

Identification of Monocarboxylate Transporter 8 as a Specific Thyroid Hormone Transporter*

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Transport of thyroid hormone across the cell membrane is required for its action and metabolism. Recently, a T-type amino acid transporter was cloned which transports aromatic amino acids but not iodothyronines. This transporter belongs to the monocarboxylate transporter (MCT) family and is most homologous with MCT8 (SLC16A2). Therefore, we cloned rat MCT8 and tested it for thyroid hormone transport in *Xenopus laevis* oocytes. Oocytes were injected with rat MCT8 cRNA, and after 3 days immunofluorescence microscopy demonstrated expression of the protein at the plasma membrane. MCT8 cRNA induced an ~10-fold increase in uptake of 10 nM ¹²⁵I-labeled thyroxine (T₄), 3,3',5-triiodothyronine (T₃), 3,3',5'-triiodothyronine (rT₃) and 3,3'-diiodothyronine. Because of the rapid uptake of the ligands, transport was only linear with time for <4 min. MCT8 did not transport Leu, Phe, Trp, or Tyr. [¹²⁵I]T₄ transport was strongly inhibited by L-T₄, D-T₄, L-T₃, D-T₃, 3,3',5-triiodothyroacetic acid, N-bromoacetyl-T₃, and bromosulphophthalein. T₃ transport was less affected by these inhibitors. Iodothyronine uptake in uninjected oocytes was reduced by albumin, but the stimulation induced by MCT8 was markedly increased. Saturation analysis provided apparent K_m values of 2–5 μM for T₄, T₃, and rT₃. Immunohistochemistry showed high expression in liver, kidney, brain, and heart. In conclusion, we have identified MCT8 as a very active and specific thyroid hormone transporter.

Thyroid hormone is important for the development of different tissues and the metabolic function of these tissues throughout life. A well known example is the critical role of thyroid hormone in brain development (1). Transport of thyroid hormone across the cell membrane is required for its metabolism and action. Thyroxine (T₄)¹ is the predominant (pro)hormone

secreted by the thyroid follicular cells, but 3,3',5-triiodothyronine (T₃) is the receptor-active form of thyroid hormone.

For its conversion to T₃, T₄ must enter cells that express the enzymes catalyzing this reaction. The three iodothyronine deiodinases (D1–D3) involved in the activation and inactivation of thyroid hormone are homologous selenoproteins with different catalytic profiles, tissue distributions, and physiological functions (2–4). D1, for example, is expressed predominantly in liver and kidney and is important for systemic T₃ production. D2, on the other hand, is mainly active in brain, pituitary, and skeletal muscle and is important for local T₃ production in tissues such as brain. D3 is expressed in brain and other tissues and is important for the degradation of T₄ and T₃. Most biological effects are initiated by the binding of T₃ to nuclear receptors, which changes the interaction of these receptors with regulatory elements of T₃-responsive genes (5). Obviously, cellular uptake of iodothyronines is critical for thyroid hormone action and metabolism.

The mechanism of cellular uptake of T₄ and T₃ has been studied in a variety of tissues and cultures (6). Functional expression studies using *Xenopus laevis* oocytes have identified two categories of transporters involved in thyroid hormone uptake in different tissues, *i.e.* organic anion transporters and amino acid transporters (7). The heterodimeric amino acid transporters consist of a common heavy chain and a variable light chain. Two of these light chains (LAT1, LAT2) are involved in transport of neutral branched-chain or aromatic (L-type) amino acids such as Leu, Phe, Tyr, and Trp. We have demonstrated that in particular LAT1 also mediates uptake of iodothyronines (8). However, there must be additional transport proteins, which contribute more substantially to thyroid hormone transport *in vivo* because LAT1 is not expressed in the liver, and the liver is the main organ for the extrathyroidal conversion of T₄ to systemic T₃.

Recently, Endou and co-workers (9, 10) have cloned a single-chain T-type amino acid transporter (TAT1) that specifically transports the aromatic amino acids Phe, Tyr, and Trp, but not iodothyronines. Previous studies by Blondeau and co-workers (11–13) have suggested the involvement of a T-type amino acid transporter in the uptake of thyroid hormone in different cell types. TAT1 was found to be a member of the so-called monocarboxylate transporter (MCT) family, different members of which have been identified as transporters for lactate and pyruvate (14). TAT1 (MCT10, SLC16A10) shows 49% amino acid identity with another member of the MCT family, *i.e.*

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¹ The abbreviations used are: T₄, thyroxine; BrAcT₃, N-bromoacetyl-3,3',5-triiodothyronine; BSA, bovine serum albumin; BSP, bromosulphophthalein; LAT, L-type amino acid transporter; MCT, monocarboxylate transporter; NTCP, Na⁺/taurocholate-cotransporting polypeptide; OATP, organic anion-transporting polypeptide; rT₃, 3,3',5'-triiodothy-

ronine; T₂, 3,3'-diiodothyronine; T₃, 3,3',5-triiodothyronine; T₄NS, N α -sulfonated T₄ (T₄ sulfamate); T₄S, 4'-OH-sulfonated T₄ (T₄ sulfate); TAT, T-type amino acid transporter; Triac, 3,3',5-triiodothyroacetic acid; TRITC, tetramethylrhodamine isothiocyanate.

MCT8 (SLC16A2), but much lower homology with other members.

Although human MCT8 was cloned in 1994 by Lafreniere *et al.* (15), its role has never been elucidated. The gene consists of 6 exons coding for a protein with 12 putative transmembrane domains, suggestive of a transporter function. MCT8 mRNA is highly expressed in liver, but also in heart, brain, placenta, lung, and kidney (15). Because the function of MCT8 is unknown, we have cloned rat MCT8 and tested it for thyroid hormone transport activity in *X. laevis* oocytes.

EXPERIMENTAL PROCEDURES

Materials—Nonradioactive L-iodothyronines, 3,3',5-triiodothyroacetic acid (Triac) and N-bromoacetyl-3,3',5-triiodothyronine (BrAcT₃) were obtained from Henning, Berlin. D-Iodothyronines, Phe, and Tyr, were purchased from Sigma, Leu was obtained from Merck, and Trp and bromosulphophthalein (BSP) were purchased from Fluka. [3',5'-¹²⁵I]T₄, [3'-¹²⁵I]T₃ and carrier-free Na¹²⁵I were purchased from Amersham Biosciences. All other ¹²⁵I-labeled compounds were prepared as described previously (16). ³H-labeled Leu, Phe, Tyr, and Trp were purchased from Amersham Biosciences. All other chemicals were of reagent grade.

Cloning of Rat MCT8—Primers for reverse transcription-PCR were designed to regions of high homology in the 5'- and 3'-untranslated region sequences of human (U05315) and mouse (AF045692) MCT8. The sense primer was 5'-AGCTCTCGAGCGCAAGCCACAGTCAG-3', corresponding to the mouse sequence from nucleotide 145 (the coding sequence starts at nucleotide 175) and contained an *Xho*I restriction site (underlined). The antisense primer was 5'-AAATGCGGCCGTCTCCGTTGGGGTCT-3', corresponding to the mouse sequence ending at nucleotide 2242 (the coding sequence ends at nucleotide 1872) and contained an *Not*I restriction site (underlined). Isolated rat liver mRNA was *in vitro* reverse transcribed and amplified using the reverse transcription system from Promega and subjected to PCR using the above primers. The product was ligated into pGEMT-Easy and sequenced.

Insertion of a FLAG-tagged MCT8 Construct into the Oocyte Expression Vector pGEM-HeJuel—For expression in oocytes it was decided to append an 8-amino acid FLAG epitope to the C terminus of MCT8 to allow detection of expression by immunofluorescence microscopy and Western blotting. For this purpose the stop codon of MCT8 was removed by performing PCR using the same sense primer as above, but with the antisense primer, 5'-ACAGCGGCCGCAAATGGGCTCTTCAGGTGTTG-3', which lacks the stop codon. This PCR product was ligated into pGEMT-Easy before being excised with *Eco*RI and ligated into the FLAG vector, pCMV-Tag4A (Stratagene), which had previously been digested with the same restriction enzyme and dephosphorylated. The FLAG epitope-tagged MCT8 was then ligated into the *Xenopus* oocyte expression vector pGEM-HeJuel which contains the 5'- and 3'-untranslated regions of the *Xenopus* β -globin flanking a multiple cloning site. The stability of the transcribed MCT8 mRNA sandwiched between the untranslated globin sequence is likely to be enhanced in the oocyte as was found to be the case for other MCTs (17).

The MCT8-FLAG insert was prepared using PCR with primers flanking the MCT8-FLAG insert in pCMV-Tag4A and containing suitable restriction sites for the insertion into the pGEM-HeJuel vector. The sense primer was 5'-GCGGGGATCCACACGTCAGTCCCCTAGCCA-3' and contained a *Bam*HI restriction site (underlined); the antisense primer was 5'-CTTATCTAGATAAGGTACCGGGCCCTACT-3' and contained an *Xba*I restriction site (underlined). Following successful PCR amplification, the product was digested with *Bam*HI and *Xba*I and ligated into the pGEM-HeJuel vector digested with the same restriction enzymes. The correct identity of the product was established using an *Eco*RI digest and confirmed with sequencing.

***X. laevis* Oocyte Expression**—MCT8 cRNA was obtained by *in vitro* transcription using the Ampliscribe High Yield T7 RNA transcription kit (Epicentre) after linearization of pGEM-HeJuel containing the MCT8 cDNA with *Not*I. Oocytes were isolated and allowed to recover overnight at 18 °C in modified Barth's solution containing 20 IU/ml penicillin and 20 μ g/ml streptomycin as described before (18). The next day, oocytes were injected with cRNA coding for MCT8 and incubated further for 3 days at 18 °C in modified Barth's solution until analysis. Uninjected or water-injected oocytes were used as controls.

Production of MCT8 Antibody—Initially, antibodies against the C terminus of MCT8 were raised in rabbits using the C-terminal 16 amino acids conjugated to keyhole limpet hemocyanin as we have done for other MCT isoforms (19). Although these antibodies were active in

enzyme-linked immunosorbent assays, they failed to detect MCT8 expressed in *Xenopus* oocytes, using either Western blotting or immunofluorescence microscopy. As an alternative strategy, a fusion protein corresponding to the C-terminal 41 amino acids of MCT8 linked to glutathione S-transferase was overexpressed in *Escherichia coli* using the pGEX-4T-3 vector (Amersham Biosciences) (20). The protein was purified using glutathione-Sepharose as described previously (20), and rabbits were inoculated with 100 μ g (four times in total) using established procedures (19). The IgG fraction of the serum from the final bleed was purified with caprylic acid, and then antibodies reactive against glutathione S-transferase were removed by passage through an affinity column (glutathione S-transferase bound to glutathione-Sepharose). Blocking peptide was produced by thrombin cleavage of the glutathione S-transferase fusion protein bound to glutathione-Sepharose.

Detection of MCT8 in Rat Tissues and *Xenopus* Oocytes by Immunofluorescence Microscopy—Oocytes were first embedded in pieces of chicken liver and then placed on pieces of cork, covered in O.C.T. embedding compound (Tissue-Tek, Sakura Finetek Europe) and frozen in liquid nitrogen-cooled isopentane. Frozen sections (5 μ m) were cut, placed on silanized slides, and air dried at room temperature for 1 h before fixing with ice-cold acetone for 10 min. Permeabilization and staining were then carried out as described previously (21) using a mouse monoclonal anti-FLAG antibody and TRITC-conjugated anti-mouse IgG secondary antibody.

Sections of rat tissues were obtained from Novagen, and after dewaxing using the protocol provided by the manufacturer, they were stained using anti-MCT8 primary antibody at a 1:100 dilution and a TRITC-labeled secondary antibody prior to visualization with a Upright Leica TCS-NT confocal laser scanning microscope. Further details of the protocol, including washing and blocking procedures, can be found elsewhere (21).

Western Blotting of Oocyte Membranes—Crude oocyte membranes were prepared using 10 oocytes harvested 3 days after microinjection with MCT8 cRNA or the equivalent volume of water. Oocytes were suspended in 500 μ l of buffer (10 mM HEPES, 83 mM NaCl, 1 mM MgCl₂, pH 7.9, containing 4 mg/ml pepstatin A, leupeptin, antipain, and 0.5 mM phenylmethylsulfonyl fluoride and benzamide) and homogenized by vigorous vortex mixing followed by several passes through an 18-gauge needle. The homogenate was centrifuged at 2,000 rpm for 5 min at 4 °C, and the supernatant was collected; the pellet was rehomogenized in 500 μ l of buffer and centrifuged as before. Both the supernatants were then mixed and centrifuged at 100,000 $\times g$ for 60 min at 4 °C and the pellet resuspended in 30 μ l of buffer and 30 μ l of SDS-PAGE sample buffer. Following separation by SDS-PAGE Western blotting was performed using mouse anti-FLAG monoclonal antibody (Sigma) with detection by enhanced chemiluminescence (ECL) as described previously (21).

Membrane fractions from the rat tissues shown were prepared as described previously (21) and subjected to SDS-PAGE and Western blotting with the MCT8 antibody at a 1:1,000 dilution. Where indicated, the MCT8 primary antibody was preincubated with blocking peptide (10 μ g/ml for 60 min at 4 °C).

Transport Measurements in Oocytes—Oocytes were washed and preincubated at 18 °C in standard uptake solution (100 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 10 mM Tris, pH 7.5). Usually, transport was tested by incubation of groups of 5–10 oocytes for 2–60 min at 25 °C with 10 nM ¹²⁵I-labeled iodothyronines or 10 μ M ³H-labeled amino acids in 0.1 ml of standard uptake solution. The possible Na⁺ dependence of transport was tested by preincubation and incubation in Na⁺-free uptake solution in which NaCl was replaced by choline chloride. The influence of the temperature on the uptake rate was tested by the incubation of oocytes with uptake solutions at different temperatures between 4 and 37 °C. The influence of albumin on uptake of T₃ was tested by the addition of 0.1% and 0.5% BSA to the standard uptake solution. The incubation was stopped by aspiration of the medium, and oocytes were washed four times at 4 °C with standard uptake solution containing 0.1% BSA.

The substrate specificity of MCT8 was investigated by incubation of oocytes with different putative radioactive ligands, including T₄, T₃, 3,3',5'-triiodothyronine (rT₃), 3,3'-diiodothyronine (T₂), Na-sulfonated T₄ (T₄ sulfamate, T₄NS), and 4'-OH-sulfonated T₄ (T₄ sulfate, T₄S), and the amino acids Leu, Phe, Tyr, and Trp. Specificity of transport of [¹²⁵I]T₄ and [¹²⁵I]T₃ was measured in the presence of putative competitors, including 10 μ M unlabeled iodothyronine derivatives such as D- and L-iodothyronines, Triac, and BrAcT₃, and 100 μ M Tyr, Trp, or BSP.

Analysis of rT₃ Metabolism in Oocytes—Groups of 10 oocytes were incubated for 2–60 min at 25 °C with 10 nM [¹²⁵I]rT₃. After incubation, medium was collected, and two groups of 5 oocytes were counted separately and homogenized in 0.1 ml of 0.1 M NaOH as described before

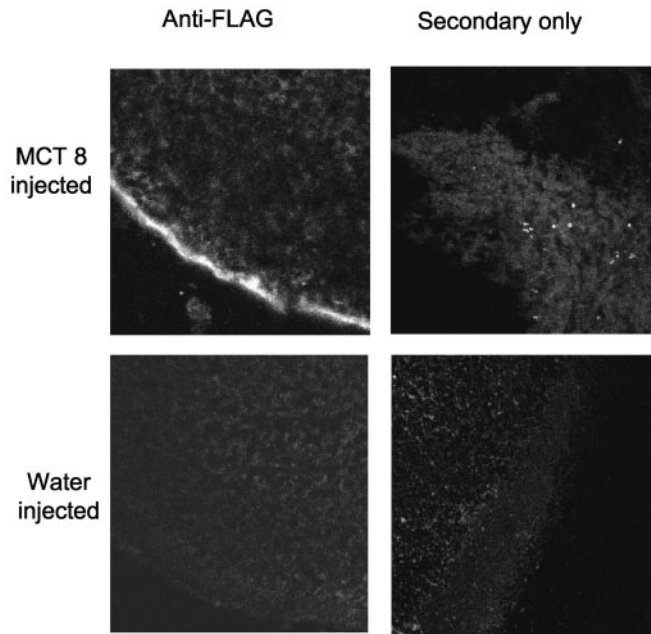


FIG. 2. **Detection of FLAG-tagged MCT8 protein at the cell surface of the oocytes.** Oocytes were fixed, sectioned, and prepared for immunofluorescence microscopy (anti-FLAG antibody) 3 days after injection with MCT8-FLAG cRNA or water (control) as described under "Experimental Procedures."

the plasma membrane. Water-injected oocytes showed no such expression, nor did the secondary antibody alone detect any protein at the plasma membrane.

Functional Characterization—Transport studies were performed to investigate the function of MCT8 expressed in *X. laevis* oocytes. Fig. 3 shows the time course of uptake of T_3 and T_4 in uninjected oocytes and in oocytes injected with 4.6 ng of MCT8 cRNA. Expression of MCT8 induced an ~10-fold increase in initial uptake of T_3 and T_4 compared with uninjected oocytes. This graph also shows that the uptake of T_3 and T_4 into MCT8 cRNA-injected oocytes was only linear for the first 4 min. Therefore, all further transport experiments were performed at 2-min incubations. The difference in uptake of T_3 and T_4 in oocytes injected with MCT8 cRNA is not statistically significant. Transport of T_3 was independent of Na^+ because the same results were obtained using medium with choline chloride instead of NaCl (Fig. 3A). However, T_4 transport by MCT8 showed a modest but consistent inhibition in the absence of Na^+ (Fig. 3B).

Fig. 4 shows the influence of the amount of cRNA injected on the uptake of 10 nM T_3 and T_4 . The results indicate that the lowest amount of MCT8 cRNA (0.23 ng) injected already induced a 5-fold increase in T_3 transport and a 7.3-fold increase in T_4 transport. Maximum induction of T_3 and T_4 transport was found after injection of 1.15–2.3 ng of MCT8 cRNA. Therefore, oocytes were further injected with 4.6 ng of MCT8 cRNA for maximum induction of iodothyronine transport.

To test the temperature dependence of MCT8-mediated iodothyronine transport, oocytes were incubated with 10 nM [^{125}I] T_3 or [^{125}I] T_4 for 2 min at 4–37 °C. Fig. 5 shows significant uptake for T_3 and T_4 into the oocytes at 4 °C, with marked increases if the temperature was increased to 15, 25, and 37 °C, which is above the usual ambient temperature for frog oocytes. Exposure to 37 °C was tolerated by the oocytes during this short incubation time; prolonged incubation at 37 °C resulted in disintegration of the cells. The results show identical temperature dependence of transport of T_4 and T_3 by MCT8.

Fig. 6 shows the effects of addition of 0.1% and 0.5% BSA on

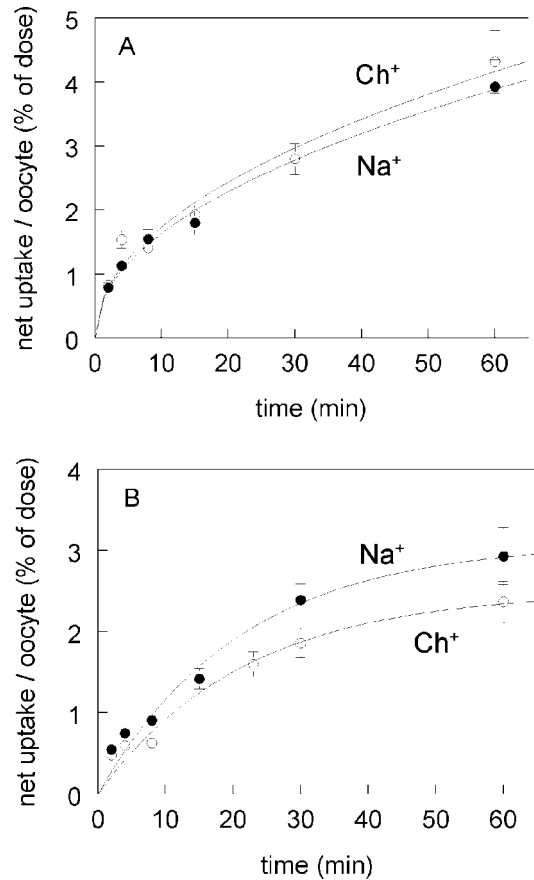


FIG. 3. **Time course of T_3 and T_4 uptake in oocytes.** Oocytes were injected with MCT8 cRNA and after 3 days incubated for 2–60 min at 25 °C with 10 nM [^{125}I] T_3 (A) or [^{125}I] T_4 (B) in medium with (●) or without (○) Na^+ . In the latter, NaCl was replaced by choline chloride.

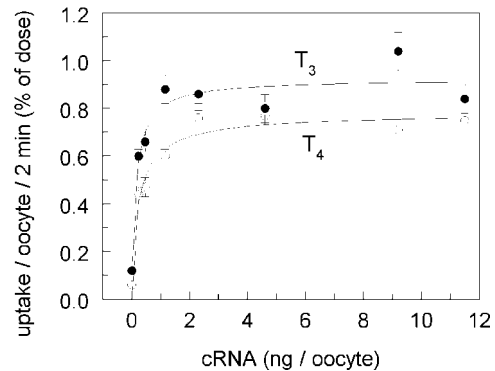


FIG. 4. **MCT8 cRNA concentration-dependent uptake of T_3 and T_4 .** Oocytes were injected with 0–11.5 ng of MCT8 cRNA and after 3 days incubated for 2 min at 25 °C with 10 nM T_3 (●) or T_4 (○).

the uptake of T_3 in oocytes. Both in uninjected oocytes and in MCT8 cRNA-injected oocytes, T_3 uptake was decreased concentration-dependently by BSA. However, the stimulation of T_3 uptake by MCT8 increased from 7.5-fold in the absence of BSA to 14-fold in the presence of 0.1% BSA and further to 25-fold in the presence of 0.5% BSA.

Substrate Specificity—The substrate specificity of MCT8 was investigated by incubation of oocytes with different putative radioactive ligands, including T_4 , T_3 , rT_3 , T_2 , T_4NS , and T_4S , and the amino acids Leu, Phe, Tyr, and Trp. Fig. 7 shows that in contrast to the rapid uptake of the different iodothyronines, T_4NS and T_4S and the amino acids are not transported by MCT8. MCT8 failed to transport the different amino acids at concentra-

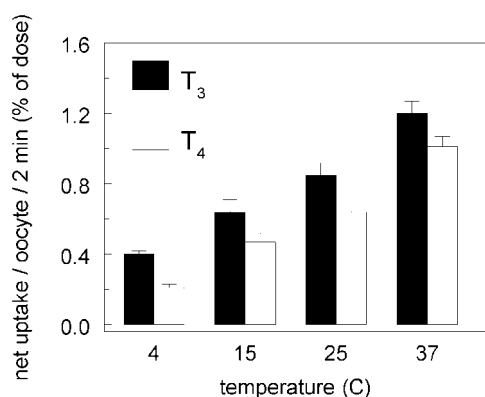


FIG. 5. **Temperature-dependent uptake of T₃ and T₄ by MCT8.** Oocytes were incubated for 2 min at 4, 15, 25, or 37 °C with 10 nM T₃ (■) or T₄ (□). Net uptake of T₃ or T₄ in MCT8 cRNA-injected oocytes is corrected for the corresponding uptake in uninjected oocytes.

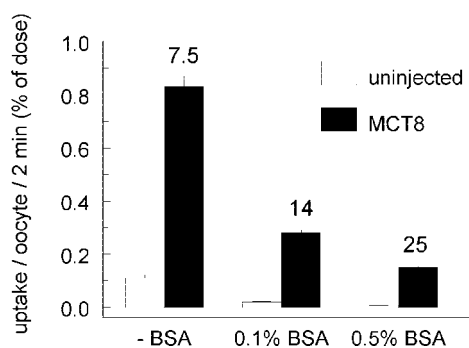


FIG. 6. **Albumin-dependent uptake of T₃.** Oocytes were incubated for 2 min at 25 °C in standard uptake solution with 10 nM T₃ without albumin or supplemented with 0.1% or 0.5% albumin (■). Uninjected oocytes were used as controls (□). Numbers indicate fold stimulation by MCT8.

tions (1–100 μM) showing facile transport by the LAT1 (8) and TAT1 (9, 10) amino acid transporters. We have also been unable to demonstrate any transport of [¹⁴C]lactate, whereas in oocytes injected with rat MCT1 cRNA, transport of [¹⁴C]lactate was greatly stimulated compared with control oocytes, but no additional uptake of [¹²⁵I]T₄ was observed (data not shown).

The specificity of iodothyronine transport by MCT8 was addressed further by investigating the uptake of [¹²⁵I]T₃ and [¹²⁵I]T₄ in the absence or presence of structurally related compounds (Table I). Uptake of labeled T₄ and T₃ by MCT8 was inhibited potently by 10 μM unlabeled L-T₄, D-T₄, L-T₃, and D-T₃, indicating that the interaction of T₄ and T₃ with MCT8 is not stereospecific. Iodothyronine uptake by MCT8 is also potently inhibited by T₃ analogs where the αNH_2 group is blocked (BrAcT₃) or deleted (Triac), indicating that this αNH_2 group is not important for interaction of iodothyronines with MCT8. The T-type amino acids Tyr and Trp hardly affected iodothyronine uptake by MCT8. The organic anion BSP proved to be a potent inhibitor. This is in contrast to the weak effects of the bile acid taurocholate and the organic anion transport inhibitor probenecid (data not shown). In general, T₃ transport by MCT8 shows less inhibition by these compounds than MCT8-mediated T₄ transport. It is remarkable that ligands and potent inhibitors of MCT8 all carry halogen atoms.

Metabolism of rT₃—Previous studies have shown that *X. laevis* oocytes actively metabolize rT₃ by sulfation. Because sulfotransferases are located intracellularly, we have used this property to investigate rT₃ internalization by oocytes. Fig. 8 shows the time course of rT₃ uptake and subsequent metabolism in native and MCT8-expressing oocytes. At several time

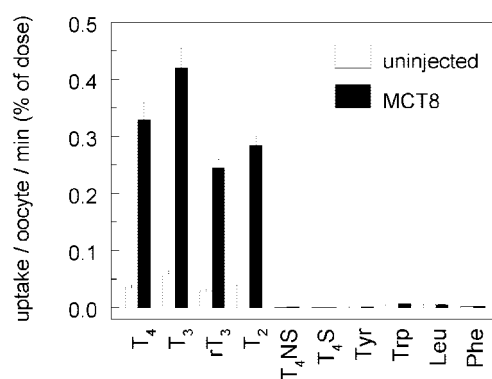


FIG. 7. **Ligand-dependent transport by MCT8.** MCT8 cRNA-injected oocytes were incubated for 2–60 min at 25 °C with 10 nM [¹²⁵I]-labeled iodothyronines or 10 μM [³H]-labeled amino acids (Tyr, Trp, Leu, and Phe) (■). Uninjected oocytes were used as controls (□). The uptake of the different putative ligands is expressed per min.

TABLE I
Inhibition of uptake of T₄ and T₃ (10 nM) in MCT8 cRNA-injected oocytes by iodothyronines or Tyr, Trp, and BSP

Inhibitor	μM	% Inhibition \pm S.E.	
		T ₄	T ₃
L-T ₄	10	69 \pm 7	26 \pm 7
L-T ₃	10	62 \pm 7	57 \pm 3
D-T ₄	10	76 \pm 7	21 \pm 5
D-T ₃	10	72 \pm 3	24 \pm 5
Triac	10	76 \pm 3	47 \pm 3
BrAcT ₃	10	90 \pm 3	67 \pm 1
Tyr	100	37 \pm 6	0 \pm 9
Trp	100	22 \pm 6	0 \pm 7
BSP	100	94 \pm 2	81 \pm 1

points, uninjected and MCT8 cRNA-injected oocytes are homogenized, and the homogenates and medium samples are analyzed for rT₃ and rT₃ sulfate (rT₃S) content. It is shown that already after 2 min of incubation, rT₃S is formed intracellularly in the MCT8 cRNA-injected oocytes. After 10 min of incubation, release of rT₃S into the medium is observed. The production rate of rT₃S is much higher in MCT8 cRNA-injected oocytes than in uninjected cells. These findings demonstrate that MCT8-mediated uptake of iodothyronines indeed represents the internalization of these compounds.

Transport Kinetics—The kinetics of MCT8-mediated iodothyronine transport were analyzed by incubation of oocytes with increasing T₄, T₃, or rT₃ concentrations (1 nM–30 μM). Net transport by MCT8 was calculated by subtracting uptake in uninjected oocytes from that in MCT8 cRNA-injected oocytes. The results showed that transport of T₄, T₃, and rT₃ by MCT8 was saturable. Michaelis-Menten analysis of the data provided apparent K_m values of 4.7 μM for T₄, 4.0 μM for T₃, and 2.2 μM for rT₃ (Fig. 9).

Tissue Distribution of MCT8 in Rat Tissues—Previous studies using Northern blot analysis suggested that MCT8 is widely distributed in human tissues with liver showing especially high levels of expression (14). However, there are no reports of expression at the protein level, and for this purpose it was necessary to raise a specific antibody against MCT8. Initial attempts to raise an antibody against the C-terminal 16 amino acids conjugated to keyhole limpet hemocyanin as we have done for other MCT isoforms (19) failed to yield a suitable antibody. Subsequently, a suitable antibody was raised using a fusion protein of glutathione *S*-transferase with the C-terminal 41 amino acids of MCT8 (see “Experimental Procedures”). Confirmation that this antibody was active in both Western blotting and immunofluorescence microscopy was first achieved using *Xenopus* oocytes expressing either MCT8 or MCT8-

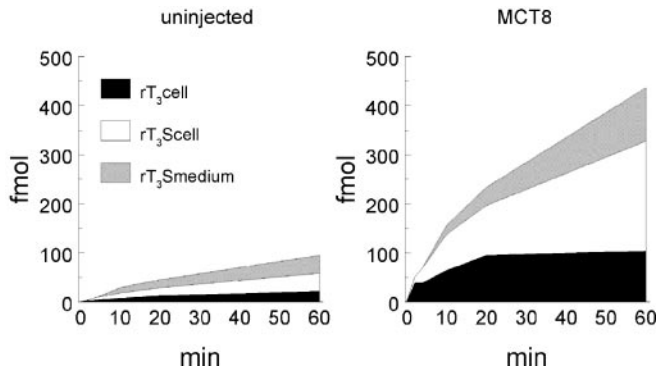


FIG. 8. **Uptake and metabolism of rT_3 .** Oocytes were incubated for 2–60 min at 25 °C with 10 nM rT_3 . At each time point, uninjected oocytes (left panel) and MCT8 cRNA-injected oocytes (right panel) were homogenized, and homogenates and medium samples were analyzed as described under “Experimental Procedures.”

FLAG. In Fig. 10A we show that membranes from oocytes expressing MCT8-FLAG gave a band of about 67 kDa on Western blotting with either the anti-FLAG antibody or the anti-MCT8 antibody that was absent in uninjected oocytes. When the oocytes were injected with MCT8 cRNA without the FLAG a band of about 63 kDa was observed only with the anti-MCT8 antibody. The lower apparent molecular mass is predicted because of the absence of the 20-amino acid FLAG sequence and linker. Preincubation of the MCT8 antibody with blocking peptide specifically removed the 63/67 kDa band as expected. In Fig. 11 we demonstrate that the antibody was also able to detect MCT8 in the *Xenopus* oocytes using immunofluorescence microscopy.

Having confirmed that the antibody was active we investigated the expression of MCT8 in crude plasma membrane preparations of a range of rat tissues by Western blotting as shown in Fig. 10B. Although several bands were apparent in the blots, only one major band of about 63 kDa was removed specifically by the peptide, and we assume this to be MCT8. Consistent with this, the highest expression was found in liver membranes. Kidney and brain also expressed significant amounts, whereas no band was visible in the testis. In the heart a very weak band at 63 kDa was visible on overexposure of the blot, but another band removed specifically by the blocking peptide was apparent at about 50 kDa. It is possible that this represents a truncated form of MCT8 with the 90-amino acid extended PEST-containing N terminus removed. That MCT8 is present in heart as well as liver and kidney is confirmed by the immunofluorescence data of Fig. 11. Confirmation of the specificity of the antibody was provided by using either secondary antibody alone or primary antibody blocked with peptide as indicated.

DISCUSSION

The *MCT8* gene consists of 6 exons coding for a protein with 12 putative transmembrane domains. The gene is located on the X chromosome (Xq13.2) and is the only MCT member that contains an N-terminal PEST domain, *i.e.* a domain abundant in Pro (P), Glu (E), Ser (S), and Thr (T) residues. This domain is probably important for the turnover of the protein (23). The human MCT8 was cloned in 1994 (15), but until now its function has not been elucidated. MCT8 is highly expressed in liver and brain but is also widely distributed in other tissues (14, 15). In this study, we have functionally characterized this orphan transporter, as the most active and specific thyroid hormone transporter known to date. The broad tissue distribution of MCT8 fits with its function as a thyroid hormone transporter because this hormone is essential for regulation of de-

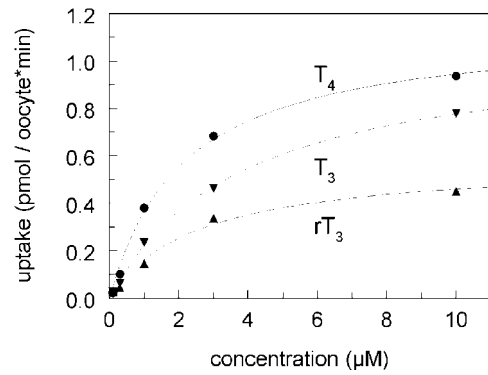


FIG. 9. **Kinetics of MCT8-mediated rT_3 , T_3 , and T_4 uptake.** Oocytes were incubated with increasing rT_3 , T_3 , or T_4 concentrations (1 nM–30 μ M). Net transport by MCT8 was calculated by subtracting uptake in uninjected oocytes from that in MCT8 cRNA-injected oocytes. Uptake is expressed as fmol/oocyte/min. Kinetics were performed using the Slide Write Plus Program.

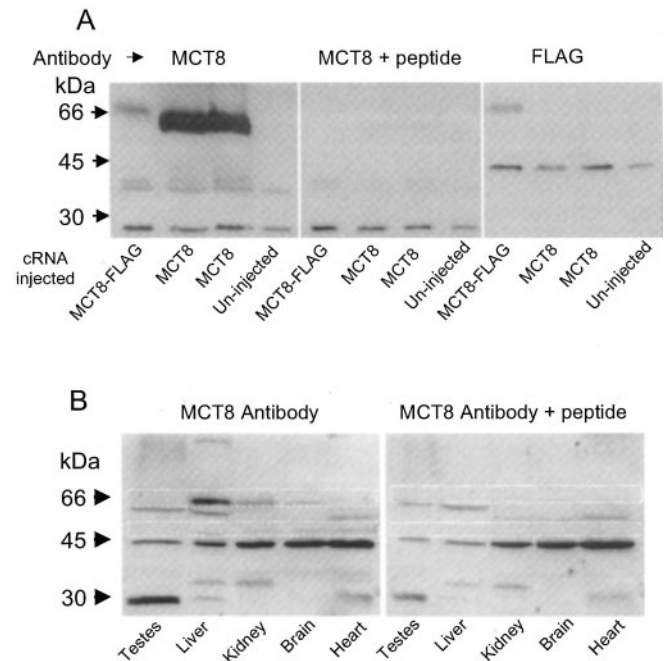


FIG. 10. **Expression of MCT8 in *Xenopus* oocytes and various rat tissues determined by Western blotting.** In A, membrane fractions were prepared from uninjected *Xenopus* oocytes and those expressing either MCT8 or MCT8-FLAG and separated by SDS-PAGE (12%) followed by Western blotting with the antibody shown at 1:1,000 dilution. Where indicated, the MCT8 primary antibody was preincubated with blocking peptide (10 μ g/ml for 60 min at 4 °C). In B, membrane fractions from the rat tissues shown were prepared as described previously (21) and subjected to SDS-PAGE and Western blotting as above. The white boxes identify bands that were specifically removed by the blocking peptide.

velopmental and metabolic processes in many tissues. The high expression of MCT8 in liver and brain is also important for the regulation of the conversion of T_4 to T_3 by the enzymes D1 and D2 expressed in these tissues, respectively.

MCT8 has the highest homology (49% identity at amino acid level) with the T-type amino acid transporter (TAT1 or MCT10), and both transporters have far less homology with the other members of the MCT family. Also functionally, MCT8 and MCT10 seem to differ from the other MCT members because MCT8 and MCT10 are capable of transporting amino acid derivatives, whereas other characterized MCTs have been shown to transport monocarboxylates (9, 10). However, it is not excluded that also other MCT transporters mediate transport

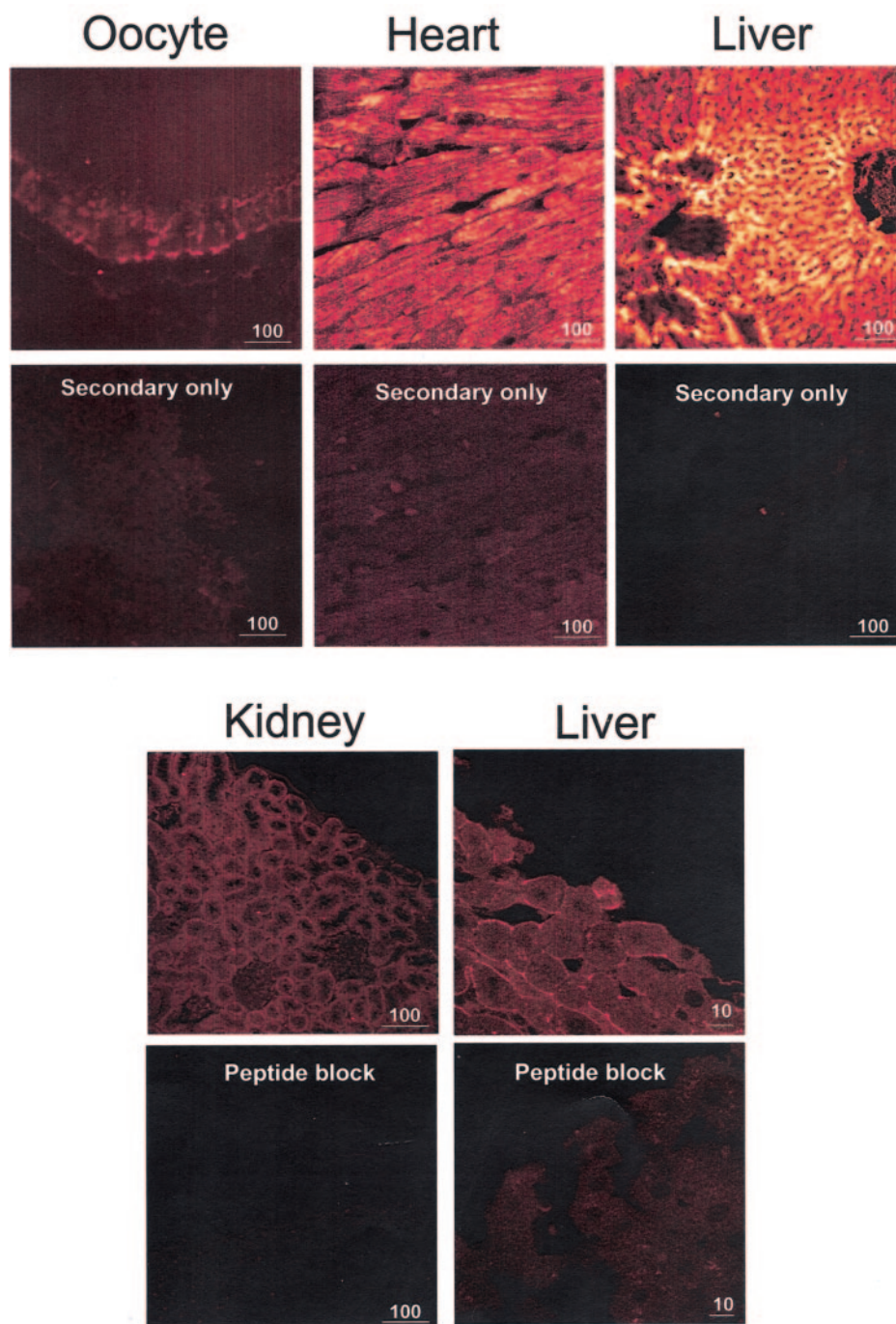


FIG. 11. Expression of MCT8 in *Xenopus* oocytes and various rat tissues and determined by immunofluorescence microscopy. Sections of the rat tissues indicated were treated with anti-MCT8 primary antibody followed by a TRITC-labeled secondary antibody as described under "Experimental Procedures" prior to confocal microscopy. Sections of *Xenopus* oocytes microinjected with MCT8 cRNA were obtained in a manner identical to those from MCT8-FLAG-expressing oocytes (see Fig. 2) and treated as above. Controls were performed either using secondary antibody alone or using primary antibody preincubated with blocking peptide (10 $\mu\text{g}/\text{ml}$ for 60 min at 4 $^{\circ}\text{C}$). Scale bars are given in μm .

of amino acids, while MCT8 and MCT10 may also transport monocarboxylates. The latter is supported by our observation that BrAcT₃ and Triac, which both lack a free αNH_2 group, are potent inhibitors of iodothyronine transport by rat MCT8. The homology in structure between MCT8 and MCT10 is also reflected in their similar preference for aromatic amino acid derivatives as ligands, although MCT10 does not transport iodothyronines, and MCT8 does not transport Tyr, Phe or Trp. The specificity of MCT8 for iodothyronines is further supported by the minor effects of large concentrations of aromatic amino acids on transport of T₄ and T₃.

In the last few years, several transporters have been shown to be capable of transporting thyroid hormone, including organic anion transporters and amino acid transporters. Among the organic anion transporters, both Na⁺/taurocholate-cotransporting polypeptide (NTCP) and various members of the Na⁺-

independent organic anion-transporting polypeptide (OATP) family mediate transport of iodothyronines as well as their sulfate and sulfamate derivatives (6, 7, 24). Recently, a new member, OATP-F, was isolated and characterized (25). OATP-F is expressed predominantly in multiple brain regions and in testis and mediates only transport of T₄ and rT₃. Expression experiments in oocytes have demonstrated that the human heterodimeric amino acid transporter consisting of the 4F2 heavy chain and the LAT1 or LAT2 light chain also mediates uptake of the different iodothyronines (8). However, the stimulation of iodothyronine uptake induced by the expression of these transporters in oocytes is limited (<2-fold), and prolonged (60-min) incubations are required to obtain reliable uptake values. In contrast, MCT8 induces an ~10-fold increase in iodothyronine uptake in oocytes, and incubations have to be limited to <4 min to prevent exhaustion of ligand. Comparison

of the present findings with those previously obtained in our laboratory indicate that the rate of transport of T_4 or T_3 by rat MCT8 is at least 10-fold higher than corresponding transport rates with rat NTCP, rat OATP1, and human 4F2/LAT1 (8, 24).

Transport of T_3 by MCT8 in oocytes is Na^+ -independent, although T_4 transport is decreased in the absence of Na^+ . This may suggest that MCT8 is not the major transporter for uptake of thyroid hormone into liver cells, as previous studies have suggested that the latter is largely Na^+ -dependent (6). However, this conclusion was largely based on the inhibition of iodothyronine uptake in hepatocytes by large concentrations (0.5 mM) of the Na^+/K^+ -ATPase inhibitor ouabain (26). We now know that ouabain may also inhibit transporters such as OATPs directly, and in this study we also noticed modest inhibition of MCT8-mediated iodothyronine uptake by 0.1 mM ouabain (data not shown). Therefore, the largely Na^+ -independent nature of iodothyronine transport by MCT8 does not exclude its role in hepatic uptake of thyroid hormone. This is further supported by the positive effects of BSA on iodothyronine uptake by MCT8-expressing oocytes, a phenomenon that has previously also been observed in hepatocytes (27). The facilitatory effect of BSA on iodothyronine uptake by hepatocytes has been explained by assuming that it provides a buffer of loosely bound ligand in the unstirred water layer surrounding the cell, from which ligand would be rapidly depleted in the absence of BSA (26). This explanation may also apply to the facilitation of MCT8-mediated iodothyronine transport in oocytes by BSA. The apparent K_m values of iodothyronines for MCT8 determined in this study (μM range) are higher than those estimated previously by uptake of these compounds in rat hepatocytes (nM range) (6). However, this may be explained at least in part by the very different conditions under which these parameters have been obtained, regarding type of cells, incubation temperature, presence of BSA, etc.

Previous studies have suggested that uptake of T_4 and T_3 by isolated rat hepatocytes is mediated by different transporters. However, MCT8 is capable of transporting both T_4 and T_3 with similar K_m values. It is not excluded that different MCT8 isoforms with different T_3 and T_4 transport efficacies may be generated by alternative exon usage, alternative splicing, and/or post-translational modification. Indeed, our results confirmed expression of multiple MCT8 protein species in different tissues, in particular the heart. It is also not excluded that MCT8 may associate with different protein partners, resulting in transport complexes with different activities toward different iodothyronines. However, we also noticed that various inhibitors show different magnitudes of inhibition of T_4 and T_3 uptake by MCT8. With the exception of L - T_3 , BrAc T_3 , and BSP, all other compounds tested inhibited the transport of [^{125}I] T_3 to a much smaller extent than transport of [^{125}I] T_4 . In addition to the differential dependence of T_4 and T_3 trans-

port by MCT8 on medium Na^+ , these results suggest different modes of interaction of T_4 and T_3 with MCT8. Obviously, this may be a direct effect of the different halogen substitution patterns of these iodothyronines, but it could also be related to the widely different pK values of the phenolic hydroxyl group which is 8.5 for T_3 and 6.5 for T_4 . At neutral pH, therefore, T_3 has no net charge, whereas T_4 is negatively charged.

In conclusion, we have characterized MCT8 to be a specific and very active thyroid hormone transporter.

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Identification of Monocarboxylate Transporter 8 as a Specific Thyroid Hormone Transporter

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