Krassas GE, Rivkees SA, Kiess W (eds): Diseases of the Thyroid in Childhood and Adolescence.
Pediatr Adolesc Med. Basel, Karger, 2007, vol 11, pp 80–103

Thyroid Hormone Transport and Actions

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Thyroid hormones (TH) are essential for normal development, differentiation growth and metabolism of every cell in the body. The pro-hormone thyroxine (T4) is synthesized by the thyroid follicles together with a small amount of the biologically active hormone triiodothyronine (T3), which derives mainly from tissue T4 deiodination. Approximately 0.03% of total T4 and 0.3% of total T3 in serum are circulating in a free or unbound form while the major part of TH is bound to circulating plasma proteins. These plasma proteins are responsible for the maintenance of the large extrathyroidal pool of TH, but their function is otherwise not quite clear, since wide differences in their concentrations do not influence the thyroid functional status of the individual to any large degree [1, 2].

Thyroid Hormone Transport

Transport in the Blood

More than 99% of the circulating thyroid hormone is bound to plasma proteins but can be liberated with great rapidity for entry into cells. The thyroid hormone-binding proteins are comprised of thyroxine-binding globulin (TBG), transthyretin (TTR or thyroxine-binding prealbumin), human serum albumin (HSA) and lipoproteins. Their functions are most probably to ensure a constant supply of TH to the cells and tissues by preventing urinary loss [3], protect the organism against abrupt changes in thyroid hormone production and degradation, protect against iodine deficiency [2] and target the amount of TH delivery by ensuring a site-specific, enzymatic alteration of TBG [4]. TBG has by far the highest affinity for T4, the result of which being that TBG binds 75% of serum T4, whereas TTR binds 20% and HSA 5% [2]. Some of the properties of the binding proteins are displayed in table 1.

Table 1. Some properties and metabolic parameters of the prinicpal thyroid hormone-binding proteins in serum

	TBG	TTR	HSA
Molecular weight, kDa	54*	55	66.5
Structure	monomer	tetramer	monomer
Carbohydrate content, %	20	–	–
Number of binding sites for T4 and T3	1	2	several
Association constant, K_a (M-1)			
For T4	1×10^{10}	2×10^{8}**	1.5×10^{6}**
For T3	1×10^{9}	1×10^{6}	2×10^{5}
Concentration in serum (mean normal, mg/l)	16	250	40,000
Relative distribution of T4 and T3 in serum, %			
T4	75	20	5
T3	75	<5	20
In vivo survival			
Half-life, days	5***	2	15
Degradation rate, mg/day	15	650	17,000

HSA = human serum albumin; TBG = Thyroxine-binding globulin; TTR = transthyretin.
* Apparent molecular weight on acrylamide gel electrophoresis 60 kDa.
** Value given is for the high affinity binding site only.
*** Longer under the influence of estrogen.
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Thyroxine-Binding Globulin

TBG carries the major part of both circulating T4 and T3 (as well as reverse T3), and therefore quantitative or qualitative changes in TBG concentration have a high impact on total serum T4 and T3. The protein is encoded by a single gene on the X-chromosome and is produced and cleared by the liver. It has a single iodothyronine-binding site with a slightly higher affinity for T4 compared to T3 [5]. When it is fully saturated it carries approximately 200 μg T4/l. The TBG concentration in serum is between 11 and 21 mg/l (180–350 nmol/l), present from 12th week of fetal life and 1.5 times higher in newborns and children until 2–3 years of age [6]. Estrogen has a marked effect on TBG by prolonging the biological half-life from the normal 5 days, thus resulting in increased plasma concentrations of TBG and total TH [7] while testosterone has the opposite effect [8]. In children and adolescents this may have an implication in diseases with a severe sex hormone overproduction related to the age, as well as oral contraceptives and pregnancy in adolescent girls.

Inherited TBG excess was first described in 1959 [9], and several familial X-chromosome-linked TBG abnormalities have been described [10, 11]. A rare TBG abnormality is seen in carbohydrate-deficient glycoprotein syndrome, which is associated with severe mental and motor retardation [12]. Acquired TBG abnormalities are mostly resulting in altered synthesis and/or degradation and caused by, e.g., severe terminal illness, hypo- and hyperthyroidism, severe liver disease and a variety of critical non-thyroidal illnesses [2, 13]. The latter may be mediated by interleukin-6 or other cytokines suppressing acute-phase reactants [14].

Transthyretin

TTR, previously called thyroxine-binding prealbumin binds only about 15–20% of the circulating TH and has a lower affinity for the hormones thus dissociating from them more rapidly and thus responsible for much of the immediate delivery of T4 and T3. Transthyretin is the major thyroid hormone-binding protein in cerebrospinal fluid. It is synthesized in the liver and the choroids plexus and secreted into the blood and cerebrospinal fluid, respectively. Only 0.5% of the circulating TTR is occupied by T4 and it has a rapid turnover of 2 days in plasma. Hence, acute reduction of the rate of synthesis results in a rapid decrease of its serum concentration [2]. Acquired abnormalities in TTR include major illness, nephrotic syndrome, liver disease, cystic fibrosis, protein fasting and hyperthyroidism. However, changes in TTR concentrations have little effect on the serum concentrations of TH [15].

Albumin

HSA binds about 5% of the circulating T4 and T3. Its affinity for the hormones is even lower, and since HSA associates with a wide variety of substances, including a number of different hormones and drugs, the association between TH and HSA can hardly be regarded specific. Even marked fluctuations in serum HSA concentrations have no effect on TH levels [16].

Lipoproteins

Lipoproteins transport a minor fraction of circulating T4 and to some extent T3 [17]. The binding site for TH on apolipoprotein A1 is distinct from that which binds to cellular protein receptors.

Consequences of Abnormal Binding Protein Concentrations

Abnormalities of the TH-binding proteins do not cause alterations in the metabolic state of the individual nor do they result in thyroid disease. Thus, abnormal concentrations of these binding proteins, due to changed synthesis, degradation or stability, result in maintaining normal free TH concentrations.

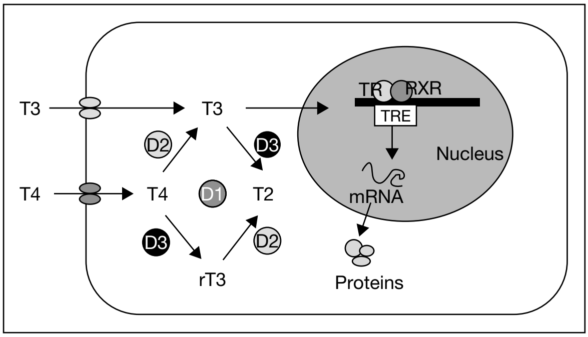

Fig. 1. Thyroid hormone transport and metabolism in a 3,3′,5-triiodothyronine (T3) target cell. Reproduced with kind permission from Jansen et al. [21].

However, they do give rise to misinterpretation of most of the measurements of serum levels of TH by available techniques. Depending on the severity of the abnormality only total TH concentrations are affected, but also the measured free TH levels by automated currently used methods give rise to incorrect results [18]. In such cases, it may be necessary to provide a free TH estimate by quantifying total hormone concentration with a subsequent estimate of the available binding places by use of a TH uptake test or direct measurement of TBG [2]. Even better is measurement of free TH concentrations by equilibrium dialysis or ultrafiltration, but not many laboratories in the world perform these measurements anymore.

Transport Across the Cell Membrane

The deiodinases involved in T4 to T3 conversion and T4 and T3 degradation as well as the T3 receptors are located intracellularly. Therefore, both action and metabolism of thyroid hormones are intracellular events requiring transport of iodothyronines across the cell membrane. For a long time it was believed that TH diffused passively over the cell membrane, but recent years of research has made it increasingly clear that cellular transmembrane transport of TH is mediated by transporters, that these transporters determine the availability of iodothyronines to the intracellular sites for metabolism and action [19], and that the TH transport is energy dependent [20] (fig. 1). Recently, specific transporters (organic anion transporters and amino acid transporters) known to facilitate cellular thyroid hormone uptake have been identified [20–22]. Hennemann and Visser [22] have defined requirements for (patho)physiological significance of thyroid hormone plasma membrane transport in the terms that it should be specific, without significant diffusion, plasma membrane transport subject to regulation, transport rate limiting on subsequent metabolism, and changes in transport should be appropriate from the (patho)physiological point of view.

Organic Anion Transporters

These mediate uptake of iodothyronines and their sulphonated derivatives and they are members of the Na^+/taurocholate cotransporting polypeptide (NTCP) and the Na^+-independent organic anion transporting polypeptide (OATP) families [23, 24]. NTCP is only expressed on hepatocytes and is the major transporter of conjugated bile acids in the liver. The OATPs are a large family responsible for transmembrane transport of a number of compounds including TH. The most interesting OATP superfamily members in terms of TH transport are OATP1C1 and OATP14. The former has been demonstrated to be widely expressed both in human brain and the Leydig cells of testis [25]. In the brain they seem to participate in maintaining the T3 concentration along with parallel changes in D2 expression. It has been demonstrated that the thyroid state modulates OATP1C1, and by doing so counteracts the effects of alterations in circulating T4 levels on brain T4 uptake [26, 27]. In humans, OATP1C1 is also expressed in the testis where also D2 expression has been demonstrated [28]. This combination supports a role of TH in development, growth and differentiation of Leydig cells. In particular T3 is very important for testosterone biosynthesis and may therefore have an important role in male puberty. Other OATPs have been demonstrated in a number of other tissues and may exert a variety of effects, but this is not well clarified, and they are possibly less tissue-specific considering the widespread expression [21]. Some characteristics of the transporters are shown in table 2 [29–39].

Amino Acid Transporters

Iodothyronines are a particular class of amino acids built from two tyrosine residues implying transport by specific amino acid transporters, in particular the L and T type amino acid transporters, which therefore are involved in TH uptake into several tissues [40–44]. Among those are members of the heterodimeric amino acid transporter (HAT) family. Their exact role is not clear, but it has been demonstrated that overexpression of the heterodimer L-type transporter in cells resulted in increased intracellular T3 availability and a consequent augmentation of T3 action [45]. Evidence has also been presented to suggest a role of members from the HAT family in supplying the placenta and developing fetus with thyroid hormone [46].

The monocarboxylate transporter (MCT) family comprise to date 14 identified members in various tissues from different species [21]. MCTs are dispersed over autosomal chromosomes, except MCT8, which is X-linked [47]

Table 2. Characteristics of thyroid hormone transporters

Gene	Protein	Species	Accession code	Chromosome	Tissue distribution	Iodothyroine transport	Ref.
SLC10A1	NTCP	human	NP_003040	14q24.1	liver	T4, T3, rT3, T2	[28, 29]
SLC10A1	NTCP	rat	NP_058743	6q24	liver, kidney, CP	T4, rT3, T3, T2	[29]
SLCO1A1	OATP1A1	rat	NP_058807	4q44			
SLCO1A2	OATP1A2	human	NP_602307	12p12	brain, kidney, liver	T3, T2, T4, rT3	[29–31]
SLCO1A4	OATP1A4	rat	NP_571981	4	liver, brain, retina	T4, T2, T3, rT3	[29, 32]
SLCO1A5	OATP1A5	rat	NP_110465	4q44	kidney, retina, liver	T3, T4	[30, 32]
SLCO1B1	OATP1B1	human	NP_006437	12p	liver	T3, T4	[30, 33]
SLCO1B2	OATP1B2	rat	NP_113838	4q44	liver	T3, T4	[34]
SLCO1B3	OATP1B3	human	NP_062818	12p12	liver	T3, T4	[30]
SLCO1C1	OATP1C1	human	NP_059131	12p12.3	brain, cochlea	T4, rT3, T3	[25]
SLCO1C1	OATP1C1	rat	NP_445893	4q44	brain	T4, rT3, T3	[26]
SLCO4A1	OATP4A1	human	NP_057438	20q13.33	multiple	T3, T4, rT3	[31]
SLCO4A1	OATP4A1	rat	NP_598292	3q43	multiple	T3 (T4, rT3 NT)	[31]
SLCO4C1	OATP4C1	human	NP_851322	5q21.2	kidney, other	T3, T4	[35]
SLCO4C1	OATP4C1	rat	AAQ04697	9		T3, (T4 NT)	[35]
SLCO6B1	OATP6B1	rat	NP_596903	9q36	testis	T4, T3	[36]
SLCO6C1	OATP6C1	rat	NP_775460	9q36	testis	T4, T3	[36]
SLC7A5	LAT1	human	NP_003477	16q24.3	multiple (not liver), tumors	T2, rT3, T3, T4	[37]
SLC7A5	LAT1	rat	NP_059049	19q12			
SLC7A8	LAT2	human	NP_036376	14q11.2	multiple, tumors	T2, rT3, T3, T4	[37]
SLC7A8	LAT2	rat	NP_445894	15p13			
SLC16A2	MCT8	human	NP_006508	Xq13.2	brain, liver, kidney, heart, thyroid, eye, pituitary, other	T3, T2, T4, rT3	[38, 39]
SLC16A2	MCT8	rat	NP_671749	Xq31			

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and a specific TH transporter [38]. Compared to other TH transporters the rate of T3 and T4 transport is much higher and follows the criteria set down for requirements of a transporter. The MCT8 gene is located in the region of the X-chromosome associated with X-linked diseases [47], and it was therefore hypothesized that a mutation in this gene would result in an X-linked form of thyroid hormone resistance. Indeed, this hypothesis was verified first in a 6-year-old boy with highly elevated serum T3 and severe psychomotor retardation of unknown origin, where a deletion of the first exon of the MCT8 gene was demonstrated [39]. Since then the same group have described 5 unrelated

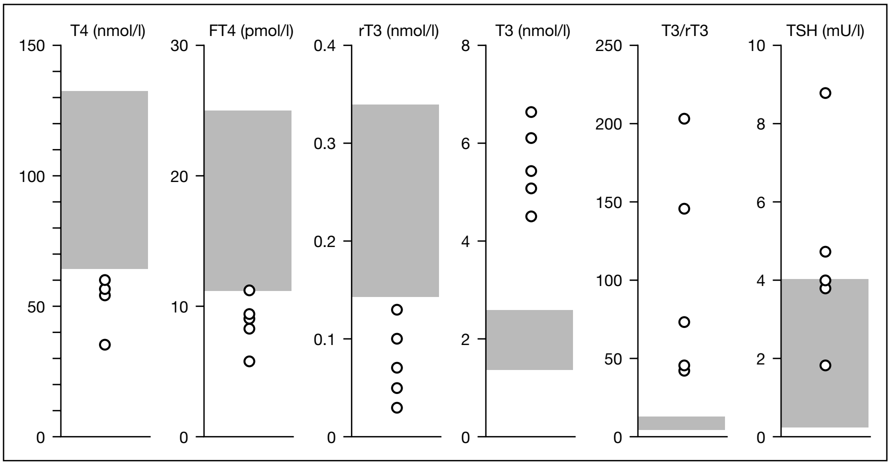

Fig. 2. Thyroid hormone serum levels in patients with mutations in MCT8. Hatched areas indicate normal reference ranges for each analyte. Reproduced with kind permission from Jansen et al. [21].

young boys aged 1.5–6 years with mutations or deletions in the MCT8 gene. They all had a uniform type of severe psychomotor retardation of hitherto unknown origin. The described phenotype comprised symptoms such as severe proximal hypotonia with poor head control and lack of verticalization, absence of targeted grasping, severe mental retardation with only rudimentary communicative skills and movement-induced increase in tone in the extremities [39]. Concerning thyroid function variables, T3 was invariably strongly elevated in all the patients, T4 and free T4 were mildly increased while thyroid-stimulating hormone (TSH) was in the normal range for age in 4 patients and increased in one (fig. 2). The various mutations have been described in more detail in a recent review [21]. All the mothers of the 5 patients were proven to be carriers, all of them with normal thyroid hormone levels and without psychomotor retardation. Another group has described two other cases with different mutations [48]. By studying the complex clinical picture of these patients it was assumed that MCT8 had an important role in TH-dependent processes of brain development. To provide a clue to the cellular function of MCT8 in brain, the expression of MCT8 mRNA in the murine central nervous system was studied by in situ hybridization histochemistry [49]. In addition to the choroid plexus structures, the highest transcript levels were found in neo- and allocortical regions (e.g. olfactory bulb, cerebral cortex, hippocampus, and amygdala), moderate

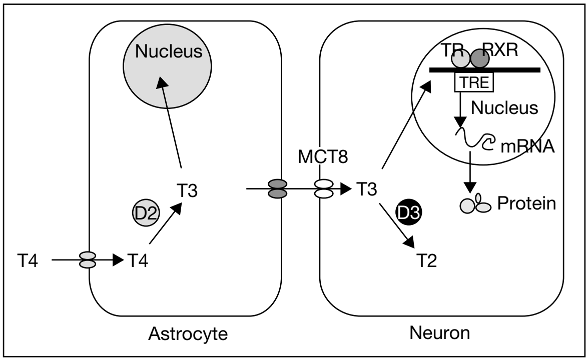

Fig. 3. Role of MCT8 in the neuronal uptake of T3. Reproduced with kind permission from Jansen et al. [21].

signal intensities in striatum and cerebellum, and low levels in a few neuroendocrine nuclei. Co-localization studies revealed that MCT8 was predominantly expressed in neurons. Together with the spatiotemporal expression pattern of MCT8 during the perinatal period, these results strongly indicated that MCT8 plays an important role for proper central nervous system development by transporting TH into neurons as its main target cells [49]. Another hypothesis raised by these clinical pictures was that MCT8 must play an essential role in the supply of T3 to neurons in the central nervous system (fig. 3). T3 binds to nuclear receptors in neurons, which are a primary action site for T3. The action of T3 is terminated by deiodination by D3, which is expressed in the neurons. However, for local production of T3 the neurons are dependent on neighboring astrocytes expressing D2, which is necessary for the local deiodination (fig. 3). Inactivation of MCT8 by mutation in the gene will result in an impaired supply of T3 to the neuron, as well as a decrease in T3 clearance due to block of T3 access to D3 with a possible subsequent increase in serum T3, consequently stimulating a further expression of D1 in the liver and kidney. The resulting increase in conversion of T4 to T3 and breakdown of reverse T3 explains the serum thyroid hormone concentrations in these patients.

The mutations in the MCT8 gene thus resulted in a severe hypothyroidism in the brain with the consequent phenotype, but other tissues and organs did not demonstrate signs of hypothyroidism e.g. bones and metabolism. It therefore seems that other tissues than the brain, are not dependent on MCT8 for uptake of TH. The elevated T3 did not exert any symptoms of hyperthyroidism in the patients, indicating that other yet unknown regulating mechanisms must be in place.