Understanding the Hypothalamus-Pituitary-Thyroid Axis in Mct8 Deficiency

Julia Müller Heike Heuer

Leibniz Institute for Age Research/Fritz Lipmann Institute, Jena, Germany

Key Words

Monocarboxylate transporter 8 (Mct8, Slc16a2) ·
Allan-Herndon-Dudley syndrome · Mct10 · Oatp1c1 ·
Deiodinases

Abstract

Thyroid hormone (TH) metabolism and action via binding to nuclear receptors are intracellular events that require the passage of TH across the plasma membrane. This process is mediated by specific TH transporters of which the monocarboxylate transporter 8 (Mct8) has received major attention. Mct8 is highly expressed in different tissues such as liver, kidney, thyroid, pituitary and brain. In humans, inactivating mutations of the MCT8 gene (SLC16A2) are associated with severe forms of psychomotor retardation and abnormal TH serum levels (Allan-Herndon-Dudley syndrome). Surprisingly, Mct8 knockout (ko) mice do not exhibit overt neurological symptoms but fully replicate the unusual serum TH profile with highly increased serum T₃ in the presence of low serum T₄. In order to evaluate the underlying mechanisms for these abnormalities, TH transport and metabolism have been intensively studied in different tissues of Mct8 ko mice. Here, we summarize the observed changes within the hypothalamus-pituitary-thyroid axis that result in altered TH production and secretion. Although analysis of Mct8 ko mice has greatly expanded our knowledge, many open questions still remain to be addressed in order to define the tissue- and cell-specific role of this important TH transporter.

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Introduction

The availability of sufficient amounts of thyroid hormone (TH) is a prerequisite for almost all physiological processes and normal tissue function. In particular, TH is essential for a normal maturation of the central nervous system (CNS). TH deprivation during critical stages of brain development will affect events such as neuronal differentiation, synaptogenesis and myelination and, therefore, can lead to irreversible brain damage [1–4].

In order to unravel the molecular actions by which TH modulates tissue function and controls brain development, TH receptors have been extensively studied and excellent reviews about TRs have been published [5–8]. Another very active field of research is to delineate the role of deiodinases (D1, D2, D3) that can activate and inactivate TH by outer- and inner-ring deiodination, respectively. These deiodinases represent important determinants in regulating systemic as well as local TH concentrations [9–12]. Most of the circulating iodothyronine T₃ is not produced by the thyroid itself but generated by deiodinases D₁ and D₂ expressed in peripheral tissues and in the brain, respectively. Deiodinase type 3 catalyzes only the inactivation of TH and is in particular strongly expressed in the CNS and in fetal tissues.

For a long time the common assumption persisted that TH can pass the phospholipid bilayer simply by passive diffusion due to the lipophilic nature of TH. However, in the last decade it has become clear that influx and efflux of TH are carrier-mediated events [13–16]. Up to now,

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Heike Heuer, PhD
Leibniz Institute for Age Research/Fritz Lipmann Institute e.V.
Beutenbergstrasse 11
DE–07745 Jena (Germany)
Tel. +49 3641 65 6021, E-Mail hheuer@fli-leibniz.de

numerous transporters have been identified that are more or less specific in transporting iodothyronines in and out of cells, namely the Na⁺-taurocholate cotransporting polypeptide, members of the Na⁺-independent organic anion transporting polypeptide (OATP) family, the heterodimeric L-type amino acid transporters Lat1 and Lat2 as well as the monocarboxylate transporters Mct10 and Mct8.

Of all these transporter candidates, Mct8 has attracted a great deal of attention as it shows a highly restricted substrate specificity [17, 18]. Both human and rat Mct8 transport iodothyronines very efficiently in a bidirectional manner, but they do not accept any other substrate tested so far. In comparison, Mct10, as another member of the monocarboxylate transporter family, is similarly effective in mediating the transport of TH [19]. However, in contrast to Mct8, Mct10 also transports aromatic amino acids.

The human MCT8 gene (SLC16A2) is located on the X chromosome (Xq13.2) and was initially cloned by Lafrenière et al. [20] in 1994. It contains 6 exons and 5 introns encoding a 60-kDa membrane-spanning protein with 12 putative transmembrane domains and both, the N- and the C-terminal domains, are located intracellularly [20, 21]. In humans, MCT8 mRNA was found to be highly expressed in liver, kidney, heart, placenta, lung, thyroid as well as in brain and pituitary [22]. Here, we will summarize the consequences of Mct8 deficiency in humans and mice with a special focus on the hypothalamus-pituitary-thyroid axis.

Phenotype of MCT8 Patients

The physiological significance of Mct8 as a transporter critical for TH homeostasis and the supply of TH to the developing brain was underscored by the identification of patients who carry inactivating mutations or deletions in the MCT8 gene (SLC16A2). Since the MCT8 gene is encoded on the X chromosome (Xq13.2) the disease was described as an X-linked psychomotor retardation syndrome [23, 24]. Subsequently, mutations in the MCT8 gene were discovered to be associated with the long known Allan-Herndon-Dudley syndrome (AHDS) [25]. Following these initial descriptions, mutations in more than 50 families have been described so far [26]. With the ongoing screenings initiated, the number of patients diagnosed with a defect in MCT8 is expected to even further increase in the upcoming years.

All patients with an inactivating MCT8 mutation suffer from a unique syndrome. They show serious mental deficits, congenital hypotonia, spastic or dystonic quadriplegia, seizures, as well as muscle hypoplasia, generalized muscle weakness with poor head control. Many of them are not able to sit, stand or walk independently, and do not develop any speech [25, 27, 28]. Interestingly, the severity of the syndrome seems to correlate with the impact of the respective mutation on the TH transport activity of MCT8. Mutations in MCT8 that still allow residual TH transport are associated with a more advanced psychomotor development, whereas mutations that lead to a complete loss of function are linked to more dramatic forms of neurological impairments [26, 29].

Information about the cellular damage in the CNS of these patients is still rather limited. Brain magnetic resonance imaging studies of young MCT8 patients (<2 years) revealed that Mct8 deficiency gives rise to anomalous myelination as well as brain atrophy [30, 31]. In addition, MCT8 mutations have been reported in patients affected by hypomyelinating leukodystrophies initially diagnosed with Pelizeus-Merzbacher-like disease [32]. A delayed myelination would also be a hallmark of patients that suffer from insufficient TH supply during critical stages of brain development. Consequently, a hypothyroid situation in the brain might be envisaged for patients with MCT8 mutations.

A second hallmark of the 'MCT8 disease' are very unusual TH parameters [21, 33]. All patients show strongly increased serum T₃ levels, while T₄ and rT₃ concentrations are rather low. TSH values are in the normal range or even slightly elevated. These findings clearly underline the physiological significance of MCT8 for TH transport *in vivo*, although, at least at first glance, the pathogenic mechanisms from which these TH abnormalities arise are not obvious.

Phenotype of Mct8 Knockout Mice

In order to get further insights into the mechanisms underlying the neurological and endocrine symptoms observed in MCT8 patients, two different Mct8-deficient mouse models have been generated and intensively analyzed [34, 35]. Mct8 knockout (ko) mice fully reproduce the TH abnormalities found in the patients and exhibit highly increased T₃ (twofold) and reduced T₄ (to 34%) and rT₃ values resulting in a strikingly increased T₃/T₄ ratio (sixfold). However, they completely lack any neurological or locomotor impairment and display a normal brain

morphology [36], suggesting that Mct8 may be of minor importance for proper development of the murine brain.

This was a rather unexpected finding since in the mouse CNS, Mct8 is highly expressed in TH-sensitive neuronal populations such as in the cerebral and cerebellar cortex as well as in the hippocampus and striatum where it has been speculated to mediate neuronal T_3 uptake [37]. Moreover, Mct8 was also localized in choroid plexus structures and in brain capillary endothelial cells where it may participate in the transport of TH via the blood-brain barrier (BBB) and/or blood-CSF barrier [38]. Indeed, *in vivo* transport studies revealed a strongly diminished uptake of T_3 into the brain of Mct8 ko mice. In contrast, the transport of T_4 was only mildly affected [35]. Thus, Mct8 is critically involved in the passage of T_3 into the brain, whereas at least one additional T_4 transporter must exist that can compensate for the absence of Mct8 in the mouse but not in the human brain. Such a candidate is represented by Oatp1c1 that preferentially transports T_4 [39, 40]. Indeed, a first analysis of Oatp1c1-deficient mice points to a role of this transporter in the uptake of T_4 at the BBB as Oatp1c1 ko animals showed a reduced brain T_4 content despite normal Mct8 expression at the BBB and normal TH levels in the circulation [41].

The significance of Mct8 in neuronal T_3 transport is less clear. Due to the low T_4 concentrations in the CNS of Mct8 ko mice, a strong compensatory upregulation of D_2 activity together with a decreased expression of D_3 could be detected indicating that the local production of T_3 within the brain is strongly induced and TH inactivation by inner-ring deiodination is downregulated [34, 35]. Apparently, this increase in T_4 to T_3 conversion by D_2 is effectual to provide most of the neuronal cells with sufficient amounts of T_3 and, hence, serious brain damages can be prevented. Some neurons in the Mct8 ko brain, however, show a reduced expression of T_3 -sensitive target genes like neurogranin (RC3) in the striatum and hairless in the cerebral cortex [35, 42]. Currently, it is unknown whether these mild alterations reflect inadequate local T_3 production or partially impaired neuronal T_3 uptake due to the missing of Mct8. Future studies using conditional Mct8 mouse mutants will hopefully help to clarify this question.

In mice, Mct8 is not the only TH transporter localized to neurons, and species differences between mice and men with respect to the cell-specific TH transporter repertoire have undoubtedly been demonstrated. As one example, the L-type amino acid transporter Lat2 that can act as a TH transporter as well is present in murine neurons and glia cells, whereas in the human brain its expres-

sion is restricted to microglia cells [36, 43]. Thus, as another hypothesis, a possible compensation for the lack of Mct8 by Lat2 has been put forward in order to explain the lack of any neurological damage in Mct8 ko mice. Again, future mouse studies using Mct8/Lat2 double ko (dko) mice are expected to respond to this question.

Role of Mct8 in the Hypothalamus

In contrast to the rather mild hypothyroid situation in the brain of Mct8-deficient animals, one specific subset of neurons appears to be more prominently affected by the absence of Mct8, namely the hypophysiotropic TRH-producing cells in the hypothalamic paraventricular nucleus (PVN). *In situ* hybridization studies revealed a strong upregulation of TRH mRNA expression in these TH-sensitive neurons indicating a pronounced hypothyroidal state [35]. This difference may have several reasons. For example, the uptake of T_3 into these neurons might be restricted due to the absence of Mct8 in these cells. However, peripheral application of pharmacological doses of T_4 are sensed by the TRH neurons and resulted in a downregulation of TRH expression. Thus, T_3 import into PVN cells must still be possible in the absence of Mct8 and the hypothyroidal state may be rather due to an insufficient local T_3 supply. In this respect, tanycytes that are specialized glia cells lining the third ventricle seem to exert an important function. With their long processes they are able to make contact with the axonal endings of TRHergic fibers in the median eminence [44]. Under normal conditions, tanycytes express high levels of D_2 as well as of Mct8 [36, 37, 45]. Based on their unique features, it is tempting to speculate that they may serve a special function within the negative feedback loop by providing the TRH neurons with T_3 , a function that may be partially impeded when Mct8 is missing. Consecutively, TRHergic neurons may secrete elevated amounts of TRH that in turn can affect pituitary function.

Such changes in hypothalamic TH sensing are most likely not specific for the mouse brain but may also occur in a similar manner in the human MCT8-deficient brain. In contrast to other areas of the human CNS, MCT8 distribution, carefully mapped in the human hypothalamus, revealed the localization of the transporter in PVN neurons as well as in glial cells [46–48]. Thus, it is tempting to speculate that patients with inactive MCT8 may also exhibit an increased hypothalamic TRH secretion.

Role of Mct8 in the Pituitary

One of the most enigmatic findings concerns the role of Mct8 in the pituitary. Both in the human and in the mouse anterior lobe, Mct8 is not expressed in thyrotrophs as one might assume but it is restricted to folliculostellate cells [48, 49]. In mice, Mct8-specific *in situ* hybridization signals were also found in pituicytes of the posterior lobe and in a thin line of cells at the border of the anterior and intermediate loop (fig. 1). The function of Mct8 in these non-hormone-producing cells is completely unclear. Determination of TSH mRNA levels in the Mct8 ko mouse models revealed slightly elevated transcript levels that also fit to the increased serum TSH values found in these animals indicating that at least the pituitary thyrotrophs are in a mild hypothyroid state [34, 50]. This assumption is supported by moderately increased activities of the enzyme D_2 [35] that in mice are localized in TSH-producing cells [51, 52]. That thyrotrophs do indeed not sense appropriately the high circulating T_3 levels could also be confirmed by TSH suppression tests [34, 35]. When Mct8-deficient mice were rendered hypothyroid by mercaptoimidazole treatment, only the application of high T_3 concentrations was successful in suppressing endogenous TSH production. Not surprisingly, hypothalamic TRH expression did not show any response towards the T_3 treatment due to the impaired T_3 passage into the CNS. A similar pituitary resistance to TH has also been observed in patients with MCT8 mutations [53]. Altogether, in the absence of Mct8, the pituitary is unable to integrate the high T_3 serum levels adequately, but the underlying mechanism of this resistance remains mysterious and requires further investigations.

Role of Mct8 in the Thyroid Gland

In addition to the hypothalamus and pituitary, the thyroid gland appears to be affected by the lack of Mct8 as well. Compared to other TH transporters in wild-type mice, Mct8 is highly expressed in follicular cells of the thyroid gland [50, 54, 55]. Based on its basolateral localization it is tempting to speculate that Mct8 may be involved in TH efflux, and the analysis of Mct8-deficient animals strongly supports this hypothesis. Moreover, due to the elevated serum TSH levels, TH production may be constantly stimulated that in turn can influence thyroid morphology.

In fact, gross histological examination revealed an enlarged thyroid gland in Mct8 ko mice. Quantification of

thyroid follicle parameters demonstrated the presence of bigger follicles in the absence of Mct8, a phenotype that even progresses with age [50, 54, 55]. Furthermore, starting from the age of 6 months, papillary structures in the thyroid gland became evident [50]. At the age of 12 months, more than 80% of Mct8 ko mice showed pathological abnormalities. In 1 patient with a MCT8 mutation who underwent thyroidectomy, follicular irregularities were diagnosed as well [50]. So far, the underlying mechanisms are rather unknown, and it also remains to be evaluated whether the age-dependent development of papillary thyroid carcinoma is a general hallmark of Mct8 deficiency.

Determination of the thyroidal TH content revealed significantly increased Tg bound and free T_3 and T_4 concentrations in the thyroids of adult Mct8 ko mice [54–56]. These alterations may be a consequence of the elevated serum TSH levels. In fact, our analysis of Mct8/Trhr1 dko mice that are characterized by a reduction in TSH activity demonstrated strongly reduced free and Tg-bound TH content in the thyroid gland compared to the single Mct8 ko mice. However, independent of the TH content in the gland, T_4 secretion is impaired in the absence of Mct8 as shown by TSH stimulation tests using Mct8 ko and Mct8/Trhr1 dko mice [55]. Moreover, Mct8 ko mice that were set on a 2-week low-iodine diet and then injected with ^{125}I , exhibited a reduced rate of radioiodine clearance from the thyroid gland and a reduced appearance of labeled iodothyronine in the serum [54], an observation that further supports a role of Mct8 in TH secretion.

In addition to the decrease in T_4 export, we observed a rather surprising finding with respect to the release of T_3 . Following TSH stimulation, both Mct8 ko mice as well as Mct8/Trhr1 dko mice displayed an enhanced thyroidal efflux of T_3 [55]. We speculated that due to a partial retention of T_4 in thyrocytes, more T_4 is accessible to thyroidal D_1 . This may lead to an increased intrathyroidal T_4 to T_3 turnover and consequently to an altered ratio of TH released from the gland. Such a shift would be further amplified peripherally by D_1 in hepatocytes and kidney cells that converts even more T_4 to T_3 . According to this hypothesis, alterations in thyroidal TH secretion would represent an important mechanism that contributes to the generation of the abnormal TH levels. A powerful approach to test this hypothesis would be the tissue-specific inactivation of Mct8 in the thyroid gland by taking advantage of conditional Mct8 mutant mice.

That the thyroid gland may contribute at least partially to the increase in serum T_3 could be also shown by a more indirect approach. By crossing Mct8 ko mice with

Fig. 1. Consequences of Mct8 deficiency on TH tissue concentrations, metabolism and transport in mice. Images illustrate Mct8 expression in different tissues as revealed by β -galactosidase immunoreactivity (hypothalamus, CNS), and radioactive in situ hybridization (pituitary, thyroid, liver, kidneys), respectively. **a** In mice, Mct8 is highly expressed in the PVN of the hypothalamus, in the anterior as well as in the posterior pituitary and in follicular epithelial cells of the thyroid gland. Absence of Mct8 results in increased hypothalamic TRH expression, a moderate increase in pituitary TSH mRNA levels and a strong elevation of free and thyroglobulin bound T₃ and T₄ content in the thyroid gland. **b** A characteristic hallmark of Mct8 deficiency are abnormal serum TH levels with elevated T₃ and reduced T₄. In the CNS, Mct8 is highly expressed in neurons as demonstrated for the hippocampal area (Hip), in endothelial cells of the BBB as

well as in tanycytes (T) that represent specialized glia cells lining the third ventricle (3V) and are in contact with the median eminence (ME). Mct8 ko mice exhibit a strongly diminished uptake of T₃ into the brain whereas transport of T₄ is not impeded. Consequently, the brain is in a hypothyroid state with increased D₂ and decreased D₃ activities as well as reduced T₃ and T₄ brain content. Liver and kidneys are also affected by Mct8 deficiency and show highly elevated D₁ expression and strongly induced T₃ tissue content. In the liver, T₄ concentrations are similar to those observed in wild-type littermates (=) indicating that Mct8 deficiency may result in a moderate retention of T₄ in the liver. In the kidneys, transport studies revealed a highly stimulated uptake of both T₃ and T₄ that in turn leads to an accumulation of TH in this tissue. As a result, liver and kidneys of Mct8 ko mice are in a thyrotoxic state.

Pax8 ko mice, we were able to produce animals that not only lack Mct8 but also do not develop a functional thyroid gland [57]. These athyroid mice do not produce any TH endogenously and are therefore perfectly suited for TH substitution experiments. Indeed, daily injection of Pax8 ko mice with 20 ng/g b.w. T_4 was sufficient to produce T_3 and T_4 serum levels similar to those found in wild-type littermates that have a functional thyroid gland [58]. When the same dose of T_4 was applied to Mct8/Pax8 dko mice during the first 3 weeks of life, serum T_3 concentrations were similar to that detected in control animals as well [55]. Thus, we concluded that in Mct8 deficiency normal circulating T_3 levels can be achieved by T_4 replacement therapy as long as the influence of the hypothalamus-pituitary-thyroid axis is abolished.

Despite the fact that T_3 was normalized in T_4 -treated Mct8/Pax8 dko mice, serum T_4 values were still strikingly low. This observation indicates that rather extra-thyroidal mechanisms must play a role in minimizing circulating T_4 , whereas an impaired thyroïdal T_4 secretion contributes only to a minor extent to this phenotype but initiates the imbalances reinforced by a vicious circle. This situation does not seem to be specific for mice but also holds true for humans as decreased serum T_4 has been observed in 1 patient who underwent thyroidectomy and was subsequently substituted with T_4 [50].

Where does T_4 disappear from the circulation? We considered a renal contribution since Mct8 is highly expressed at the basolateral site of the proximal tubule cells. Moreover, Mct8 ko mice show an increased renal uptake of T_3 and T_4 suggesting that Mct8 may primarily act as an TH export system. As a consequence, kidney TH content and even more so urinary TH concentrations are pronouncedly elevated [59]. In addition, D_1 activity in the kidneys is strongly induced indicating a thyrotoxic state of this tissue. Why renal TH transport is enhanced in the absence of Mct8 is another important question to be addressed in further studies. It remains to be investigated

to which extent Mct8 plays a role in the hepatic TH efflux as well since recent data suggest an impairment in hepatic T_4 release [56]. Such changes may also contribute to the hyperthyroidal state of the liver that has been described in the Mct8 ko mouse. Recent studies have demonstrated that alterations in D_1 activities are involved in the generation of the abnormal serum profile as Mct8/ D_1 dko mice show a partial normalization in serum TH values. Although a major impact of increased hepatic D_1 activities has been discussed, final proof for this assumption is still missing since in addition to the liver, D_1 is also present in the kidneys and in the thyroid [56].

Conclusion and Outlook

Overall, Mct8 deficiency results in robust tissue-specific alterations in TH transport, metabolism and action. The analysis of Mct8 ko mice has significantly expanded our knowledge how different tissues are affected by Mct8 deficiency. A simplified cartoon illustrating the alterations described so far is depicted in figure 1. We speculate that many of these alterations take place in patients as well. As one example, patients show elevated levels of sex hormone-binding globulin, an indicator for liver thyrotoxicosis [60, 61]. However, the studies of Mct8 ko mice have also revealed the limitations of this animal model. Due the vast array of changes caused by the ubiquitous inactivation of Mct8, it is almost impossible to define the exact cell-specific function of the transporter. In this respect, we await the generation and analysis of tissue-specific Mct8 mutant mice that will shed further light on the function of this unique TH transporter.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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