Chapter 3B. CELLULAR UPTAKE OF THYROID HORMONES

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ABSTRACT

The biological activity of thyroid hormone (TH) is regulated at the target tissue level by two important processes, *i.e.* deiodination and plasma membrane transport. The first process involves the expression of the deiodinase D2, which converts the prohormone T4 to bioactive T3, and/or of the deiodinase D3 which converts both T4 and T3 to inactive metabolites. Intracellular metabolism and action of TH in target cells as well as transcellular TH transport across, for instance, the blood-brain barrier (BBB) and the intestinal wall depends on the expression of transporters facilitating uptake and/or efflux of iodothyronines.

Recently, several important TH transporters have been identified, including monocarboxylate transporter 8 (MCT8), MCT10 and organic anion transporting polypeptide 1C1 (OATP1C1). The physiological relevance of MCT8 has been demonstrated in studies of male patients with the Allan-Herndon-Dudley syndrome, characterized by severe psychomotor retardation and abnormal TH levels caused by hemizygous mutations of the X-linked *MCT8* gene. In human brain, MCT8 appears to be important in particular for T4 and T3 transport across the BBB and for T3 uptake in neurons, whereas OATP1C1 is predominantly involved in T4 uptake in astrocytes to allow its conversion to T3 by D2 also expressed in these cells. MCT10 also transports aromatic amino acids and its physiological role in tissue TH transport remains to be established. For complete coverage of this and related areas of Endocrinology, visit our free web-book, www.thyroidmanager.org .

INTRODUCTION

It was thought for a long time that thyroid hormone (TH) crosses the plasma membrane of tissue cells by simple diffusion since iodothyronines are lipophilic compounds which would easily pass the lipid bilayer of the plasma membrane. However, it has become increasingly clear that diffusion plays a minor role, if any, in TH transport across the plasma membrane. Rather, TH is transported into cells by specific carrier-mediated mechanisms. In the second half of the 20th century, many studies have been published about the biochemical characterization of TH transport mechanisms in a variety of cell types. In general, these studies have indicated that cellular TH transport is a saturable process which in liver cells may be Na^+ dependent and in other cell types may be inhibited by aromatic and/or aliphatic amino acids. Early studies of cellular TH transport have been extensively reviewed in 2001 (1) and only some of them will be mentioned here.

About 80% of circulating T3 is produced outside the thyroid gland by peripheral conversion of T4, and only 20% is directly secreted by the thyroid gland (2). T3 is considered to be the major bioactive TH, whereas T4 is mainly a prohormone that becomes activated upon its conversion to T3 (2). Most TH actions are initiated by binding of T3 to its nuclear receptors in target cells (3,4). Therefore, the biological activity of TH is determined largely by the intracellular T3 concentration, which depends on a) the circulating concentration of T3 and its precursor T4; b) the activities of deiodinases that catalyze the production (D1, D2) or degradation (D1,D3) of T3; and c) the activities of transporters which mediate the cellular uptake or efflux of T3 and T4 (Fig. 1). It should be noted that TH bioactivity may be regulated in an autocrine fashion as shown in Fig. 1, or in a paracrine fashion, where T3 production and action take place in the same cell or in separate cells, respectively.

Recently, three relatively specific TH transporters have been identified. OATP1C1 is a member of the organic anion transporting polypeptide family, which shows preference for T4 above T3, and is expressed almost exclusively in brain (5). In human brain, OATP1C1 is

importantly expressed in astrocytes, where it facilitates entry of T4 allowing its conversion to T3 also expressed in these cells (6). MCT8 and MCT10 are members of the monocarboxylate transporter family. While MCT10 is also known to transport aromatic amino acids, no other substrates have been identified for MCT8 than iodothyronines and iodotyrosines (7-9). MCT8 and MCT10 are expressed in various tissues (10). In human brain, MCT8 is importantly expressed in endothelial cells of the blood-brain barrier (BBB) as well as in neurons (6). Mutations in the MCT8 transporter have been identified in male patients with the Allan-Herndon-Dudley syndrome, characterized by severe X-linked psychomotor retardation and elevated serum T3 levels (11-14).

THYROID HORMONE TRANSPORTERS

Organic Anion Transporters

Organic anion transporting polypeptides (OATPs) represent a large family of homologous proteins, many of which have been shown to transport different iodothyronines and their sulfate conjugates (Table 1) (15-17). The genes coding for these transporters are now referred as the SLCO family. The OATPs accept a wide range of substrates, not only anionic but also neutral and sometimes even cationic compounds. Some members are expressed in a single tissue, whereas others have a wider tissue distribution. The SLCO1A2, 1B1, 1B3 and 1C1 genes are clustered together with a related pseudogene on human chromosome 12p12 (17). The encoded OATPs have all been shown to transport iodothyronines (18). Of these, OATP1B1 and 1B3 are expressed specifically in the liver, OATP1C1 is expressed only in brain and testis, and OATP1A2 is expressed in brain, liver and kidney. In terms of TH transport, OATP1C1 is the most intriguing as it shows a high specificity and affinity towards T4 and rT3. In mouse brain, Oatp1c1 is localized both in capillary, the choroid plexus, and astrocytes, but in human brain localization of OATP1C1 in endothelial cells is negligible (5,19-22). Therefore, the primary role of OATP1C1 in human brain appears to be the transport of T4 into astrocytes to allow its conversion to T3 by D2 that is also expressed in astrocytes.

It should be realized that the organization of the OATP1 subfamily is very different in humans than in mice and rats (Fig. 2) (23). Although human, mouse and rat OATP1C1 are clearly orthologues, the OATP1A branch has only 1 member in humans (1A2) but 4 members in mice (1A1,4-6) and 5 members in rats (1A1,3-6), whereas the OATP1B branch has 2 members in humans (1B1,3) and one member in rats and mice (1B2). Therefore, mice and rats are not good animal models for TH transport by members of the OATP1A/B subfamily. Considering the different cellular distribution of OATP1C1 in human and mouse brain, the same precaution may also apply to this transporter.

OATPs transport their substrates in a Na-independent manner. The solute carrier family 22 (SLC22) also contains many organic anion transporters (OATs) and organic cation transporters (OCTs) (24), but information about the possible transport of TH by any of these transporters has not been published. We have demonstrated that the Na-taurocholate co-transporting polypeptide (NTCP, SLC10A1) facilitates uptake of the different iodothyronines as well as their sulfates (25,26). The SLC10 family contains 7 members of which NTCP is expressed exclusively in liver (27-29). SLC10A2 is another bile acid transporter expressed in the intestine and kidney. SLC10A6 transports different organic anions but substrates for the other SLC10 transporters have not yet been identified. NTCP is the only SLC10 family member capable of transporting TH (26).

Interestingly, NTCP has recently been identified as the receptor involved in the infection of liver cells by hepatitis B virus (HBV) and hepatitis D virus (HDV) (30). This NTCP-mediated internalization of HBV and HBD is specifically inhibited by the novel drug Myrcludex B, a synthetic peptide derived from the HBV/HBD surface protein preS1 (30). Myrcludex B also inhibits bile acid transport by NTCP (31,32) and it would be interesting to know if it affects TH transport into the liver. Clinical trials are now conducted with this drug in hepatitis B and D patients (33).

Amino Acid Transporters

Iodothyronines are a particular class of amino acids built from two tyrosine residues. Therefore, it is no surprise that amino acid transporters, in particular the L and T-type amino acid transporters, are involved in TH uptake into several tissues (34-38). L-type transporters mediate uptake of large neutral, branched-chain and aromatic amino acids, whereas T-type transporters are specific for the aromatic amino acids Phe, Tyr and Trp.

Four L-type transporters (LAT1-4) have been identified, two of which (LAT1,2) belong to the heterodimeric amino acid transporter family. These transporters consist of a heavy chain and a light chain, linked through a disulfide bond (39). There are 2 possible heavy chains, SLC3A1 (rBAT) and SLC3A2 (4F2hc or CD98), and in humans there are 13 possible light chains belonging to the SLC7 gene family. The 4F2 or CD98 cell surface antigen is expressed in many tissues, especially on activated lymphocytes and tumor cells. 4F2hc is a glycosylated protein with a single transmembrane domain, whereas the light chains are not glycosylated and have 12 transmembrane domains (40). LAT1 and LAT2 consist of the SLC3A2 heavy chain in combination with the SLC7A5 and SLC7A8 light chain, respectively. They are obligate exchangers, implying that the cellular uptake of extracellular substrates is tightly coupled to the efflux of intracellular substrates.

Significant Na-independent transport of iodothyronines has been observed in *Xenopus* oocytes expressing heterodimeric transporters consisting of human SLC3A2 and either human SLC7A5 (LAT1) or mouse SLC7A8 (LAT2) (Table 1) (41). The rate of iodothyronine uptake by the 4F2hc/LAT1 transporter decreased in the order 3,3'-T2 > T3 ~ rT3 > T4. Apparent K_m values were found to be in the micromolar range, being lowest for T3 (1.5 μM) (41).

Ritchie *et al.* have reported on the stimulation of T3 transport in oocytes injected with mRNA for 4F2hc and for the IU12 *Xenopus* LAT1 homolog (42). They have also shown that overexpression of the heterodimeric L type transporter in cells results in increased intracellular T3 availability and, thus, augmented T3 action (43). Furthermore, they demonstrated T3 uptake via the 4F2hc/LAT1 transporter into human BeWo placental choriocarcinoma cells, suggesting that this transporter plays a role in the trans-placental transfer of maternal TH to the fetus (44). Indeed both LAT1 and LAT2 have been localized in the human placenta, in particular in cytotrophoblasts (45-48).

TH transport by LAT1 and LAT2 has recently been characterized in more detail, and a structural model of LAT2 has been obtained (49-51). LAT1 facilitates cellular uptake of all iodothyronines tested with a clear preference for 3,3'-T2 and rT3. LAT2 also shows significant cellular uptake of T3 and, in particular, 3,3'-T2. Both LAT1 and LAT2 also markedly facilitate cellular entry of 3-iodotyrosine (MIT) (51).

In addition to LAT1 and LAT2, two other L-type amino acid transporters have recently been identified in the SLC43 family. LAT3 (SLC43A1) and LAT4 (SLC43A2) are monomeric proteins containing 12 trans-membrane domains, which apparently do not require ancillary proteins for proper expression in the plasma membrane (52). Both LAT3 and LAT4 especially facilitate the cellular efflux of their substrates, including 3,3'-T2 and MIT (51). The function of the third member of this small family (SLC43A3) is unknown.

A T-type amino acid transporter (TAT1) has been cloned from rats and humans, showing transport of Phe, Tyr and Trp (53,54). This protein is a member of the monocarboxylate transporter family, and is also called MCT10 (SLC16A10). The MCT family consists of 14 members, and earned its name because MCT1-4 have been characterized as monocarboxylate transporters (55). Endogenous substrates are being recognized for other MCT family members, such as β-hydroxybutyrate for MCT7 (56), carnitine for MCT9 (57), and carnitine for MCT12 (58) The degree of homology between MCT proteins is especially high for MCT1-4 and for MCT8/10. In contrast to the initial failure to demonstrate TH transport by MCT10, we have demonstrated that both MCT8 and MCT10 are highly effective iodothyronine transporters (Table 1) (7-9).

MCT8 and MCT10

MCT8 and *MCT10* have identical gene structures; both consist of 6 exons and 5 introns, with a particularly long first intron (~100 kb). The *MCT10* gene is located on human

chromosome 16q21-q22 and codes for a protein of 515 amino acids. The *MCT8* gene is located on human chromosome Xq13.2 and has 2 possible translation start sites, coding for proteins of 613 or 539 amino acids (Fig. 2). The significance of the N-terminal extension of long *versus* short human MCT8 (indicated in yellow in Fig. 2) remains to be investigated. A patient has been reported with psychomotor retardation associated with a Met to Leu mutation (M1L) at the upstream translation start site. However this mutation was also identified in a healthy relative (59), suggesting that the long MCT8 protein does not have a crucial physiological function. Moreover, a recent study indicated that if the long MCT8 protein is generated it undergoes effective ubiquitination and proteasomal degradation (60). Most species, including mice and rats, only express the short MCT8 protein as they lack the first translation start site. Functional studies of human MCT8 have been carried out so far by expression of the short protein.

Both MCT8 and MCT10 proteins have 12 putative transmembrane domains, with both N- and C-terminal ends located on the inside of the plasma membrane. The common amino acids in MCT8 and MCT10 are indicated in green in Fig. 2, showing a high degree of homology in particular in the transmembrane domains. Unique to the MCT8 structure is the presence of PEST domains in the N-terminal intracellular part of the protein, rich in Pro (P), Glu (E), Ser (S) and Thr (T) residues. The function of these domains is unknown. MCT8 is expressed in many tissues, including human liver, kidney, heart, brain, placenta, adrenal gland, skeletal muscle, and thyroid. MCT10 also shows a wide tissue distribution, with particularly high expression in human skeletal muscle, intestine, kidney and pancreas (10).

After the cloning of *MCT8* in 1994 (61), no reports on the biological function or the transported substrates have been published until Friesema *et al.* identified rat MCT8 as a specific TH transporter (8). Expression of rat Mct8 in *Xenopus* oocytes induced a ~10-fold increase in iodothyronine uptake, much greater than that induced by any other transporter, including rat NTCP, rat OATP1A1 and human LAT1 (8). Although rat MCT8 does not discriminate between T4, T3, rT3 and 3,3'-T2, it does not transport iodothyronine sulfates, the amino acids Phe, Tyr, Trp and Leu, or the monocarboxylates lactate and pyruvate. Apparent Km values amount to 2-5 μM for the different iodothyronines in the absence of protein in the medium. T4 and T3 transport are largely Na^+ independent (8).

Subsequent studies in mammalian cells transfected with human MCT8 or MCT10 cDNA have demonstrated that both transporters effectively facilitate transmembrane TH transport (7,9). Co-transfection of the high-affinity cytoplasmic TH-binding protein mu-crystallin (CRYM) strongly augments the cellular accumulation of iodothyronines compared with cells transfected with MCT8 or MCT10 alone. These and other findings suggest that MCT8 and MCT10 facilitate both cellular uptake and efflux of T4 and T3. MCT10 appears to transport T3 better than MCT8 whereas the opposite is true for T4. Transfection of MCT8 or MCT10 into cells that express D1, D2 or D3 results in a marked increase in the intracellular metabolism of different iodothyronine substrates (7,9).

CLINICAL RELEVANCE OF MCT8

Worldwide, over 100 families have been reported where males are affected by severe psychomotor retardation associated with a particular combination of abnormal serum TH levels. A large family with this X-linked mental retardation (XLMR) syndrome was first reported in 1944 by Allan, Herndon and Dudley (62,63). Since then, this disorder is usually referred to as the Allan-Herndon-Dudley syndrome (AHDS). Only 60 years later it was realized that patients with AHDS also have abnormal TH levels (11,12,64).

Usually, patients with AHDS are born at term following an uncomplicated pregnancy with a normal birthweight, body length and head circumference. During the first 6 months a general hypotonia is noticed. During development the truncal hypotonia remains, whereas the distal hypotonia progresses into dystonia and spasticity. The truncal hypotonia results in poor head control. Growth is relatively normal, but final body length is reduced and body weight is usually extremely low with obvious signs of muscle wasting. There is also progressive microcephaly. In the first 2 years of life, brain MRI shows clearly delayed myelination. Although myelination improves in subsequent years, it never really normalizes.

This is supported by a recent study of post-mortem brains from a fetal and a 11-year old AHDS patient (65). Based on observations of delayed myelination, AHDS has also been referred to as a Pelizaeus-Merzbacher-like disorder (PMLD) (66).

Although in some families the clinical phenotype is somewhat milder, AHDS patients are usually incapable of sitting, standing or walking independently, and do not develop any speech. They are severly mentally retarded with IQ values <40. Feeding is a problem in AHDS patients as they have difficulties swallowing; aspiration is a frequent cause of pneumonia. For a detailed description of the clinical features of patients with AHDS, the reader is referred to recent literature (66-69).

AHDS patients have a characteristic combination of abnormal serum TH levels (68). Both T4 and FT4 levels are low-normal to clearly reduced, whereas serum T3 and FT3 are markedly elevated. Serum rT3 is always low. Consequently, the serum T3/T4 and T3/rT3 ratios are strongly elevated. Serum TSH is usually within the normal range, but the mean serum TSH level in AHDS patients is about twice that in healthy controls. Serum SHBG levels are markedly elevated, and several studies have reported on elevated serum lactate levels in young patients (70,71).

In 2004, it was demonstrated by the group of Refetoff and by our group that AHDS represents a TH resistance syndrome caused by a defect in TH uptake in target cells due to mutations in *MCT8*. Since then, *MCT8* mutations have been identified in over 100 families with AHDS. These mutations include 1) deletions affecting one or more exons, 2) frame-shift mutations resulting in scrambled and often truncated proteins, 3) splice site mutations, 4) nonsense mutations resulting in truncated proteins; 5) deletions or insertions of one or more codons and, thus, amino acids, 6) missense mutations asociated with single amino acid substitutions. A list of missense mutations is provided in Table 2.

The larger deletions, frame shift mutations and nonsense mutations are obviously deleterious for MCT8 function. The functional consequences of single amino acid substitutions, deletions or insertions have been investigated in cells transfected with wild-type or mutated MCT8. Most mutations were found to result in an amost complete loss of TH transport by MCT8. However, the extent to which these mutations affect MCT8 function depends on the type of cell used for transfection for reasons which need to be fully explored (72-75). Studies of the localization of wild-type and mutant MCT8 protein have indicated two distinct pathogenic mechanisms, in that certain mutations interfere with the trafficking of the transporter to the plasma membrane, while other mutations allow proper routing of MCT8 but interfere with the substrate translocation process (73,76,77). The functional consequences of MCT8 mutations have also been demonstrated using fibroblasts cultured from skin biopsies, showing that T4 and T3 uptake by cells from AHDS patients is markedly reduced compared with cells cultured from healthy controls (75,78-82).

ANIMAL STUDIES

Studies in humans and animals have indicated that MCT8 is expressed in a variety of tissues, including brain. The distribution of MCT8 expression in mouse brain has been studied in detail by Heuer *et al* (83). These studies have demonstrated that MCT8 is predominantly expressed in neurons in different brain areas, including hippocampus, cerebral cortex, striatum, hypothalamus and cerebellum. In addition, MCT8 is importantly expressed in capillary endothelial cells, the choroid plexus, and tanycytes which line the third ventricle (83,84). MCT8 expression in neurons coincides with expression of D3. D2 is largely expressed in adjacent astrocytes. In mouse brain, the T4 transporter OATP1C1 is expressed in capillaries, in the choroid plexus and in astrocytes (83-86). However, in primate brain localization of OATP1C1 in capillaries appears to be negligible (22,87).

MCT8 (KO) mice have been studied in the laboratories of Heuer (88-90), Refetoff (91-94), Bernal (95-97) and Schweizer (98-100). In contrast to the severe neurological phenotype in male patients with MCT8 mutations, neither hemizygous MCT8 KO male mice nor homozygous MCT8 KO female mice show an obvious phenotype. However, they show the same abnormal serum thyroid parameters as patients with MCT8 mutations, *i.e.* a large decrease in T4, a large increase in serum T3, and slightly elevated TSH levels. In addition,

MCT8 KO mice show the following features: 1) normal brain T4 uptake but impaired brain T3 uptake, 2) decreased brain T4 and T3 contents, 3) increased D2 and decreased D3 activities in brain, 4) normal liver T4 and T3 uptake, 5) increased kidney T4 and T3 uptake, 6) increased kidney T4 and T3 contents, and 7) increased D1 activity in both liver and kidney (90).

The paradoxical increase in renal T4 and T3 uptake in MCT8 KO mice is unexplained, but the increase in renal T4 content in combination with the increased D1 expression may account for an enhanced renal T4 to T3 conversion, and thus contribute to the decrease in serum T4 and increase in serum T3 (90). In addition, there is evidence suggesting that thyroidal hormone secretion is affected by MCT8 inactivation, perhaps leading to preferential T3 secretion (89,92). Since MCT8 is expressed in the hypothalamus, inactivation of MCT8 is associated with an impaired feedback of TH at the hypothalamic level, contributing to the slightly increased serum TSH level (88,101).

In addition to MCT8 KO mice, a number of other interesting mouse models have been generated which in addition to MCT8 are also deficient in other TH-related genes. Liao *et al* (102) have studied the effects of the deletion of D1 and/or D2 on serum and brain TH levels in MCT8 KO and WT mice. The results indicate that D1 plays an important role in the altered TH homeostasis in MCT8 KO mice, probably involving and increased T4 to T3 conversion in the thyroid and the kidneys (89,90,92,103). In a recent study, the effect of MCT8 deletion was investigated in D3 deficient mice (104). Inactivation of D3 in mice is associated with marked morbidity and mortality, which is largely prevented by deletion of MCT8. The mechanism by which MCT8 deletion improves the phenotype of D3 deficient mice is unknown.

Of special interest are studies using mice which in addition to MCT8 are also deficient in other TH transporters, such as MCT8/MCT10 (105) and MCT8/LAT2 (106) double knockout (DKO) mice. The additional deficiency of LAT2 or MCT10 results in interesting changes in TH levels compared with MCT8 only KO mice, although deletion of MCT10 or LAT2 alone have little effect on TH homeostasis (105-107). Perhaps most interesting are the findings obtained in MCT8/OATP1C1 DKO mice (21). In contrast to the only mild reduction in brain T3 levels and the lack of an obvious phenotype in mice deficient in MCT8 alone or OATP1C1 alone (108), MCT8/OATP1C1 DKO mice show a dramatic decrease in brain T3 content associated with a markedly impaired neurodevelopment (21). These findings suggest overlapping activities of MCT8 and OATP1C1 in TH transport in the brain, as they are both capable of transporting T4 across the BBB. Apparently, development of the human brain is more vulnerable to mutations in MCT8, since OATP1C1 is not significantly expressed in the human BBB and thus cannot compensate for the loss of MCT8.

Figure 3 shows a schematic of the regulation of T3 supply to neuronal target cells, based largely on studies by Heuer *et al* (21) and Bernal *et al* (109). The steps involved in this process include 1) TH transport across the BBB by both OATP1C1 and MCT8 in mice and by MCT8 alone in humans, 2) uptake of T4 in astrocytes by OATP1C1, 3) conversion of T4 to T3 by D2 in astrocytes, 4) release of T3 from the astrocytes by an unidentified transporter, 5) uptake of T3 in neurons by MCT8. These neurons may also express D3 for termination of T3 activity. MCT8 may also be involved in T3 uptake by oligodendrocytes, but this remains to be established. This schema is an oversimplification as, for instance, it ignores the importance of TH transport across the blood-CSF barrier by MCT8 and OATP1C1.

PATHOGENESIS OF AHDS BY MCT8 MUTATIONS

TH plays an essential role in brain development. This requires optimal spatio-temporal regulation of T3 supply to brain target cells, in particular neurons. MCT8 is supposed to be crucial for T4 and T3 transport across the BBB and may also play an important role in T3 uptake by neurons. Inactivation of MCT8 results in an impaired development of the central nervous system and thus in severe psychomotor retardation. It is also possibe that MCT8 is more important for T3 uptake in certain subsets of neurons than for others. This may result in a dysbalance of T3 supply to different neuronal populations and thus in a defect in the coordinated development of neuronal networks in the brain.

In addition to the TH dysregulation in the brain, the effects of MCT8 mutations on the thyroid state of peripheral tissues should also be considered. Usually, the heart appears to function normally in MCT8 patients despite exposure to highly elevated serum T3 levels. This suggest a partially impaired cardiac T3 upake in case of a MCT8 mutation, implying the involvement of additional TH transporters in the heart. MCT8 patients show extensive muscle wasting and increased serum SHBG levels, which likely reflect a hyperthyroid state of the skeletal muscles and liver, respectively (hepatic SHBG production is increased by TH). This would indicate that MCT8 inactivation does not impair muscle and liver T3 uptake, suggesting a more important role of other transporters. Finally, findings in MCT8 KO mice suggest that the kidneys are also in a hyperthyroid state in MCT8 patients, but there is no direct evidence for this assumption.

CONCLUSIONS AND PERSPECTIVES

Much progress has been made in recent years with the identification of TH transporters and their role in the tissue-specific regulation of TH bioactivity in health and disease. However, it is likely that important TH transporters still remain to be discovered. For instance, none of the TH transporters characterized recently at the molecular level have the properties of transporters involved in TH uptake in liver cells, such as nanomolar affinities, ATP and Na^+ dependence, as determined in previous studies (1). Further, the physiological relevance of OATP1C1 and MCT10 need to be demonstrated. Although it has been demonstrated that mutations in MCT8 cause severe psychomotor retardation, the pathogenic mechanism has not been established. For this it is essential to know exactly where MCT8 is expressed in the human brain and other tissues. A beginning has been made with the treatment of AHDS patients with T3 analogues which do not require MCT8 for cellular uptake, such as DITPA (110) and Triac (https://clinicaltrials.gov/ct2/show/NCT02060474), but further work is needed to develop an optimal therapy for these patients.

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Table 1. Characteristics of human thyroid hormone transporters

Gene	Protein	Chr	Tissue distribution	Ref.
SLC10A1	NTCP	14q24.1	Liver	(26)
SLCO1A2	OATP1A2	12p12	Brain, kidney, liver	(18,111,112)
SLCO1B1	OATP1B1	12p12	Liver	(112-114)
SLCO1B3	OATP1B3	12p12	Liver	(18,112)
SLCO1C1	OATP1C1	12p12	Brain, cochlea, testis	(5,115)
SLCO3A1	OATP3A1	15q26	Brain, testis	(116)
SLCO4A1	OATP4A1	20q13.33	Multiple	(111)
SLCO4C1	OATP4C1	5q21.2	Kidney, other	(117)
SLC7A5	LAT1	16q24.3	Multiple, tumors	(41)
SLC7A8	LAT2	14q11.2	Multiple, tumors	(41)
SLC16A2	MCT8	Xq13.2	Brain, liver, kidney, heart, thyroid etc	(8,9)
SLC10A10	MCT10	16q21-q22	Multiple.	(7)

Table 2. Missense MCT8 mutations in AHDS patients

Exon 1	Exon 2	Exon 3	Exon 4	Exon 5	Exon 6
c.563T>A p.I188N (118)	c.661G>A p.G221R (64,66,119)	c.812G>A p.R271H (72,120-124)	c.1298T>A p. L433H (NP)	c.1412T>C p.L471P (11,125,126)	c.1658C>A p.A553D (NP)
c.575A>G p.H192R (66)	c.670G>A p.A224T (120,127)	c.826G>A p.G276R (128)	c.1301T>G p.L434W (64)	c.1475T>C p.L492P (80)	c.1673G>A p.G558D (59)
c.575A>C p.H192P (2,129)	c.671C>T p.A224V (11,72,118,130, 131)	c.844G>T p.G282C (132)	c.1333C>T p.R445C (79,133)	c.1481T>C p.L494P (65)	c.1690G>A p.G564R (75,134)
c.581C>T p.S194F (64,73)	c.671C>A p.A224E (2)	c.869C>T p.S290F (81,135)	c.1333C>A p.R445S (136)	c.1484G>C p.G495A (137)	c.1691G>A p.G564E (NP)
c.587G>A p.G196E (138)	c.703G>A p.V235M (64,73)	c.872T>G p.L291R (139)	c.1358A>T p.D453V (NP)	c.1492G>A p.D498N (140)	c.1703T>C p.L568P (64,73)
		c.911T>C p.L304P (141)		c.1535T>C p.L512P (12)	
		c.962C>T p.P321L (66,142)		c.1610C>T p.P537L (143)	
		c.1061A>G Y354C (144)		c.1621G>T p.G541C (145)	
		c.1163G>A p.R388Q (146)			
		c.1201G>A p.G401R (147)			

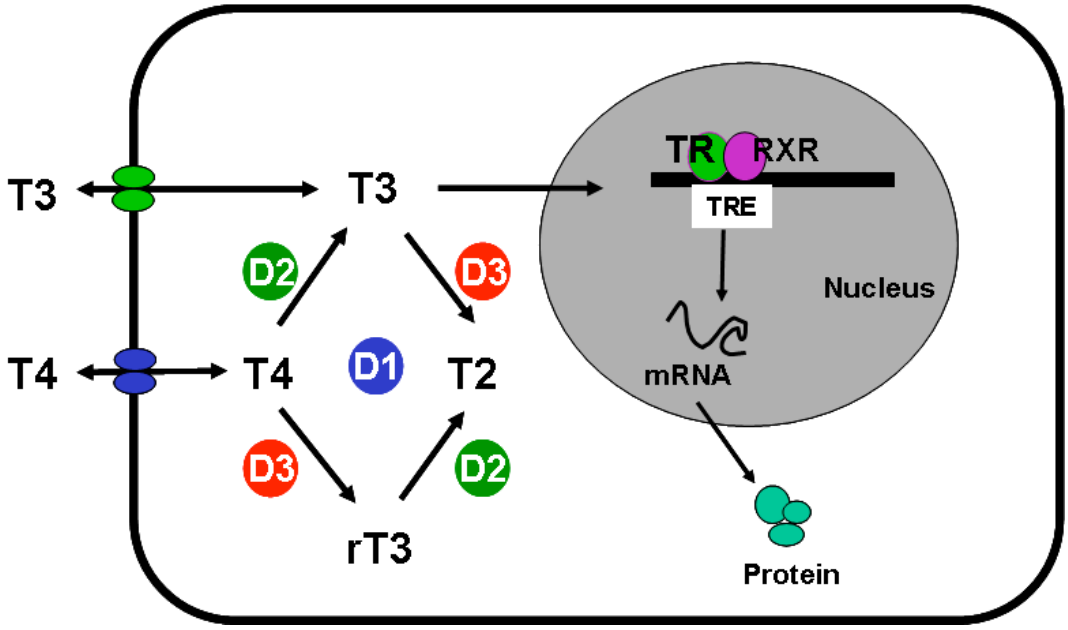

Fig. 1. Thyroid hormone transport, metabolism and action in a target cell.

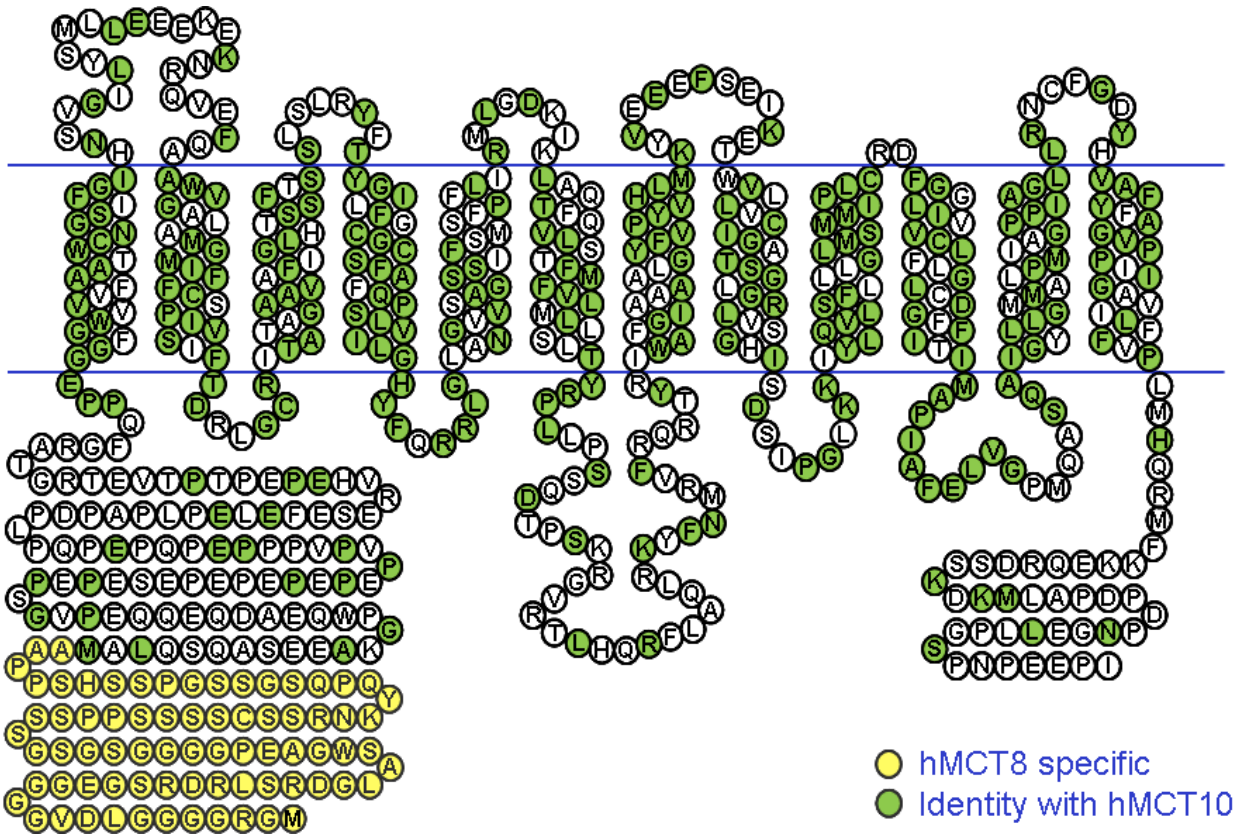

Fig. 2. Structure of the human MCT8 protein with 12 transmembrane domains. The blue lines represent the plasma membrane. The N- and C-terminal domains are located in the cytoplasm. The N-terminal extension of long *vs.* short MCT8 protein generated using the first or the second translation start site is indicated in yellow. The amino acid identity with human MCT10 is indicated in green.

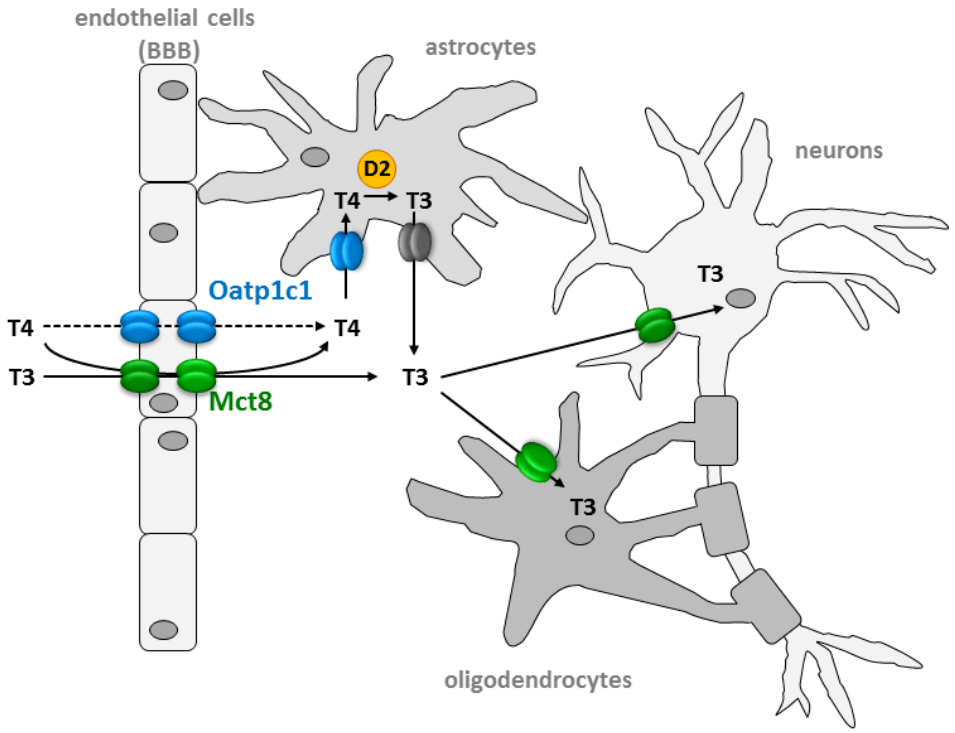

Fig. 3. Schematic of steps involved in the supply of bioactive T3 to target cells in the brain. In contrast to the mouse brain, OATP1C1 does not seem to play an important role in T4 transport across the blood-brain barrier. (*Courtesy of Drs. Steffen Mayerl and Heike Heuer*).