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Transport of thyroid hormones is selectively inhibited by 3-iodothyronamine

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Abstract

Thyroid hormone transporters are responsible for the cellular uptake of thyroid hormones, which is a prerequisite for their subsequent metabolism and action at nuclear thyroid hormone receptors. A recently discovered thyroid hormone derivative, 3-iodothyronamine (T₁AM), has distinct biological effects that are opposite those of thyroid hormone. Here we investigate the effects of T₁AM on thyroid hormone transporters using COS-1 cells transfected with the multispecific organic anion transporting polypeptides (OATPs) 1A2, 1B3, and 1C1, as well as the specific thyroid hormone transporters MCT8 and MCT10, and show that T₁AM displays differential inhibition of T₃ and T₄ cellular uptake by these transporters. T₁AM inhibits T₃ and T₄ transport by OATP1A2 with IC₅₀ values of 0.27 and 2.1 μM, respectively. T₄ transport by OATP1C1, which is thought to play a key role in thyroid hormone transport across the blood-brain barrier, is inhibited by T₁AM with an IC₅₀ of 4.8 μM. T₁AM also inhibits both T₃ and T₄ uptake *via* MCT8, the most specific thyroid hormone transporter identified to date, with IC₅₀ values of 95 and 31 μM, respectively. By contrast, T₁AM has no effect on thyroid hormone transport by OATP1B3 and MCT10. Given that OATP1A2, OATP1C1, and MCT8 are all present in the brain, T₁AM may play an important role in modulating thyroid hormone delivery and activity in specific target regions in the central nervous system.

Introduction

Thyroid hormone has many important physiological roles and is critical for growth, development, and regulation of metabolic processes throughout life.¹ Entry of thyroid hormone into the cell is required for its action and metabolism, since it acts by binding to nuclear thyroid hormone receptors to regulate transcription of target genes. The deiodinases involved in T₄ conversion to the active hormone T₃ or in T₃ degradation are also located intracellularly. Although the lipophilic hormone was originally believed to enter cells by passive diffusion, several plasma membrane transporters responsible for thyroid hormone uptake have recently been identified.^{2–4}

Some of the transporters now known to be involved in the uptake of thyroid hormones include members of the polyspecific organic anion transporting polypeptide (OATP) family⁵ and the monocarboxylate transporter (MCT) family, in particular the specific thyroid

hormone transporters MCT8 and MCT10.⁶ Some examples of OATPs capable of thyroid hormone transport include OATP1A2, which is expressed in the brain, liver, and kidney and likely plays a role in the delivery of T₄ to the kidney and across the blood-brain barrier, the liver-specific OATP1B3 involved in the uptake of T₄ into hepatocytes and the release of T₃ into blood plasma, and OATP1C1, the OATP with the highest affinity and specificity for T₄ and expressed in brain capillaries, suggesting a particular importance for transport of T₄ across the blood-brain barrier.²

In contrast to the numerous OATPs, which transport a wide variety of compounds aside from thyroid hormone, MCT8 is the most active and specific thyroid hormone transporter identified to date⁷ that has also been clearly linked to human disease. Mutations in this transporter result in a severe X-linked mental retardation syndrome with dramatic neurological deficits^{6,8,9} due to impaired delivery of T₃ to neurons in the brain, underscoring a crucial role for MCT8 in the development of the central nervous system. Aside from expression in the brain, MCT8 is also widely distributed in other tissues and likely plays an essential role in thyroid hormone delivery for regulation of developmental and metabolic functions throughout the body. Within the MCT family, MCT10 is most homologous with MCT8. MCT10 has been characterized as a T-type amino acid transporter, which facilitates cellular uptake and efflux of aromatic amino acids such as Trp, Phe, Tyr, and DOPA.^{10,11} MCT10 is expressed in different tissues like intestine, kidney, liver and placenta. Recent studies have demonstrated that MCT10 is also a thyroid hormone transporter with preference for T₃ over T₄.¹²

Aside from enabling intracellular delivery of thyroid hormones, thyroid hormone transporters are also required for the metabolism of thyroid hormones. Further enzymatic reactions could lead to another class of compounds, known as thyronamines, which arise from the decarboxylation of thyroid hormone.¹³ One of these thyronamines, 3-iodothyronamine (T₁AM), is a biogenic amine that is found in vertebrate tissues as well as in the circulatory system. T₁AM has many intriguing pharmacological actions, including effects on thermal regulation, cardiac performance, and metabolism.^{13,14} For example, T₁AM administration triggers a shift in fuel usage toward lipids and away from carbohydrates in both mice and Siberian hamsters.¹⁵ The molecular target(s) of T₁AM action is not entirely clear at present.

Like other trace amines, T₁AM is a potent agonist of the rat and mouse trace amine associated receptors 1 (TAAR1), members of the G-protein-coupled receptor (GPCR) family.¹³ T₁AM may also have a neuromodulatory role as an inhibitor of the dopamine and norepinephrine transporters responsible for the reuptake of these classical neurotransmitters, as well as the vesicular monoamine transporter VMAT2, an intracellular transporter which packages monoamines into synaptic vesicles.¹⁶ While TAAR1 signaling mechanisms and modulation of monoamine transport may help explain some of the pharmacological effects of thyronamines *in vivo*, a greater understanding of the actions of T₁AM is needed.

The importance of thyroid hormone transporters in normal thyroid hormone physiology raises the possibility that the modulation of transporter function may serve to regulate the actions of thyroid hormone. Since T₁AM has been detected in the circulation,¹³ and because the physiological actions of T₁AM are opposite those associated with excess levels of thyroid hormone, we hypothesized that T₁AM may in some way directly oppose the effects of thyroid hormone. Just as T₁AM has been found to inhibit monoamine transport, it is conceivable that T₁AM may also have an effect on the cellular uptake of thyroid hormones and thus play a role in regulation of thyroid hormone activity and metabolism.

With the goal of expanding our knowledge of the molecular mechanisms underlying T₁AM action, the aim of this study was to determine whether T₁AM influences the activity of thyroid hormone transporters. In particular, we were interested in determining whether T₁AM could inhibit thyroid hormone uptake by a selection of thyroid hormone transporters and thereby limit the access of thyroid hormone to the intracellular thyroid hormone deiodinases and receptors. By overexpressing several thyroid hormone plasma membrane transporters in mammalian cells, we developed an assay method to investigate the effect of increasing concentrations of T₁AM on the consequent accumulation of radiolabeled thyroid hormones intracellularly.

Results

T₁AM inhibits thyroid hormone uptake by OATP1A2 and OATP1C1

To examine the effect of T₁AM on transport of thyroid hormone by several plasma membrane transporters of the OATP superfamily, COS-1 cells transiently transfected with constructs containing the entire coding region of OATP1A2, OATP1B3, and OATP1C1 were incubated with the radiolabeled substrates ¹²⁵I-T₃ and ¹²⁵I-T₄, either alone or in the presence of various concentrations of unlabeled T₁AM. For all thyroid hormone uptake studies, cells were cotransfected with μ -crystallin (CRYM), a high-affinity cytosolic thyroid hormone -binding protein that prevents the efflux of internalized iodothyronines.^{17,18} T₁AM inhibited OATP1A2-mediated transport of both thyroid hormones, showing greater potency towards T₃ as revealed by a nearly ten-fold lower IC₅₀ value of 0.27 μ M as compared to an IC₅₀ of 2.1 μ M for T₄ transport (Fig. 1). The observed high basal uptake levels of both ¹²⁵I-T₃ and ¹²⁵I-T₄ in cells transfected with empty vector and CRYM is probably due to thyroid hormone uptake by endogenous transporters present in COS-1 cells.

By contrast, T₁AM did not inhibit uptake of either T₃ or T₄ by OATP1B3. OATP1B3 expression did not enhance uptake of T₃ much above the basal level, and the presence of T₁AM had a similar effect on T₃ uptake in both OATP1B3-transfected and empty vector-transfected cells (Fig. 2A, Fig. S1†). Although T₄ uptake was stimulated over basal levels to a greater degree in OATP1B3-overexpressing cells, the addition of increasing concentrations of T₁AM had only minor effects on uptake in the experimentally accessible concentration ranges (Fig. 2B, Fig. S1†).

OATP1C1 is known to transport T₄ but not T₃,¹⁸ and indeed we also observed increased uptake levels of T₄ in cells transfected with OATP1C1 and CRYM as compared to cells transfected with empty vector and CRYM. Moreover, T₁AM inhibited T₄ uptake in a dose-dependent manner, with an IC₅₀ of 4.8 μ M (Fig. 3).

T₁AM inhibits thyroid hormone uptake by MCT8

We next determined the effect of T₁AM on thyroid hormone uptake by MCT8, the most specific thyroid hormone transporter identified to date.⁷ COS-1 cells were transiently transfected with a construct containing the complete coding region of MCT8 and subsequently incubated with the radiolabeled substrates ¹²⁵I-T₃ and ¹²⁵I-T₄, either alone or in the presence of various concentrations of unlabeled T₁AM. As in the transport studies involving OATPs, cells were cotransfected with CRYM. T₁AM inhibited the uptake of both thyroid hormones but showed an approximately three-fold higher potency for T₄ transport inhibition, with an IC₅₀ of 30.8 μ M for T₄ as compared to an IC₅₀ of 94.9 μ M for T₃ uptake (Fig. 4).

Inhibition of thyroid hormone uptake by iodothyronines or thyronamines: comparison of MCT8 and MCT10

To examine the effect of all of the different iodothyronines and thyronamines on T_3 uptake, COS-1 cells were transiently transfected with either MCT8 or MCT10 cDNA. Cells were incubated for 5 min with 1 nM ^{125}I - T_3 with or without 10 μM of each iodothyronine or thyronamine. Inhibition by iodothyronines is shown in Fig. 5A (MCT8) and 5B (MCT10), and inhibition by thyronamines is shown in Fig. 5C (MCT8) and 5D (MCT10). Comparison of Fig. 5A versus 5B shows that in general the uptake of T_3 by MCT10 is inhibited by iodothyronines to a larger degree than uptake by MCT8. T_3 uptake by MCT10 is inhibited most strongly by 3,5- T_2 and only slightly by 3',5'- T_2 and T_0 . On the other hand, T_3 uptake by MCT8 is inhibited most strongly by T_4 , followed by rT_3 , T_3 and 3',5'- T_2 . Some iodothyronines do not inhibit T_3 uptake by MCT8 at all. On the other hand, Fig. 5C and D show that T_3 uptake by MCT10 is not inhibited by any of the thyronamines, whereas uptake by MCT8 is inhibited most strongly by 3,3'- $T_2\text{AM}$ and to a lesser extent by the other thyronamines.

$T_1\text{AM}$ is not a substrate of the thyroid hormone transporters OATP1A2, OATP1B3, OATP1C1, and MCT8

To verify that $T_1\text{AM}$ is not a substrate of the thyroid hormone transporters tested, we incubated COS-1 cells transiently transfected with each thyroid hormone transporter or empty vector and CRYM with ^{125}I - T_3 , ^{125}I - T_4 , and ^{125}I - $T_1\text{AM}$. As previously observed, T_3 uptake is not significantly enhanced in OATP1B3-expressing cells, and as has been previously reported,¹⁸ T_3 is not transported by OATP1C1 (Fig. 6A). On the other hand, T_4 uptake levels are increased in cells overexpressing OATP1A2, OATP1B3, OATP1C1, and MCT8 (Fig. 6B). However, $T_1\text{AM}$ uptake levels in cells transfected with the thyroid hormone transporters are not enhanced over the basal uptake levels seen in cells transfected with empty vector (Fig. 6C), revealing that $T_1\text{AM}$ is not a substrate of these transporters. Also, radioactive 3,3'- $T_2\text{AM}$ is not a substrate for the thyroid hormone transporters MCT8 or MCT10 (data not shown).

Discussion

$T_1\text{AM}$ is a recently discovered endogenous derivative of thyroid hormone with dramatic pharmacological actions when administered *in vivo*, including hypothermia, bradycardia, hyperglycemia, and general behavioral inactivity, that are in general opposite those effects associated with excess levels of thyroid hormone. Little is known about the mechanism of action of $T_1\text{AM}$. As a trace amine, it is a potent agonist of TAAR1.¹³ $T_1\text{AM}$ has also been found to inhibit the dopamine and norepinephrine plasma membrane transporters, as well as the vesicular monoamine transporter VMAT2.¹⁶ Specific cellular uptake of $T_1\text{AM}$ has also been identified in various cell lines, although the particular plasma membrane transporters involved remain unknown.¹⁹

In the current study, we describe an additional action of $T_1\text{AM}$ in the differential inhibition of thyroid hormone transport by several thyroid hormone transporters. A broad range of transporter types mediate intracellular entry of thyroid hormones, including classic multispecific organic anion/cation transporters such as several OATP family members and monocarboxylate transporters such as MCT8 and MCT10.^{5,6} The transport of thyroid hormones into their target tissues by saturable mechanisms is critical for proper physiological control of both their action and metabolism. Thus, the potential modulation of thyroid hormone transport and subsequent delivery to intracellular targets by $T_1\text{AM}$ provides important additional insight into the role of this relatively new class of signaling molecules.

Of the wide variety of known thyroid hormone transporters, we chose to focus on three members of the OATP superfamily as well as the more specific thyroid hormone transporters MCT8 and MCT10. Of the eleven different OATPs, OATP1A2, 1B1 and 1B3 are believed to be involved in overall body detoxification, and OATP1C1 is a high-affinity T₄ transporter and appears to be important for thyroid hormone metabolism.⁵ While OATP1A2 has been shown to transport a broad range of organic anionic, neutral, and cationic compounds,²⁰ it also transports T₃ and T₄. Because of its expression in the endothelial cells of the blood-brain barrier, and in the kidney and liver, OATP1A2 is thought to be involved in the delivery of thyroid hormones to the kidney and across the blood-brain barrier, as well as function in the removal of thyroid hormone from peripheral tissues for elimination by the liver.⁵ OATP1B3 also has wide substrate specificity aside from thyroid hormones, and is expressed specifically in the liver. On the other hand, OATP1C1 is perhaps the most interesting OATP because it is the most thyroid hormone -specific transporter of the OATP superfamily and is also the thyroid hormone transporter with the highest affinity towards T₄.² OATP1C1 is expressed only in brain and testis, and because of its preferential localization in brain capillaries may be important for T₄ transport across the blood-brain barrier. We also chose to study the effect of T₁AM on MCT8 transport function, as this transporter shows the highest specificity towards thyroid hormones and has also been linked to human disease, underscoring a particular importance of MCT8 for proper thyroid hormone physiology. With its high expression in many tissues, including liver, kidney, heart, brain, placenta, lung, and skeletal muscle,⁶ MCT8 is likely to be important for thyroid hormone delivery to target tissues throughout the body and particularly crucial to thyroid hormone transport into neurons, since mutations in the transporter result in severe neurological deficits.⁸ We also tested the thyroid hormone transporter MCT10, another member of the MCT family with the highest homology with MCT8. MCT10 also shows a wide tissue distribution with particularly high expression in intestine, kidney, liver and placenta,¹¹ but does not appear to be sensitive to transport inhibition by T₁AM or any of the other thyronamines, a notable distinction from MCT8.

The inhibition profile of T₁AM towards thyroid hormone uptake by the transporters studied is interesting in its selectivity for certain transporters and differing potencies for T₃ and T₄ (Table 1). Although T₁AM has no effect on transport by the liver-specific OATP1B3 and MCT10, T₁AM inhibits thyroid hormone uptake by the other three transporters to varying degrees. Interestingly, OATP1A2, OATP1C1, and MCT8 are all expressed in the brain, and at least two of these transporters are believed to play an essential role in thyroid hormone delivery to the brain. Taken together with the previous finding of T₁AM as a potential neuromodulator in its inhibition of monoamine transport,¹⁶ the possible function of T₁AM in regulation of thyroid hormone transport in the brain underscores an intriguing role of this thyroid hormone metabolite in development and proper functioning of the central nervous system. Although the micromolar potencies of T₁AM inhibition of the thyroid hormone transporters are higher than their endogenous circulating levels, active trafficking and subsequent metabolism of thyroid hormone in brain synaptosomes could conceivably result in local concentrations of T₁AM in certain regions of the brain that may far exceed circulating levels.^{21–23} In addition, with the exception of OATP1C1, the reported K_m values for thyroid hormone transport by the various thyroid hormone transporters are also in the micromolar range, far above their endogenous circulating levels, but have nevertheless been shown to be essential for thyroid hormone delivery into target organs and tissues.^{5,6}

In conclusion, we have demonstrated that T₁AM is an inhibitor of thyroid hormone uptake by several of the identified thyroid hormone transporters. Although T₁AM has profound effects when administered in vivo, much remains to be discovered regarding the mechanisms by which it exerts these effects. A role in regulation of thyroid hormone delivery to target sites presents a novel mechanism of action for this endogenous thyroid

hormone metabolite. Further studies of T1AM and its effect on thyroid hormone transport and action would be critical to our understanding of not only T1AM action but also its potential implications for thyroid hormone regulation and possible involvement in certain endocrine disorders.

Materials and methods

Constructs

The plasmids OATP1A2_pSG5,24 OATP1B3_pSG5,24 OATP1C1_pcDNA3.1,18 MCT8_pcDNA3,25 MCT10_pcDNA3.1,12 and CRYM_pSG5,12 were described previously and used for transient transfection of COS-1 cells for thyroid hormone transport studies.

Cell transfection and uptake studies (protocol UCSF and ErasmusMC, respectively) COS-1 cells were maintained in DMEM of high glucose or DMEM/F12, supplemented with 10% FBS, 100 U ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin at 37 °C with 5% CO₂ and 95% humidity. The day before transfection, cells were seeded into 24-well or 6-well tissue culture plates in complete media lacking antibiotics. Cells were transfected with either 200 ng of transporter construct or empty vector and 200 ng of CRYM (24-well plates, protocol UCSF) or only 300 ng of transporter construct or empty vector (6-well plates, protocol ErasmusMC), using FuGENE6 Transfection Reagent (Roche) according to manufacturer's protocols. 24 h after transfection, cells in 24-well plates were washed and preincubated with prewarmed HBSS for 15 min at 37 °C. Cells in 6-well plates were only washed once with incubation medium (Dulbecco's PBS supplemented with 0.1% D-glucose and 0.1% BSA) at room temperature. For inhibition studies in 24-well plates, all experiments were performed in cells transfected with either transporter construct or empty vector and CRYM, as described above; uptake was initiated by the addition of a tracer amount of 125I-T3 or 125I-T4 (Perkin Elmer), with or without various concentrations (100 µM–10 nM) of unlabeled T1AM diluted in HBSS. In 6-well plates, the comparative thyronine/thyronamine inhibition studies were started by adding 1.5 ml of incubation medium with 1 nM (2 × 10⁵ cpm) 125I-T3, with or without 10 µM of unlabeled iodothyronines or iodothyronamines.

For uptake studies, tracer amounts diluted in HBSS of 125I-T3, 125I-T4 (Perkin Elmer), or 125I-T1AM, synthesized as described previously,²⁶ were each added alone to the cells. Uptake in 24-well plates was terminated after 60 min at 37 °C, the cells were washed twice with cold HBSS and solubilized in 1% SDS, and the accumulated radioactivity was determined by scintillation counting. Uptake in 6-well plates was terminated after 5 min at 37 °C, the cells were washed once with incubation medium at room temperature, lysed with 0.1 M NaOH, and the accumulated radioactivity was counted.

Statistical analyses

Statistical analyses were performed with the GraphPad Prism version 4.00 software, with values expressed as means ± SD. For all thyroid hormone transport inhibition curves, data was fitted according to a sigmoidal dose response equation, $y = \text{bottom} + (\text{top} - \text{bottom}) / (1 + 10^{[\text{caret (insertion mark)}](\log \text{IC}_{50} - x)})$, where x is the logarithm of the concentration of T1AM and y is the amount of thyroid hormone transported. Total uptake of thyroid hormone is expressed as “relative uptake,” which is determined by normalizing the 125I-T3 or 125I-T4 uptake values to the maximum uptake signal (DPM radioactivity counts) obtained for each experiment.

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Fig. 1.

Thyroid hormone transport by OATP1A2. (A, B) T_3 uptake by COS-1 cells transfected with OATP1A2 (solid circles) or empty vector (empty circles) and CRYM, an intracellular thyroid hormone-binding protein. Cells were incubated for 60 min at 37 °C with ^{125}I -labeled T_3 in the presence of various concentrations of T_1AM . Total uptake (A) and net uptake levels corrected for background uptake observed in cells transfected with empty vector (B) are shown. Relative uptake is determined by normalizing the ^{125}I - T_3 uptake values to the maximum uptake signal (DPM radioactivity counts) obtained with ^{125}I - T_3 incubation in the presence of the lowest concentration of T_1AM . (C, D) T_4 uptake by COS-1 cells transfected with OATP1A2 (solid circles) or empty vector (empty circles) and CRYM, an intracellular thyroid hormone-binding protein. Cells were incubated for 60 min at 37 °C with ^{125}I -labeled T_4 in the presence of various concentrations of T_1AM . Total uptake (C) and net uptake levels corrected for background uptake observed in cells transfected with empty vector (D) are shown. Relative uptake is determined by normalizing the ^{125}I - T_4 uptake values to the maximum uptake signal (DPM radioactivity counts) obtained with ^{125}I - T_4 incubation in the presence of the lowest concentration of T_1AM .

Fig. 2.

Thyroid hormone transport by OATP1B3. Total uptake levels of T_3 (A) and T_4 (B) by COS-1 cells transfected with OATP1B3 (solid circles) or empty vector (empty circles) and CRYM, an intracellular thyroid hormone -binding protein. Cells were incubated for 60 min at 37 °C with ^{125}I -labeled T_3 or T_4 in the presence of various concentrations of T_1AM .

Fig. 3.

Thyroid hormone transport by OATP1C1. T_4 uptake by COS-1 cells transfected with OATP1C1 (solid circles) or empty vector (empty circles) and CRYM, an intracellular thyroid hormone-binding protein. Cells were incubated for 60 min at 37 °C with ^{125}I -labeled T_4 in the presence of various concentrations of T_1AM . Total uptake (A) and net uptake levels corrected for background uptake observed in cells transfected with empty vector (B) are shown.

Fig. 4.

Thyroid hormone transport by MCT8. (A, B) T₃ uptake by COS-1 cells transfected with MCT8 (solid circles) or empty vector (empty circles) and CRYM, an intracellular thyroid hormone -binding protein. Cells were incubated for 60 min at 37 °C with ¹²⁵I-labeled T₃ in the presence of various concentrations of T₁AM. Total uptake (A) and net uptake levels corrected for background uptake observed in cells transfected with empty vector (B) are shown. (C, D) T₄ uptake by COS-1 cells transfected with MCT8 (solid circles) or empty vector (empty circles) and CRYM, an intracellular thyroid hormone -binding protein. Cells were incubated for 60 min at 37 °C with ¹²⁵I-labeled T₄ in the presence of various concentrations of T₁AM. Total uptake (C) and net uptake levels corrected for background uptake observed in cells transfected with empty vector (D) are shown.

Fig. 5. Inhibition of T₃ uptake by 10 μ M iodothyronines (A, B) or thyronamines (C, D): comparison between MCT8 (A, C) and MCT10 (B, D) in COS-1 cells transfected with transporter construct or empty vector. Cells were incubated for 5 min at 37 °C with ¹²⁵I-labeled T₃ in the presence of the different inhibitors. T₃ uptake without the addition of inhibitor is expressed as 100% control (ctrl). Ti = iodothyronine, TiAM = thyronamine.

Fig. 6. Uptake of thyroid hormones and T₁AM by thyroid hormone transporters. Transport of T₃ (A), T₄ (B) and T₁AM(C) in COS-1 cells transfected with either OATP1A2, OATP1B3, OATP1C1, MCT8, or empty vector and CRYM, an intracellular thyroid hormone -binding protein. Cells were incubated for 60 min at 37 °C with the corresponding ¹²⁵I-labeled substrate. Uptake levels are expressed relative to uptake observed in empty vector-transfected cells.

Table 1Summary of T₁AM inhibition profile

Transporter	IC ₅₀ (T ₃)	IC ₅₀ (T ₄)
OATP1A2	0.27 μ M	2.1 μ M
OATP1B3	No inhibition	No inhibition
OATP1C1	N/A	4.8 μ M
MCT8	95 μ M	31 μ M