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Finding the Way into the Brain without MCT8

AQ: 1

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How does thyroid hormone (TH) find its way into the brain? Although it has been known for a long time that TH is crucial for normal brain development, the exact molecular mechanisms involved in TH transport in the brain have remained elusive until recently. Early studies showed selective and saturable accumulation of TH in particular brain regions, suggesting that active transport processes are required for TH entry across the blood-brain barrier (BBB) and into brain cells (1). The discovery that TH transporter proteins located in the plasma membrane are required for cellular entry of the hormone has advanced our understanding of TH physiology. Thus, TH transporters mediate transport not only across the BBB but also into each individual cell of the brain.

Many transporters have been identified that accept a wide range of substrates, including TH (2). Monocarboxylate transporter 8 (MCT8) and its homolog, MCT10, are important exceptions, showing a high activity and specificity for TH (3, 4). MCT10 shows preference for T_3 over T_4, but also transports aromatic amino acids. MCT8 transports different iodothyronines (T_4, T_3, rT_3, 3,3′-T_2) with similar efficiencies but does not appear to transport aromatic amino acids.

The biological importance of TH transporters was clearly established by the discovery of patients harboring mutations in the *MCT8* gene, which is located on the X-chromosome (5, 6). Affected male patients suffer from severe psychomotor retardation and also show abnormal serum TH levels. They have severe cognitive deficits, with IQ values mostly below 40. Speech development is mostly severely hampered. Newborns with *MCT8* mutations present with global hypotonia. The central hypotonia that is associated with poor head control persists throughout life, whereas the peripheral hypotonia usually progresses into spastic quadriplegia. Most patients are unable to sit, stand, or walk independently.

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Patients with *MCT8* mutations demonstrate typical abnormal thyroid function tests. Serum T_3 levels are elevated, and both serum T_4 and rT_3 levels are decreased. TSH levels range from normal to moderately elevated, with mean values twice that in controls. Because the clinical phenotype resembles that in subjects with the Allan-Herndon-Dudley syndrome (AHDS), Schwartz *et al.* (7) discovered that mutations in *MCT8* represent the genetic basis of this syndrome originally described in 1944. The pathogenicity of most mutations has been documented by assessing the TH transport capacity of the mutants by *in vitro* assays.

The pathogenesis of the AHDS is incompletely understood. The current hypothesis holds that brain of AHDS patients is deprived of TH and, thus, is in a hypothyroid state. This concept is based on the expression of MCT8 in the BBB, in the choroid plexus (blood-cerebrospinal fluid interface), and in neuronal cells (8). Myelination, which depends on TH, is delayed in AHDS patients, suggesting also an important role of MCT8 in oligodendrocytes. Unfortunately, *Mct8* knockout (KO) mice lack overt neurological features, limiting the use of this model to study the pathogenesis of the neurological phenotype of AHDS patients (9, 10). Nevertheless, it has been clearly established that cerebral T_3 and T_4 levels are diminished in *Mct8* KO mice. Available evidence suggests that brain T_4 is low because of the low supply of serum T_4, and not so much because of decreased T_4 transport into the brain, whereas the low brain T_3 despite the high serum T_3 is caused by impaired T_3 transport into the brain (11).

The reduced cerebral TH levels in *Mct8* KO mice still appear sufficient to support expression of T_3 target genes and relatively normal brain development. Adaptive regulation by the deiodinases D2 and D3 probably contributes to maintain sufficient brain T_3 levels in *Mct8* KO mice (9, 10). Another transporter may provide the mouse brain

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Abbreviations: AHDS, Allan-Herndon-Dudley syndrome; BBB, blood-brain barrier; DITPA, 3,5-diiodothyropropionic acid; KO, knockout; MCT8, monocarboxylate transporter 8; PTU, propylthiouracil; TH, thyroid hormone.

specifically with T_4 for local conversion to T_3. The organic anion transporting polypeptide Oatp1c1, which transports T_4, but not T_3, may well fulfill this role. *Oatp1c1* KO mice display decreased T_3 and T_4 levels in brain, despite the presence of Mct8 (12). If OATP1C1 is expressed at much lower levels in the human BBB as one study suggests (8), the human brain may thus be much more dependent on MCT8 for its TH supply. In addition to possible differences in the role of MCT8 in transporting TH into the human or mouse brain, there may also be important species differences in the function of MCT8 in the transport of T_3 in target cells, in particular neurons and oligodendrocytes. Obviously, we need a much better understanding of the exact cellular localization of MCT8 in the human brain.

The low body weight, in particular low muscle mass, of AHDS patients as well as the elevated levels of SHBG, a marker of liver thyroid state, likely result from the effects of elevated serum T_3 levels on peripheral tissues. Thus, depending on the expression MCT8 and other TH transporters, different tissues are either deprived of TH or exposed to excess TH, resulting in hypothyroid (brain) and hyperthyroid (liver, muscle) tissues, respectively.

Several mechanisms may contribute to the abnormal TH levels in MCT8-deficient subjects. Increased D1 activities documented in liver and kidney of *Mct8* KO mice account for increased peripheral T_4 to T_3 conversion, which is further enhanced by the renal trapping of T_4 in *Mct8* KO mice (9, 10, 13–15). The importance of D1 in producing the biochemical phenotype is underscored by the observation that thyroid parameters are nearly normalized in *Mct8*/*Dio1* double KO mice (16). Importantly, MCT8 facilitates TH secretion by the thyroid gland (17, 18). Impaired thyroidal secretion of T_4 and preferential T_3 secretion likely contribute to the low T_4 and high T_3 levels.

Unfortunately, therapeutic options for AHDS patients are limited. Combined treatment with propylthiouracil (PTU) and LT_4 (block-and-replace therapy) has been applied to two AHDS patients, aiming to reverse the deleterious effects of excess T_3 on peripheral tissues (13, 14). Normalization of T_3 and T_4 levels was readily achieved, and beneficial effects were reflected in increased body weight and reduction of SHBG levels. Hypothetically, the brain may also benefit from this treatment because it results in an increased supply of serum T_4 and, thus, in an increased local T_3 production, although it is offset by a decreased serum T_3 supply. However, treatment with PTU and LT_4 did not result in an improvement of cognitive functions.

Theoretically, compounds that mimic T_3 action but rely on other transporters than MCT8 for cellular entry are suited to reverse or prevent the neurological phenotype in AHDS patients. Studies in *Mct8* KO mice with the T_3 analog 3,5-diiodothyropropionic acid (DITPA) by Refetoff and coworkers (19) demonstrated the normalization of TH parameters and attenuation of the thyrotoxic state of peripheral tissues. Importantly, an improvement of several indices of TH action in brain was observed. The group of Refetoff pursued these observations and describe their experience with DITPA therapy in AHDS patients in this issue of the *JCEM* (20). Four children, two of whom were twins, were treated for over 2 yr with DITPA. The main consistent findings were a significant decrease in serum T_3 and SHBG levels. Subtle increases in serum rT_3 and T_4 and slight decreases in serum TSH were also observed. Heart rate decreased in three patients. None of the patients showed improvement in psychomotor development.

These data suggest that DITPA treatment may be used to normalize T_3 levels in AHDS patients. Apparently, this is beneficial for the liver and heart, as suggested by the decrease in SHBG levels and heart rate. Although other thyroid parameters changed moderately, T_4 and rT_3 levels remained low or low-normal. The effects on other parameters of TH action showed a substantial variation among the different patients. Significant weight gain was noted in the twin patients but also a progressive weight loss in another patient of similar age. Patient-specific differences in nutritional supply and supportive care may also contribute to the different results observed in the patients treated with DITPA. The lack of an obvious beneficial effect on brain development may be explained by an irreversible damage caused by the inappropriate TH supply to the brain in the fetal and neonatal period. At the doses used, DITPA may have insufficient access to the human brain. Because TSH levels only decreased moderately, there was apparently little effect of DITPA on hypothalamus and the pituitary. Higher DITPA doses were required to suppress TSH in normal adult subjects (21). In addition, AHDS may not be caused by a decreased brain T_3 supply alone, but in addition by the deficiency of another, unidentified ligand for MCT8, which is not replaced by DITPA.

The present study is an important and rational approach toward an effective therapy for patients with AHDS. It also raises several questions. Is DITPA the optimal therapy to normalize serum TH levels and, thus, to improve the thyrotoxic state of peripheral tissues? Should PTU with its intrinsic D1-inhibitory effect be part of the treatment, given the prominent role of D1 in the derangement of TH levels? Should treatment aim at normalization of T_4 levels? Will other T_3 analogs be able to prevent or reverse the brain phenotype? What are the prospects for gene therapy?

Future strategies to develop optimal treatment for AHDS patients may explore the use of alternative T_3 an-

alogs. A possible candidate is the naturally occurring TH metabolite 3,3′,5-triiodothyroacetic acid (Triac), which has a high affinity for the T_3 receptors. Although it is unclear whether low T_4 levels in AHDS patients are harmful, one may argue that providing the brain with normal T_4 levels better reflects physiology. This is only achieved by adjuvant treatment with LT_4, as has been done in patients treated with PTU (13, 14).

Because *Mct8* KO animals are not suited to study neurological consequences of AHDS, other models should be investigated. A recent study indicates that brain development is markedly affected in the *Mct8*/*Oatp1c1* double KO mice, which therefore appear to be a better model for AHDS patients than *Mct8* KO mice (22). To investigate treatment options in large, random controlled trials is almost impossible because AHDS is rare. Nevertheless, these patients deserve a collaborative effort to develop treatment strategies for this severe disease. This is very much the purpose of regular meetings where scientists and professionals providing care for AHDS patients discuss these strategies.

The treatment studies carried out in AHDS patients all have in common that they were initiated in patients in whom at least part of the brain damage is irreversible. Therefore, irrespective of the therapy, it is of the utmost importance to detect MCT8 mutations as early as possible. In several AHDS patients where this has been documented, neonatal T_4 levels were found to be low (23). If additional determination of T_3 and rT_3 in the neonatal screening for congenital hypothyroidism would produce the typical endocrine fingerprint, diagnosis and treatment may be much advanced.

Much insight has been achieved in the understanding of the AHDS phenotype since the discovery of MCT8 mutations less than a decade ago. The initiatives to search for a therapy have yielded benefits without apparent harm for the patients (13, 14, 19, 20). Future studies of the pathogenesis of AHDS in animal models and of pharmacological interventions in patients will certainly continue the progress that has been made, as illustrated by the present study in this issue of the *JCEM*. Hopefully, these studies will find a way for TH or analogs into brains lacking MCT8.

Acknowledgments

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