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.
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.

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

<table>
<thead>
<tr>
<th></th>
<th>TBG</th>
<th>TTR</th>
<th>HSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight, kDa</td>
<td>54*</td>
<td>55</td>
<td>66.5</td>
</tr>
<tr>
<td>Structure</td>
<td>monomer</td>
<td>tetramer</td>
<td>monomer</td>
</tr>
<tr>
<td>Carbohydrate content, %</td>
<td>20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Number of binding sites for T4 and T3</td>
<td>1</td>
<td>2</td>
<td>several</td>
</tr>
<tr>
<td>Association constant, Kₐ (M⁻¹)</td>
<td>1 × 10¹⁰</td>
<td>2 × 10⁸***</td>
<td>1.5 × 10⁶***</td>
</tr>
<tr>
<td>Concentration in serum (mean normal, mg/l)</td>
<td>16</td>
<td>250</td>
<td>40,000</td>
</tr>
<tr>
<td>Relative distribution of T4 and T3 in serum, %</td>
<td>T4 75</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>T3 75</td>
<td>&lt;5</td>
<td>20</td>
</tr>
<tr>
<td>In vivo survival</td>
<td>Half-life, days</td>
<td>5***</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Degradation rate, mg/day</td>
<td>15</td>
<td>650</td>
</tr>
</tbody>
</table>

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.

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.
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

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*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].
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 \( \text{Na}^+ / \text{taurocholate} \) cotransporting polypeptide (NTCP) and the \( \text{Na}^+/\text{H}^+ \)-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].
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
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. 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].
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.

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