of MCT8-expressing cells and a Michaelis-Menten transport mechanism was assumed for calculations (▽) using GraphPad 4.0. Data correspond to a representative experiment performed in triplicate. A K_m value of 2.6 μM was determined. **e** Inhibition on MCT8-mediated 3,3′-T_2 uptake by 10 μM iodothyronines. Uptake of pcDNA3-transfected cells was considered as background and subtracted. Uptake without an inhibitor was set to 100% (control).



human CD98 in *X. laevis* oocytes, but only to a small extent the uptake of T_3 and T_4 [13]. As previously shown, thyroid hormones T_3 and T_4 show a better competition for leucine uptake in LAT1 than in LAT2 [18, 19]. In both studies the uptakes of L-Leu by LAT1 were markedly inhibited by T_3 and to a smaller extent by T_4. In comparison, T_3 and T_4 showed a milder effect on L-Leu uptake and no inhibition on L-Ala transport by LAT2, respectively [18, 19].

In the present study, the injection of oocytes with Lat2 cRNA alone does not induce the transport of the 3,3′-T_2, indicating that expression of a CD98-like heavy chain by

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native oocytes is negligible. However, if oocytes are coinjected with cRNA for both CD98 and Lat2, uptake of the 3,3′-T_2 is markedly stimulated above that seen after injection of CD98 cRNA alone, which is characteristic for the heterodimeric transporter Lat2 [11, 20].

Interestingly, the uptake by noninjected oocytes was significantly lower when cells were incubated in choline chloride-containing buffer. This observation is in line with findings from Friesema et al. [13], who reported that uptake of iodothyronines by endogenous transporters in oocytes seems to be at least partially sodium dependent. In addition, this correlates to the already mentioned findings where the presence of a sodium-dependent LAT-like transporter in oocytes was reported [21].

For comparison, the substrate affinity of 3,3′-T_2 was also analyzed in uptake measurements of the MCT8 transporter. Visser et al. [4] showed that MCT8 transports 3,3′-T_2 using *X. laevis* oocytes as the expression system. The thyroid hormones K_m values were calculated in a low micromolar range, for example LAT1, MCT8 and OATP1C1 [4, 15, 22, 23].

Next we asked whether the known LAT inhibitor BCH [24] influences the 3,3′-T_2 uptake by Lat2. Coincubation of 3,3′-T_2 with BCH of noninjected oocytes resulted in no significant decrease in the uptake of hormone, suggesting that there is no endogenous expression of LATs in *X. laevis* oocytes (data not shown).

In addition, we used the 3,3′-T_2 uptake for inhibition studies with known Lat2 substrates like L-Leu, L-Ile, L-Met and L-His [18, 20]. All four amino acids resulted in a high competing inhibition of 3,3′-T_2 uptake (fig. 4), which is comparable with previously observed effects of L-Leu on iodothyronine transport by LAT1 [19].

Finally, we tested thyroid hormones, which differ in their number and position of iodine atoms, for inhibition of 3,3′-T_2 transport. T_0 and also T_1 and T_2 derivatives showed a strong inhibitory effect (fig. 4). In contrast, 3,3′-T_2 transport by MCT8 was only inhibited by iodothyronines carrying at least one iodine atom per aromatic ring like 3,3′-T_2, and T_3 (fig. 5). The competition studies on 3,3′-T_2 uptake by MCT8 are comparable with previous findings [15].

Our results are likely influenced by the fact that this transporter is bidirectional. It may also happen that the efflux will increase over time with the amount of imported T_2/T_3.

So far, the focus in thyroid research has mainly been directed towards the classical thyroid hormones T_3 and T_4. Interestingly, diiodothyronines, which are mostly regarded as a mere degradation product, also trigger cellular responses, e.g. cell metabolism. For example, 3,3′-T_2 binding sites were discovered in rat mitochondria, suggesting a role in metabolic regulation of the cell [25]. Also, stimulation of the cytochrome oxygenase was noted, but this data has not yet been independently confirmed. While only a few possible biological effects of 3,3′-T_2 and 3′,5′-T_2 have been reported, there is more information available for 3,5-T_2, which circulates in human serum [26] and might rapidly and directly act on mitochondrial function and at higher concentrations also mimics T_3 effects via modulation of TR-dependent transcription in many target cells [27–31]. Moreover, it was recently demonstrated that 3,5-T_2 influences the volume of adipocytes by inducing lipolysis and increases the mitochondrial content in brown adipocytes and thermogenesis, thus demonstrating an influence of 3,5-T_2 on the metabolism [32]. Recent findings in fish even suggest an activating role of 3,5-T_2 by binding to the thyroid hormone receptor β1, thus remodeling the role of T_2 in the mode of action of thyroid hormones [33].

Taken together, we not only affirmed previous findings pointing towards an involvement of Lat2 in the uptake of thyroid hormones, but, as the most important finding, we showed that Lat2 mainly transports 3,3′-T_2, less T_3 and no rT_3 and T_4. One of the questions that remains to be clarified is the extent to which cellular uptake of iodothyronines through Lat2 is stimulated by countertransport of different intracellular iodothyronines or amino acids. This is because Lat2 was described as an amino acid exchanger and could also be involved in the efflux of these substrates [34].

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Disclosure Statement

The authors have nothing to disclose.

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