Biochimica et Biophysica Acta 1830 (2013) 3974–3978

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

The pathophysiological consequences of thyroid hormone transporter deficiencies: Insights from mouse models☆

Heike Heuer [a,*], Theo J. Visser [b]

[a] Leibniz Institute for Age Research/Fritz Lipmann Institute, Jena, Germany
[b] Dept. Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 7 March 2012
Received in revised form 4 April 2012
Accepted 11 April 2012
Available online 20 April 2012

Keywords:
Allan–Herndon–Dudley syndrome
Monocarboxylate transporter 8
Slc16a2
L-type amino acid transporter 2
Organic anion transporting polypeptide 1c1

ABSTRACT

Background: As a prerequisite for thyroid hormone (TH) metabolism and action TH has to be transported into cells where TH deiodinases and receptors are located. The trans-membrane passage of TH is facilitated by TH transporters of which the monocarboxylate transporter MCT8 has been most intensively studied. Inactivating mutations in the gene encoding *MCT8* are associated with a severe form of psychomotor retardation and abnormal serum TH levels (Allan–Herndon–Dudley syndrome). In order to define the underlying pathogenic mechanisms, Mct8 knockout mice have been generated and intensively studied. Most surprisingly, Mct8 ko mice do not show any neurological symptoms but fully replicate the abnormal serum thyroid state.
Scope of review: We will summarize the findings of these mouse studies that shed light on various aspects of Mct8 deficiency and unambiguously demonstrated the pivotal role of Mct8 in mediating TH transport in various tissues. These studies have also revealed the presence of the complex interplay between different pathogenic mechanisms that contribute to the generation of the abnormal TH serum profile.
Major conclusions: Most importantly, studies of Mct8 ko mice indicated the presence of additional TH transporters that act in concert with Mct8. Interesting candidates for such a function are the L-type amino acid transporters Lat1 and Lat2 as well as the organic anion transporting polypeptide Oatp1c1.
General significance: Overall, the analysis of Mct8 deficient mice has greatly expanded our knowledge about the (patho-) physiological function of this transporter and established a sound basis for the characterization of additional TH transporter candidates. This article is part of a Special Issue entitled Thyroid hormone signalling.

1. Introduction

Elucidating the biological function of thyroid hormones (TH) requires not only an in depth knowledge of the molecular events underlying thyroid hormone receptor (TR) activation but also includes a detailed analysis of those mechanisms that locally control the amount of TH available for cell- and tissue-specific action. Deiodinases are important components in determining tissue-specific TH availability and have been analyzed in great detail. Type 1 (D1) and Type 2 (D2) deiodinases play an important role in the conversion of T4, the main product of the thyroid gland, to the receptor active form T3 by outer ring deiodination whereas type 3 (D3) deiodinase activity is restricted to inner ring deiodination, a process that results in the formation of the inactive TH metabolites rT3 and T2. Intriguingly, D1 shows inner-ring deiodination activity as well suggesting that this enzyme exhibits two different functions. All three deiodinases display a differential distribution pattern and are tightly regulated by TH, with D1 and D3 in a positive and D2 in a negative manner. These features indicate specialized functions of these enzymes in TH metabolism, and more information is found in excellent reviews published elsewhere [1–4] (Fig. 1).

As additional components critical for intracellular TH metabolism and action TH transporters have recently attracted a great deal of attention. Despite the long-fostered idea of TH crossing cell-membranes just by passive diffusion a wealth of evidence has accumulated demonstrating that TH as amino acid derivates need transporters for entering cells [5]. Indeed, various transporter candidates have been studied and have been shown to transport TH among a vast array of other compounds [6–8]. However, with the characterization of the monocarboxylate transporter 8 (Mct8) as the first specific TH transporter [9] and, subsequently, with the identification of patients with inactivating mutations in the MCT8 gene a new chapter in the field of TH research has been opened. In particular, the generation and analysis of mouse mutants deficient in Mct8 have turned out to be a valuable approach in order to study the (patho-) physiological significance of this transporter though still many

☆ This article is part of a Special Issue entitled Thyroid hormone signalling.

* Corresponding author at: Leibniz Institute for Age Research/Fritz Lipmann Institute e.V., Beutenbergstr. 11, D-07745 Jena, Germany. Tel.: +49 3641 65 6021; fax: +49 3641 65 6335.
E-mail address: hheuer@fli-leibniz.de (H. Heuer).

0304-4165/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagen.2012.04.009

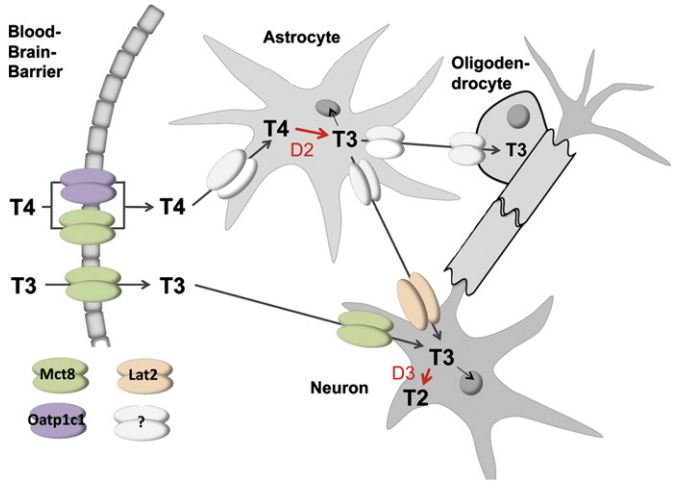

Fig. 1. Schematic illustration of thyroid hormone (TH) traffic in the mouse brain. T4 can pass the blood–brain barrier via the monocarboxylate transporter Mct8 and the organic anion transporting polypeptide Oatp1c1. Uptake of T3 into the CNS is dependent on Mct8. The transporters involved in glial TH passages are still largely unknown although subtypes of astrocytes have been reported to express Mct8 or Oatp1c1. Neurons can import T3 via Mct8 and the L-type amino acid transporter Lat2. Activation of T4 to T3 is mediated by type 2 deiodinase (D2) localized in astrocytes. In contrast, inactivation of TH takes place in neurons where type 3 deiodinase (D3) is expressed.

open questions remain to be addressed. Here, we aim to provide a summary of the results from studies with Mct8 ko and compound mutant mice and we will also address the phenotype of mouse mutants deficient in other TH transporters such as Lat2 and Oatp1c1.

2. Clinical symptoms of patients with inactivating MCT8 mutations

Patients with inactivating mutations in the X-linked gene encoding MCT8, first identified in 2004 [10,11], show a unique set of symptoms combining severe neurological impairments with high serum T3 concentrations. Meanwhile, MCT8 mutations in more than 75 families have been reported. Even the clinical findings in patients already described in 1944 by Allan, Herndon and Dudley have been related in retrospect to mutations in the Mct8 genes [12,13]. Based on the initial description, MCT8 deficiency is also known as the Allan–Herndon–Dudley syndrome.

Characteristic features of Mct8 deficiency are a severe form of mental retardation (often accompanied by a lack of speech development and poor communication skills), proximal hypotonia and spastic paraplegia [14–17]. Many patients suffer from seizures and exhibit pronounced feeding difficulties. Consequently, malnutrition in association with low body weight is another predominant hallmark of this disease. Low body fat content and changes in blood parameters such as increased SHBG values point to a thyrotoxic situation in peripheral organs as a result of the high serum T3 concentrations [18]. Indeed, reduction of serum T3 concentrations as achieved by PTU and T4 treatment, was accompanied by weight gain and normalization of heart parameters in one patient but did not result in any improvement of the neurological symptoms [19].

Why patients with MCT8 mutations show the severe neurological phenotype is still a matter of speculation. Some clinical observations such as delayed myelination and decreased TH concentrations in the CSF point to a hypothyroid situation in the CNS and may suggest a pivotal role of MCT8 in providing access of TH to the developing brain/neurons [20–23]. However, we still cannot rule out that MCT8 transports other substrates not identified yet and thereby exerts additional functions critical for proper brain development.

3. Analysis of Mct8 ko mice

3.1. General appearance

In order to study the pathogenic mechanisms that cause the severe symptoms diagnosed in patients with MCT8 mutations, two different Mct8 ko mouse models have been generated and extensively studied [24–26]. Mct8 ko mice are born with the expected Mendelian frequency and develop indistinguishably from their wild type littermates. Males and females are fertile and do not show any overt neurological symptoms such as ataxia and locomotor impairment, common features in hypothyroid mouse models [27]. Even when Mct8 deficient animals were subjected to an extensive battery of histochemical, neurological and behavior tests only subtle changes were observed such as hyperalgesia and a decreased anxiety-related behavior [28].

In contrast to the unexpected mild CNS phenotype, Mct8 ko mice replicate very nicely the abnormal thyroid parameters found in patients with MCT8 mutations. They show highly increased serum T3 values in the presence of low T4 and slightly elevated TSH. These changes have robust consequences for TH metabolism and action in various organs. Moreover, in vivo transport studies revealed that on top of the abnormal serum TH concentrations the inactivation of Mct8 also interferes with TH transport in certain tissues. Overall, despite a seemingly normal appearance, Mct8 ko mice show striking abnormalities that are associated with tissue-specific changes in TH availability.

3.2. Brain abnormalities in Mct8 ko mice

Despite the lack of severe neurological impairments, the mouse CNS is affected by the absence of Mct8. In vivo transport studies revealed that Mct8 ko mice show a strongly reduced transport of radiolabeled T3 into the brain whereas the uptake of radiolabeled T4 was not impeded [25]. Consequently, T3 and T4 brain content is moderately decreased in the absence of Mct8. In line with these observations Ceballos et al. reported a diminished response of neurons towards peripherally applied T3 in hypothyroid Mct8 ko mice [29]. These findings point to an important role of Mct8 in the transport of T3 across the blood–brain and/or blood–CSF barrier.

These data, however, also imply that at least in mice, one additional, T4 specific transporter must exist that can compensate for the absence of Mct8 and thereby prevent neurological damage caused by pronounced TH deprivation. We even speculate that this T4 specific transporter may not be present in the human brain since such a scenario would provide the explanation for the rather mild brain phenotype in the Mct8 ko mice. An attractive candidate that fulfills the criteria of T4 selectivity and differential expression in the mouse and human CNS is Oatp1c1, and indeed, first analysis using respective knockout mice support this hypothesis (see below).

In addition to the choroid plexus and capillary endothelial cells, Mct8 is strongly expressed in different neuronal populations in the mouse brain [30], and it was therefore of utmost interest to study the intracellular thyroidal state of these neurons in the absence of Mct8. Unfortunately, this information can be only obtained by indirect means, such as e.g. by determining the transcript levels of well-established T3 target genes. As one example, the expression of the synaptic protein neurogranin/ RC3 is controlled by T3 specifically in the striatum, an important brain area for motor control [31,32]. Analysis of Mct8 ko mice indeed revealed slightly reduced RC3 expression levels indicative of decreased intracellular T3 levels [25]. Expression of type 3 deiodinase (D3) as another neuronal target of T3 action was found to be decreased in the absence of Mct8 as well [25]. It is, however, rather difficult to discriminate between a reduced neuronal T3 uptake due to the missing Mct8 or insufficient local T3 production by D2 in astrocytes. In order to solve this problem, Morte and colleagues analyzed the gene expression pattern of TH target genes in the cerebral cortex of Mct8 ko