Biochimica et Biophysica Acta 1830 (2013) 3965–3973

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Function of thyroid hormone transporters in the central nervous system ☆

Ulrich Schweizer *, Josef Köhrle

Institut für Experimentelle Endokrinologie, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

ARTICLE INFO

Article history:
Received 4 April 2012
Received in revised form 12 July 2012
Accepted 30 July 2012
Available online 7 August 2012

Keywords:
Brain
Neuron
Astrocyte
Blood–brain-barrier
Allan–Herndon–Dudley syndrome

ABSTRACT

Background: Iodothyronines are charged amino acid derivatives that cannot passively cross a phospholipid bilayer. Transport of thyroid hormones across plasma membranes is mediated by integral membrane proteins belonging to several gene families. These transporters therefore allow or limit access of thyroid hormones into brain. Since thyroid hormones are essential for brain development and cell differentiation, it is expected that genetic deficiency of such transporters would result in neurodevelopmental derangements.
Scope of review: We introduce concepts of thyroid hormone transport into the brain and into brain cells. Important thyroid hormone transmembrane transporters are presented along with their expression patterns in different brain cell types. A focus is placed on monocarboxylate transporter 8 (MCT8) which has been identified as an essential thyroid hormone transporter in humans. Mutations in *MCT8* underlie one of the first described X-linked mental retardation syndromes, the Allan–Herndon–Dudley syndrome.
Major conclusions: Thyroid hormone transporter molecules are expressed in a developmental and cell type-specific pattern. Any thyroid hormone molecule has to cross consecutively the luminal and abluminal membranes of the capillary endothelium, enter astrocytic foot processes, and leave the astrocyte through the plasma membrane to finally cross another plasma membrane on its way towards its target nucleus.
General significance: We can expect more transporters being involved in or contributing to in neurodevelopmental or neuropsychiatric disease. Due to their expression in cellular components regulating the hypothalamus–pituitary–thyroid axis, mutations and polymorphisms are expected to impact on negative feedback regulation and hormonal setpoints. This article is part of a Special Issue entitled Thyroid hormone signalling.

1. The emerging roles of transporters in brain function

1.1. Genetic deficiencies for metabolite plasma membrane transporters are the basis for many rare diseases

An increasing number of rare diseases involve mutations in transmembrane transport proteins that mediate uptake or release of metabolic intermediates, minerals, vitamins or signaling molecules. Many of these syndromes involve neurodevelopmental, neurological or psychiatric conditions. Even if an essential metabolite is involved and a disease is severe, redundancy is a common theme in the transporter field. Many substrate molecules are transported by several different transporters which are expressed in a cell type-specific, sometimes overlapping, and often developmentally regulated pattern. Since many cerebral transmembrane transporters are also expressed in peripheral tissues, other organ systems may be affected by mutations as well. On the other hand, expression of a transporter in the kidney often allows diagnosis based on biochemical measurements in the urine.

Amino acids, sugars, nucleotides, and carboxylic acids are water soluble metabolites which cannot pass the plasma membrane passively. Their cellular uptake and release is often mediated by integral transmembrane proteins which are members of large gene families and can be grouped according to their energy- or co-substrate dependence or independence.

Mutations in amino acid, glucose, and vitamin transporters span a spectrum of mild to fatal diseases, including unexpected phenotypes like e.g. obsessive–compulsive disorder caused by mutations in *EAAC1*/*SLC1A1* and associated with dicarboxylic aminoaciduria.

Apparently more lipophilic compounds including cholesterol, bile acids, and fatty acid-derived molecules also depend on transmembrane transporters. Mutations in such transporters include Niemann–Pick disease or Tangier disease. Both diseases are caused by defective proteins involved in transport of cholesterol, a membrane lipid. *Expect the unexpected*![1]

1.2. Genetic deficiency in a thyroid hormone transporter leads to a syndrome of severe mental retardation

Iodothyronines are hydrophobic and tend to stick to laboratory glassware and plastic. Researchers have therefore assumed until the 1970s that these molecules are sufficiently hydrophobic to passively

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

* Corresponding author. Tel.: +49 30 450 524 080; fax: +49 30 450 524 922.
E-mail address: ulrich.schweizer@charite.de (U. Schweizer).

[1] Martin Evans, Nobel laureate 2010, Lindau conference.

0304-4165/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2012.07.015

cross the lipid bilayers of cellular membranes. Then, the groups of Georg Hennemann and Govind Rao put forward the hypothesis that thyroid hormones, as any amino acids, should need transport mechanisms to cross the plasma membrane. They started to systematically study thyroid hormone uptake into cells, while this concept still played no role in clinical research (see excellent review by [1]) and awaits more research and acceptance also in the area of steroid hormones [2].

From the 1990s, individual transporter molecules capable of iodothyronine transport were being identified: Initially, high-affinity active uptake mechanisms for thyroid hormones into liver cells were studied. Later work characterized thyroid hormone uptake into brain cells. After the first thyroid hormone transporter, rat Oatp 2/Slco1a4, was identified by expression cloning in *Xenopus* oocytes [3], more thyroid hormone transporters from other gene families were found [4]. The group of Theo Visser took a systematic candidate approach [5,6] and finally identified monocarboxylate transporter 8 (MCT8, SLC16A2) as a highly active thyroid hormone transporter [7]. Shortly afterwards, mutations in *MCT8* were found to cause severe X-linked psychomotor retardation [8,9]. Subsequently, mutations in MCT8 were found to underlie the Allan–Herndon–Dudley syndrome, an X-linked mental retardation syndrome described already in 1944 [10]. Early in-situ hybridization studies detected *Mct8* mRNA in neurons of the mouse brain, but also along the ventricles and in the choroid plexus [11]. These findings were then supported and extended by immunohistological studies which allowed to assign MCT8 protein to distinct membranes or e.g. tanycyte processes in the hypothalamus [12]. These new concepts tantalized the thyroid hormone field: There was now clinical evidence that transmembrane transport of iodothyronines unequivocally required transport proteins! Moreover, MCT8 emerged as the most import of iodothyronine transporter for brain function [13].

2. Thyroid hormone transport into the brain

2.1. Thyroid hormones are hydrophobic molecules, which associate with binding proteins in plasma and cerebrospinal fluid

To fully appreciate the intricacy of iodothyronine transport mechanisms in the brain, soluble iodothyronine transfer proteins need to be included in the discussion. Owing to their hydrophobicity, thyroid hormones associate with plasma carrier proteins. Non protein-bound thyroid hormones infused via the portal vein into the liver immediately associate with vessel walls and become completely depleted from the perfusate [14]. Only through reversible binding to plasma transfer proteins, thyroid hormones are able to circulate through the blood stream. In fact, while the plasma total concentration of T_3 and T_4 is 1.8 nM and 100 nM, respectively, the free hormone concentrations are only 5 pM and 20 pM. Thyroxine hormone-binding globulin (TBG) has the highest affinities among the binding proteins for T_3 ($5\times10^8 M^{-1}$) and T_4 ($1\times10^{10} M^{-1}$). Consequently, TBG binds up to 80% and 68% of plasma T_3 and T_4, respectively. Transthyretin (TTR) has lower affinities in the range of $10^8\ M^{-1}$ for both thyroid hormones, and thus, despite its higher capacity for binding, contains only about 10% of each hormone in plasma. The highest capacity of thyroid hormone binding, although at low affinity ($10^6\ M^{-1}$), has serum albumin, which binds the remaining 10–20% of T_3 and T_4. The three carrier proteins, which are functionally redundant, thus buffer a thyroid hormone binding range over six orders of magnitude from the picomolar free hormone concentrations to the limit of thyroid hormone solubility in the micromolar range [15]. The binding sites of the carrier proteins are not saturated in the circulation and several aromatic, phenolic and hydrophobic drugs compete with thyroid hormone binding sites, thus altering free thyroid hormone concentrations.

TTR is a major protein constituent of and the only thyroid hormone binding protein in human cerebrospinal fluid (CSF). It is synthesized in the choroid plexus epithelium from early development onwards and directionally secreted into the CSF [16,17]. T_4 and rT_3 are present in CSF at about 2–3 nM and 10–230 pM, respectively. T_3 is detectable at approximately 50–270 pM [18]. Free thyroid hormone concentrations and their relative abundance differ in CSF from serum. While total T_4 in CSF is lower than in plasma (ca. 100 nM), free T_4 concentrations are higher (70 pM) than in plasma (20–30 nM) and about 1.4% of total T_4 is free in CSF but only 0.02% in plasma [19,20]. Free rT_3 in CSF is higher (10–15 pM) [21,22] than free T_3 concentration (3 pM) which is in a similar range as in plasma [23]. Limited data on free rT_3 concentration in human CSF [21,22] indicate 20–25 fold higher levels compared to serum, and authors suggested either restricted passage of rT_3 across the blood–brain or blood–CSF-barriers or local rT_3 production in the brain contributing to this marked rT_3 gradient. Whether altered protein composition of CSF, which lacks TBG compared to serum, is involved in these free rT_3 differences require more research.

Interestingly, unlike in other tissues, brain T_3 content (3 pmol/g wet tissue) is similar to T_4 (5 pmol/g wet tissue) suggesting that T_3 is mainly present within cells [24]. Also high 3,5-T_2 concentrations (70–150 fmol/g) have been reported for several brain areas and in human brain tumors 3,5-T_2 concentrations were equal to the low T_3 content [25]. Mammalian TTR has a higher T_4 affinity and lower T_3 affinity than TTR in birds and reptiles, which do not express a high affinity TBG. This evolutionary adaptation enables a higher extent of local T_3 formation in those brain regions reached by CSF containing TTR-bound T_4. Also total protein concentration in CSF accounts only for 0.5% of that in plasma with a markedly different composition. With increasing brain size TTR became the most relevant thyroid hormone binding protein in brain.

2.2. Thyroid hormones are transported across the blood–brain- and blood–CSF-barriers

Early experiments aimed at determining the mechanism of thyroid hormone uptake were designed along three basic methods: (i) injection (*intravenous* or *intra-carotic*) of radiolabelled thyroid hormone and counting of radioactivity in dissected brain tissue, (ii) injection of radiolabelled thyroid hormone followed by autoradiography of brain sections, and (iii) studies involving perfused choroid plexus, mostly in sheep. These studies were complemented by cell culture studies involving endothelial cells. At a time when transmembrane transporters were not yet cloned, a role for TTR was assumed in brain thyroid hormone uptake [26]. This hypothesis was eventually not supported by gene inactivation of *TTR* gene targeting in mice [27], which led to minor changes in plasma free thyroid hormones, but did not result in brain developmental defects such as observed in hypothyroidism. Apparently, successful evolution of the fine tuned and redundantly controlled permissive thyroid hormone system rather tolerates complete deficiency of single components than expression of functionally deficient mutants as impressively illustrated by TRα and TRβ mutations, which lead to tissue-specific thyroid hormone resistance [28,29]. However, genetic *TTR* inactivation presents with a decreased thyroid hormone availability of the population of progenitor cells and the stem cell niche in the subventricular zone of the developing mouse brain [30].

While choroid plexus epithelial cells with their tight junctions form the blood–CSF-barrier and ependymal cells have no tight junctions [31], the 'backdoor-entry' of thyroid hormones to the brain via vectorial secretion of TTR-T_4 complex into the CSF remains an attractive hypothesis. This would require that T_4 (and T_3) is imported into choroid plexus epithelial cells, associates there with newly synthesized TTR and is secreted into the CSF bound to TTR. Alternatively, a second transporter might export T_4 (and T_3) into the CSF, where TTR and its