Review

Thyroid hormone action during brain development: More questions than answers

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ABSTRACT

Thyroid hormone is essential for proper brain development since it acts on processes such as neuronal migration and differentiation, myelination and synaptogenesis. In this review, we summarize the consequences of thyroid hormone deficiency for brain development with special focus on the cerebellum, an important target of thyroid action. In addition, we discuss the role of iodothyronine deiodinases and thyroid hormone transporters in regulating local thyroid hormone concentrations as well as current knowledge about the function of thyroid hormone receptors and their target genes during brain maturation. Despite considerable progress in recent years in deciphering thyroid hormone signaling pathways we still know very little on the molecular level by which mode of action thyroid hormone exerts its cell-specific effects. Hence, we will particularly address the open questions that remain to be addressed in order to better understand the role of thyroid hormone in brain development.

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Contents

1. Introduction

Thyroid hormone (the prohormone thyroxine T4, and its active metabolite 3,5,3′-triiodothyronine T3) is an essential factor during all stages of brain development. Children who develop under condition of severe thyroid hormone deprivation suffer after birth from severe mental retardation, deaf-mutism, spastic diplegia and extrapyramidal rigidity (DeLong et al., 1985). The most prevalent cause for thyroid hormone insufficiency particularly during gestation is the lack of dietary iodine that prevents thyroid hormone

synthesis and may cause these neurological symptoms also known as neurological cretinism (Berbel et al., 2007). Even a mild reduction in maternal thyroxine production during gestation (maternal hypothyroxinemea) greatly increases the risk for neurodevelopmental abnormalities and may lead to a decreased IQ in the progeny (de Escobar et al., 2004). Children can also develop hypothyroidism after birth due to an agenesis or dysgenesis of the thyroid gland (De Felice and Di Lauro, 2004). Congenital hypothyroid newborns are usually diagnosed within the first weeks of life during a neonatal TSH screening which is common practice in many countries (American Academy of Pediatrics et al., 2006). However, if thyroid hormone replacement therapy is not instituted immediately after birth, these children will also exhibit cognitive impairment demonstrating that even during postnatal periods of brain

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maturation thyroid hormone must be present to ensure normal development.

Despite the wealth of clinical findings underscoring the importance of thyroid hormone for brain development it is surprising that we know very little about the cellular and molecular mechanism by which thyroid hormone influences structure and function of the central nervous system (CNS). The limitations in defining the exact role of thyroid hormone are due to a rather complex interplay of thyroid hormone with thyroid hormone receptors that includes genomic actions as well as rapid activation of cytosolic signaling cascades. Other reasons for the limitations include cell-specific differences in cellular availability that are largely determined by the activities of thyroid hormone activating and inactivitating enzymes (in particular the iodothyronine deiodinases type 2 (D2) and type 3 (D3) as well as by the cell-specific repertoire of thyroid hormone transporters, and also challenges in discriminating cell-autonomous effects of thyroid hormone versus indirect effects that might be due to a systemic action of thyroid hormone that in turn affects the CNS.

This review will briefly assess the current knowledge as well as the open questions that still need to be addressed in order to better understand the role of thyroid hormone during brain development with a special focus on the cerebellum. For more in-depth description the reader is referred to excellent reviews published elsewhere (Anderson, 2008; Bernal, 2005; Bernal, 2007; Nunez et al., 2008; St Germain et al., 2009). Moreover, we will not discuss the role of thyroid hormone in retina and cochlea development which has been addressed in a number of interesting papers published elsewhere (Ng et al., 2004; Roberts et al., 2006; Lu et al., 2009; Ng et al., 2009).

2. Ontogenesis of thyroid hormone action

A wealth of studies have revealed that thyroid hormone already acts during early stages of development. In humans as well as in rodents, thyroid hormones as well as their receptors are present in the fetus prior to the onset of fetal thyroid hormone production underscoring the relevance of maternal thyroid hormone production for fetal development (Bernal and Pekonen, 1984; Obregón et al., 1984; Contempré et al., 1993; de Escobar et al., 2004). For instance, already at week 12 of gestation both T4 and T3 can be detected in the human developing cortex but not in the serum and other fetal tissues. At week 18 when fetal thyroid hormone synthesis has just started thyroid hormone levels in the cerebral cortex reach peak concentrations that are comparable to those found in the adult cortex (Kester et al., 2004). Local concentrations of thyroid hormones are controlled at various levels of synthesis and secretion. In this respect, the ontogenic profile of deiodinases may play an important role by modulating T3-bioavailability in a time- and region-specific manner. Whether the transport of thyroid hormones is also spatially and temporally controlled during ontogenesis still remains to be elucidated. However, the severe neurological phenotype of patients in which the thyroid hormone transporter MCT8 is inactive (see below) indicates an important role of thyroid hormone transporters in regulating the access of thyroid hormone to its target cells during prenatal stages.

Studies on thyroid hormone action in brain development have been performed mainly in rodents in which thyroid hormones, receptors and deiodinases are also expressed in the developing brain well before the onset of the fetal thyroid gland activity. However, when comparing thyroid hormone action in human and rodent brain development one has to keep in mind that the rat brain at birth approximates the developing human brain at 6 months of gestation whereas the human brain at birth exhibits a stage of differentiation that is similar to a rat brain at postnatal day P6–P10 (Porterfield and Hendrich, 1993; Oppenheimer et al., 1995). (A detailed comparison of the developmental timing between rodents and humans is displayed at http://www.translatingtime.net.) As a consequence, brain development may be influenced by the maternal thyroidal state more in humans than in rodents. For a more detailed time-line describing the ontogenesis of the thyroid hormone system in relation to human and rodent brain development the reader is referred to excellent reviews published elsewhere (Anderson et al., 2003; Bernal, 2007).

In order to study the consequences of severe hypothyroidism, pregnant rats were rendered hypothyroid by the treatment with n-propylthiouracil (PTU) which blocks thyroid hormone synthesis by inhibiting the iodination of thyroglobulin and by decreasing the activity of deiodinase D1. Alternatively, animals can be treated with MMI (1-methyl-2-mercapto-imidazol)/Perchlorate in order to decrease significantly thyroid hormone production. For monitoring thyroid hormone effects during postnatal neurodevelopment the Pax8 ko mouse represents a very suited animal model (Mansouri et al., 1998). Pax8 is an essential transcription factor for the development of thyroid follicular structure and the expression of thyroid-specific genes. As a consequence, Pax8 ko mice born to euthyroid Pax8 heterozygous mothers are completely athyroid after birth. Neither in tissues nor in the serum thyroid hormones can be detected indicating that at least in mice maternal thyroid hormones are not provided via the milk (Friedrichsen et al., 2003). Due to their athyroidism Pax8 ko mice are strongly growth retarded, deaf and exhibit an ataxic phenotype indicating that among other brain structures particularly the maturation of the cerebellum is impaired. However, since in utero Pax8 ko mice are provided with maternal thyroid hormone this animal model is not suited to assess the consequences of thyroid hormone deficiency for prenatal neurodevelopmental events.

3. Metabolism of thyroid hormones by brain deiodinases

Since the major thyroid hormone produced by the thyroid gland is the receptor inactive T4, extrathyroidial conversion of T4 to T3 is needed for any action that is signalled via the classical thyroid hormone receptors. Studies in animal models have revealed that approximately up to 80% of the active thyroid hormone T3 is produced locally in the CNS (Crantz et al., 1982) suggesting a prominent role of the activating enzyme D2 which exclusively catalyses outer ring deiodination. In contrast to thyroid hormone receptors that are highly enriched in oligodendrocytes and neurons, D2 is predominantly found in astrocytes throughout the brain (Guadano-Ferraz et al., 1997). In the rat, D2 expression is first detectable at E16.5 and increases successively until postnatal day 15. Ontogenic profiling of D2 in the fetal human brain revealed the occurrence of D2 in the developing cerebral cortex during the first trimester of pregnancy just at a time point when also the cortical T3 concentration can first be detected (Chan et al., 2002). The similar ontogenic profile of D2 expression and T3 content in different developing brain structures has led to the hypothesis that D2 is particularly important in providing developing brain structures with T3 produced from maternally derived T4. Another remarkable feature of D2 is that its activity is highly regulated by the thyroid status via both pre- and post-translational mechanisms. Hypothyroidism results in markedly upregulated D2 activities and hyperthyroidism leads to a decrease in D2 expression levels (Burmeister et al., 1997; Escobar-Morreale et al., 1997). These changes have been interpreted as a protective mechanism to maintain the brain T3 content as normal as possible in light of altered serum thyroid hormone levels.

Recently, the generation and analysis of a D2 deficient mouse model revealed surprising results that challenged the current concept of D2 as an essential gate keeper in the CNS (Galton et al., 2007). D2 knockout mice exhibit normal serum T3 but increased

serum T4 and TSH levels indicating that D2 is critically involved in the negative feedback regulation within the HPT axis. In the brain, T4 content was found to be elevated whereas T3 concentrations were substantially decreased to 50% reflecting a diminished local T3 production. Despite the low T3 levels, locomotor activity, learning and memory skills which are severely affected in hypothyroid animals were seemingly normal in D2 knockout mice suggesting that a deficiency of local T3 production can largely be compensated by an uptake of T3 from the circulation (St Germain et al., 2009). In fact, even mice mutants that are devoid of any 5′-deiodinase activities (D1/D2 double knockout mice) show along with unexpected normal serum T3 levels only very mild neurological impairments (Galton et al., 2009).

Thus, at least in the mouse brain, D2 is not essential for all thyroid hormone-dependent developmental processes as it has been postulated in previous models.

In addition to D2, iodothyronine deiodinase Type 3 (D3) represents another substantial factor for thyroid hormone metabolism in the brain. This enzyme is strongly expressed in neurons (Tu et al., 1999) where it catalyzes inner ring deiodination by which thyroid hormones are inactivated and thereby local thyroid hormone levels are controlled. The activities of this enzyme are also regulated by thyroid hormone though in an opposite manner compared to D2. As a consequence, thyroid hormone deficiency in the brain is not only accompanied by increased local T3 production but also by a decreased local T4 and T3 inactivation.

The significance of D3 has been highlighted by the generation of a respective knockout mouse (Hernandez et al., 2006; Hernandez et al., 2007). Among the most striking phenotypical abnormalities of these animals are a significant perinatal mortality and a strongly perturbed HPT axis that is characterized by a thyrotoxic state of the D3 knockout newborns and a central hypothyroid state of adult animals. As a consequence, the expression levels of T3-responsive genes in the CNS of D3 deficient animals are higher than normal during neonatal stages and lower than normal later in life. The functional consequences of this altered gene expression pattern, however, have still to be defined.

4. Role of thyroid hormone transporters

As another prerequisite for thyroid action and metabolism thyroid hormone has to enter the target cell in order to bind to its receptors, and/or to activate cytosolic processes. Extensive studies within the last decade irrevocably revealed that the transmembrane passage of thyroid hormone does not occur via passive diffusion but is rather mediated by transporters (Hennemann et al., 2001). For regulating brain maturation, several membrane barriers have to be taken until T3 finally binds to its receptor in the CNS. At first, thyroid hormone has to be taken up into the brain either via the blood–brain barrier or the choroid plexus-cerebrospinalfluid (CSF) barrier. In rodents, entry of T4 and T3 via the blood–brain barrier appears to be the preferred route for the overall distribution of thyroid hormone in the brain whereas the uptake via the choroid plexus-CSF barrier may be especially important to provide circumventricular areas with thyroid hormone (Dratman et al., 1991). The monocarboxylate transporter MCT8 has been shown to be critically involved in the transport of T3 into the brain as in MCT8 deficient mice uptake of radiolabeled T3 is completely abolished (Trajkovic et al., 2007). However, T4 can still enter the brain even in the absence of MCT8 suggesting that at least in mice another transporter must exist for mediating the uptake of T4 at the blood–brain barrier. A good candidate for this transit is Oatp1c1, a member of the organic anion transporting polypeptide family which not only exhibits a high degree of substrate specificity towards T4 (and the inactive metabolite rT3) but is also strongly expressed in capillary endothelial cells in the CNS (Sugiyama et al., 2003; Tohyama et al., 2004). Whether Oatpc1c1 is indeed the predominant T4 transporter at the blood–brain barrier can only by proven after the successful generation and characterization of a respective knockout mouse.

After being released from endothelial cells into the brain parenchyma, T4 has to be taken up by astrocytes where it can then be converted to T3 by D2. The transporters involved in astrocytic thyroid hormone processes are still unknown. In certain astrocytes the uptake of T4 might be mediated by Oatp1c1 since analysis of mice expressing enhanced green fluorescent protein under the Oatp1c1 promoter revealed expression of this transporter in a subset of astroglial cells in addition to its capillary localization (http://www.gensat.org).

Following its production in astrocytes T3 is thought to be transported into the brain parenchyme from which it can be taken up by neurons and oligodendrocytes. MCT8 is thought to mediate the transport of T3 in certain neuronal populations where high MCT8 expression was detected by in situ hybridization (Heuer et al., 2005) as well as by immunohistochemistry (unpublished observations). However, at least for the murine CNS one has to postulate the existence of additional neuronal transporters, since neuronal T3 transport was only mildly affected in the MCT8 null mice (Trajkovic et al., 2007). Putative candidates include the member of the L-type amino acid transporter family of which particularly h4F2hc/hLAT1 and h4F2hc/mLAT2 transport T4 and T3 when overexpressed in Xenopus oocytes (Friesema et al., 2001). Moreover, as another interesting candidate the contribution of the monocarboxylate transporter MCT10 to thyroid hormone transport in the CNS needs to be evaluated particularly because MCT10 does not only very efficiently transport thyroid hormones (Friesema et al., 2008) but is also highly expressed in murine oligodendrocytes (unpublished observations). Again, functional analyses of respective mouse mutants are expected to provide further information as to the importance of this transporter.

5. Role of the thyroid hormone transporter MCT8

The physiological significance of thyroid hormone transport for proper brain development and function was highlighted by the identification of patients exhibiting inactivating mutations in the X-linked MCT8 gene (Dumitrescu et al., 2004; Friesema et al., 2004; Schwartz et al., 2005). All patients suffered from a unique syndrome consisting of a severe form of psychomotor retardation in combination with abnormal serum thyroid hormone levels (Friesema et al., 2006). Serum T3 levels were found to be highly elevated whereas T4 concentrations were rather low. MRI analysis in several patients revealed a persistent delay in myelination suggesting a hypothyroid situation of the brain (Namba et al., 2008). Though MCT8 deficient mice exhibit the same abnormal serum thyroid hormone levels they do not replicate the severe neurological symptoms of patients with MCT8 mutations presumably due to the presence of additional thyroid hormone transporters in mice but not in human (Dumitrescu et al., 2006; Trajkovic et al., 2007). Nevertheless, the uptake of T3 into the brain of MCT8 null mice is severely impaired, a finding demonstrating the importance of MCT8 for the transport of T3 via the blood–brain barrier. Since at least in mice T4 can still enter the brain even in the absence of MCT8, an increase in local T3 production by an upregulation of D2 expression seems to prevent severe neurological damage despite the T3 content in the brain being decreased by roughly 40%, thus comparable to the T3 content found in D2 ko mice. Obviously, different compensatory mechanisms exist by which at least the mouse brain can cope with disturbances in the thyroid hormone metabolism. It will be very interesting to see whether the concomitant deletion of D2 and MCT8 in mice will lead to a more drastic neurological phenotype.

As another important aspect it still needs to be addressed whether the thyroid hormone transporter repertoire differs significantly between mice and humans. Murine neurons may express other transporters that prevent thyroid hormone deprivation in the absence of MCT8 whereas neurons in the human CNS may be more dependent on a functional MCT8 due to the lack of additional thyroid hormone transporters. Alternatively, MCT8 might transport other, still unknown substances that are required for proper brain development in humans but not in mice. Though the latter hypothesis can currently not be excluded, the severe mental retardation, the MRI findings as well as the motor abnormalities of patients with MCT8 mutations are also observed in endemic cretinism suggesting that patients with MCT8 mutations suffer primarily from an impaired thyroid hormone transport into the brain already during early stages of development.

6. Structural alterations in the brain as a consequence of thyroid hormone deficiency

How thyroid hormone deficiency interferes with the formation of the CNS has been addressed by Eayrs, Legrand and their co-workers already half a century ago (Eayrs, 1966; Legrand, 1984). In their pioneering studies in rats that were neonatally thyroidectomized, the density of cell bodies and axons and the degree of dendritic branching was found to be compromised to such an extent that a more than 50% decrease in the probability of dendritic and axonal interactions was estimated for the somatosensory cortex. Impaired neuronal differentiation including abnormal spine formation was also noted for the hippocampus and the caudate–putamen. The most detailed histological examination of the consequences of postnatal hypothyroidism has been performed on the cerebellar cortex based on its simplicity and the homogeneity of its structure. As another advantage, the rodent cerebellum develops mainly during the first three postnatal weeks thus providing a convenient opportunity for studying the effects of thyroid hormone dysfunction on neuronal proliferation, migration and differentiation. Morphological analyses indeed revealed that basically all cerebellar cell types are affected by the absence of thyroid hormone (reviewed in Legrand, 1984). A schematic representation of the predominant alterations in the cerebellar differentiation program caused by congenital hypothyroidism is depicted in Fig. 1. Granule precursor cells exhibit a prolonged proliferation period and start their differentiation program at a later time point; Purkinje cells exhibit stunted dendrites that carry immature spines; the afferent climbing fibers that under euthyroid conditions contact the proximal dendrites of Purkinje cells do not climb up but rather build up contacts with the Purkinje cell bodies; cerebellar glomeruli, which are complex synaptic structures between granule cells, Golgi interneurons and mossy fiber terminals showed a delayed differentiation pattern; the number of basket neurons is decreased whereas the number of later forming stellate cells is increased. Even Bergmann glia cells, the principle radial glia cell of the cerebellar cortex, which not only mediate granule cell migration but are also critically involved in directing Purkinje cell dendrites exhibits strongly reduced protrusions. All these morphological alterations can be prevented as long as hypothyroid animals are treated with thyroid hormone before the end of the second postnatal week. Without any replacement therapy functional deficits concerning neuronal connectivity will permanently persist though histologically the overall maturation of cerebellar cells will eventually reach its final stage.

Is every single cerebellar cell type a direct target of thyroid hormones or do indirect actions of thyroid hormone that are mediated via cell–cell interactions or secreted factors play a pivotal role in orchestrating cerebellar differentiation? *In vitro* experiments have
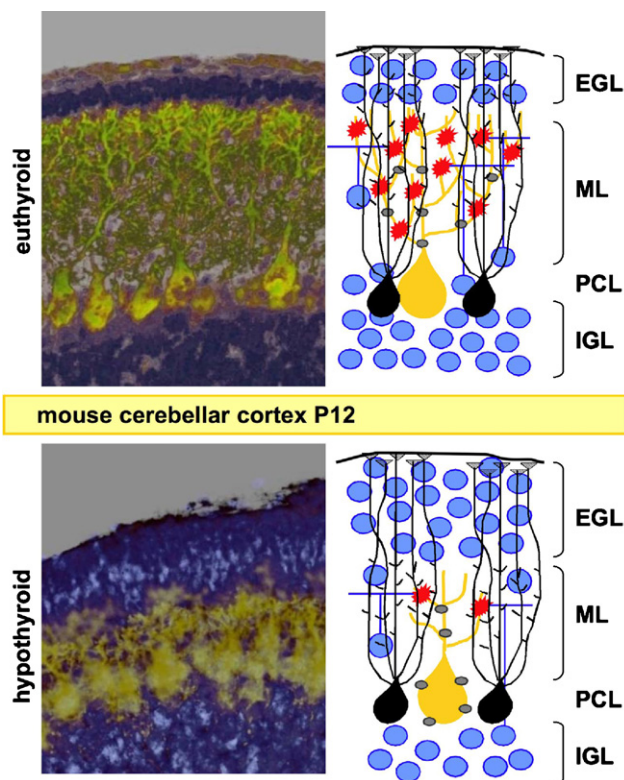

Fig. 1. Consequences of thyroid hormone deficiency for cerebellar Purkinje cell differentiation. Congenital hypothyroidism interferes with the highly orchestrated differentiation pattern of cerebellar neurons as exemplary shown for postnatal day 12: Purkinje cells (yellow) exhibit stunted dendrites; as a consequence, the thickness of the molecular layer (ML) is strongly reduced; Bergmann glia fibers (black) exhibit reduced protrusions; granule cell proliferation is prolonged leading to an increased thickness of the external granule cell layer (EGL) and a decreased number in mature granule cells in the internal granule cell layer (IGL). As a consequence, synaptic contacts (red) of granule cells with Purkinje cells are less numerous. Afferent fiber systems are affected as well: climbing fibers that build up synaptic contacts with Purkinje cell dendrites (grey) do not climb up but rather remain in contact with the cell bodies of the Purkinje cell layer (PCL) in hypothyroid animals. Schematic drawing was inspired by Koibuchi and Chin, 2000. Granule cells are shown in blue (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

revealed evidence for cell-autonomous action of thyroid hormone at least for Purkinje cells, granule cells and astrocytes (Trentin et al., 2001; Heuer and Mason, 2003). In addition, various growth factors important for cerebellar development such as the neurotrophins BDNF and NT3 (Alvarez-Dolado et al., 1994; Koibuchi et al., 1999), Igf1 (Elder et al., 2000), extracellular matrix proteins involved in axonal guidance such as laminin (Farwell and Dubord-Tomasetti, 1999), tenascin (Alvarez-Dolado et al., 1998) and reelin (Alvarez-Dolado et al., 1999), as well as cell adhesion molecules such as NCAM and L1 (Iglesias et al., 1996; Alvarez-Dolado et al., 2000) are produced in a first or even second order response to thyroid hormone indicating a plethora of possibilities by which thyroid hormone directs neuronal differentiation. In addition, thyroid hormone cannot only act in a so-called "genomic" manner by regulating gene expression via nuclear thyroid hormone receptors but it can also exert very rapid effects e.g. by activating cytosolic signaling cascades (see Fig. 2) which makes it even more complicated to elucidate its action.

7. Role of thyroid hormone receptors

The predominant mode of thyroid hormone action includes its signaling via thyroid hormone receptors. Two genes (THRa and
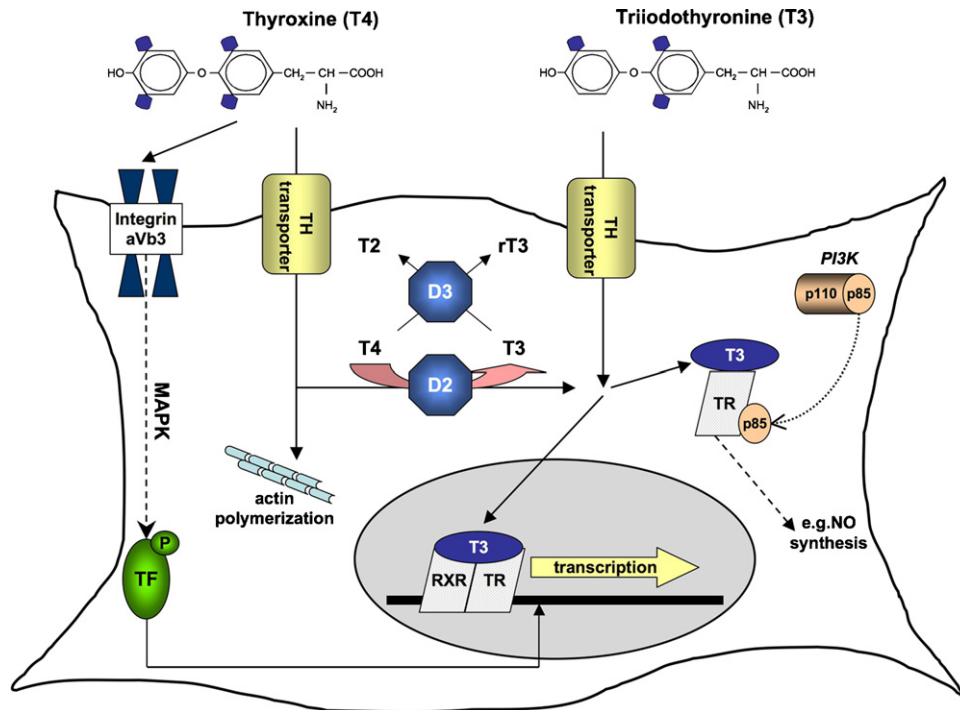
Fig. 2. Pleitrophic modes of thyroid hormone action on target cells. The classical way of thyroid hormone action includes the transport of T3 into the target cell by thyroid hormone transporters and the binding of T3 to thyroid hormone receptors (TR) that in turn regulate as heterodimers with retinoic acid receptors (RXR) the gene expression of target genes. Thyroid hormone receptors are also found in the cytosol where they can interact upon T3 binding with the regulatory subunit (p85) of PI3 kinase (PI3K) thereby activating downstream signaling cascades. In addition to T3, the prohormone T4 can be taken up by the target cell and can be intracellularly converted to T3 by the enzyme deiodinase type 2 (D2). Other actions of T4 include direct effects on the actin polymerisation state. Moreover, T4 can bind to integrin alphaVbetaIII on the cell surface of target cells. As a consequence, MAP kinase pathways are activated that may finally lead to the phosphorylation and activation of transcription factors (TF).

THRb) have been identified that give rise to several protein isoforms by alternative splicing and promoter usage (Bassett et al., 2003; Flamant and Samarut, 2003). Among these different products only TRalpha1, TRbeta1, TRbeta2 and TRbeta3 can be considered as thyroid hormone receptors as they contain both a T3- and a DNA binding domain whereas the physiological function of the non-receptor proteins that are also encoded by THRa and THRb is still unclear.

The best studied function of thyroid hormone receptors is to bind as homodimers or heterodimers with retinoic acid receptors to thyroid hormone-responsive elements (TRE) in the promoter regions of target genes (see Fig. 2). Notably, thyroid hormone receptors are able to bind to TRE's even in the absence of the ligand and to regulate gene expression by interacting with cofactors. In addition to this aporeceptor activity, both repression as well as activation of gene expression can occur upon binding of the receptor to a TRE. Which factors actually determine whether repression or activation takes place is still a matter of intensive studies.

For thyroid hormone action in the CNS, TRalpha1 is thought to play a prominent role in the CNS based on its high expression throughout the brain, accounting for approximately 70–80% of total receptor T3 binding (Ercan-Fang et al., 1996), and its occurrence already at early stages of brain development. In rodents, TRalpha1 transcripts can already be detected at E11.5 in the neuronal tube and at E12.5 in certain areas of the diencephalon and the ventral rhombencephalon (Bradley et al., 1992). In the developing cerebellum, TRalpha1 is also the predominant receptor isoform which is rather ubiquitously expressed (Bradley et al., 1992; Mellstrom et al., 1991). In contrast, expression of TRbeta is much more restricted and mainly found postnatally in selected neuronal populations such as hippocampal pyramidal and granule cells, paraventricular hypothalamic neurons and cerebellar Purkinje cells (Bradley et al., 1989; Strait et al., 1991). Analysis of receptor deficient mouse

mutants revealed that TRbeta is predominantly mediating thyroid hormone effects during the development of retina photoreceptors and of the auditory system (Jones et al., 2003).

Despite the widespread distribution of thyroid hormone receptors in the CNS mice deficient in specific receptor isoforms or even in all thyroid hormone receptors do not replicate the strong phenotype of hypothyroid animals indicating that the lack of the ligand T3 has much stronger detrimental consequences than the lack of its receptors (for reviews on the phenotype of thyroid hormone receptor mutant mice see (Forrest and Vennstrom, 2000; O'Shea and Williams, 2002; Flamant and Samarut, 2003; Bernal, 2007). According to the current model that was put forward to explain these discrepancies the structural defects associated with hypothyroidism are primarily caused by unliganded receptors (particularly TRalpha1) that exert aporeceptor activity and thereby repress a program of differentiation. According to this model thyroid hormone would release the brake by terminating aporeceptor activity, and thyroid hormone deficiency would not affect brain development in the absence of thyroid hormone receptors.

This hypothesis has been supported by several studies. For instance, Bernal and co-workers could show that the cerebellar development in TRalpha1 null mice is not altered even when the animals are rendered hypothyroid (Morte et al., 2002). Moreover, in primary cerebellar cultures, dendritic development of Purkinje cells that do not express TRalpha1 was equally advanced independent of the presence of thyroid hormone whereas Purkinje cells of wildtype animals exhibit only poor dendritic growth if thyroid hormone is not added to the medium (Heuer and Mason, 2003). A more complex picture emerged with the analysis of mouse mutants expressing dominant negative thyroid hormone receptors due to mutations in the T3 binding domain (TRbeta delta337T; TRalpha1R384C). While TRbeta delta337T mutant mice showed an impaired cerebellar Purkinje cell development (Hashimoto et al.,

2001) Purkinje cell and granule development was only slightly retarded in TRalpha1R384C mutant mice (Venero et al., 2005). However, in none of these mutant animals can the individual functions of thyroid hormone receptors in a given cell type be assessed since implementation of the mutant form always occurred ubiquitously. Only recently has the generation of a mouse mutant been reported that allows the expression of a mutated receptor form in a cell-specific manner. These mice mutants carry a floxed point mutation in the activation domain of TRalpha1 (TRalpha1L400R) that is only active after a Cre-induced recombination event (Flamant and Quignodon, 2009; Quignodon et al., 2007b). This mutation favours the permanent recruitment of corepressors by interfering the interaction with histone acetyl transferase. Therefore, such a mutated receptor form may act in a dominant negative manner. Indeed, inducing the early expression of this mutated receptor results in a phenotype that recapitulates many aspects of congenital hypothyroidism excluding those effects that are due to a repressed activation of thyroid hormone receptor beta forms (Quignodon et al., 2007b). Noteworthy is the fact that the cerebellar granule cell differentiation pattern resembles that of hypothyroid animals whereas Purkinje cell dendritogenesis is only temporarily delayed suggesting that the developmental increase in TRbeta expression in Purkinje cells might counterbalance to a certain degree the repressive mode of TRalpha1. Since this mouse model will allow a Purkinje, granule or glia cell-specific expression of the dominant TRalpha1 mutant form dependent on the Cre line used, it appears to be an attractive tool to finally dissect cell-autonomous versus indirect effects of thyroid hormones during cerebellar development.

8. Molecular targets of thyroid receptors

Several attempts to identify target genes that are directly regulated by thyroid hormone during brain development have been made with limited success (Dong et al., 2005; Quignodon et al., 2007a; Diez et al., 2008; Royland et al., 2008). As one obstacle, the (G/A)GGT(C/G)A consensus sequence of the TRE that usually contains this motif as two half sites arrayed as either direct repeats spaced by four nucleotides, inverted repeats spaced by six nucleotides or as palindromes (reviewed in Yen, 2001) is quite frequent, and bioinformatic approaches usually will reveal a large number of them. Moreover, TRE's that have been well-characterized in functional studies are often far away from the transcriptional start site and may even be localized in intronic sequences. In a recent study on thyroid hormone receptor beta binding sites and target genes using a ChIP-on-Chip approach in the developing mouse cerebellum (Dong et al., 2009) almost 50% of the identified TR bindings sites do not even represent any of the classical TRE forms. Finally, even genes that contain a well-characterized TRE, may be regulated by thyroid hormone only in a temporally and spatially very restricted manner. For instance, the expression of RC3/neurogranin, a neuron-specific calmodulin binding protein which is assumed to be important for synaptic plasticity and spatial learning, is controlled by thyroid hormone only in the striatum but not in the cerebral cortex (Iniguez et al., 1996) indicating the presence of other still unknown regulatory mechanisms that are involved in thyroid hormone induced gene expression.

One possibility by which thyroid hormone could exert its plethora of action during brain development is by controlling the expression of transcription factors that in turn regulate a specific set of genes important for neuronal differentiation. Candidates for such a function are BTEB (Denver and Williamson, 2009), hairless (hr) (Potter et al., 2002), NeuroD (Chantoux and Francon, 2002), RORalpha (Koibuchi and Chin, 1998) and NGFI-A (Egr1) (Mellstrom et al., 1994). Moreover, recent approaches to identify further thyroid hormone regulated gene products in the brain using microarray

analysis revealed additional T3 target genes (Poguet et al., 2003; Dong et al., 2005; Quignodon et al., 2007a; Diez et al., 2008). However, their exact function during neuronal differentiation still needs to be established.

9. Rapid effects of thyroid hormone

In addition to an activation of nuclear thyroid hormone receptors there are also other mechanisms known by which thyroid hormone can influence brain function. Thyroid hormone receptors were found not only to act in the nucleus but also to be localized in the cytosol where they can interact with p85, the regulatory subunit of phosphatidyl inositol 3-kinase (Moeller et al., 2006). T3 induced interaction of cytosolic thyroid hormone receptors with PI3 kinases was found in central endothelial cells in response to middle cerebral artery occlusion. This activation results via the Akt-pathway in a rapid induction of NO synthesis and a reduction in cerebral infarct size (Hiroi et al., 2006).

Very rapid effects are also exerted by iodothyronines that do not bind to thyroid hormone receptors. Preferentially, T4 can bind to the membrane receptor formed by the integrin aVb3 which then transduces the hormonal signal to the mitogen-activated protein kinase (MAPK) signaling cascade. As a consequence of this membrane action of thyroid hormone other transcription factors are phosphorylated and changes in gene expression occur (Davis et al., 2005). As another way of action, T4 and its metabolite reverse T3 (but not T3) profoundly stimulate actin polymerization in cultured astrocytes and cerebellar granule cells thereby promoting neuronal outgrowth (Farwell et al., 2005; Leonard, 2008). Whether even the recently identified thyronamine (ToAM) and 3-iodothyronamine (T1AM) which represent decarboxylated derivatives of thyroid hormone may act during brain development still needs to be investigated (Scanlan, 2009).

10. Future perspectives

Despite intensive studies on the role of thyroid hormones for proper brain development there are still many open questions remaining. To better understand thyroid hormone action a combined effort is needed not only to identify the molecular targets of thyroid hormone but also to unravel the reasons why specific genes respond to thyroid hormone only in a temporally and spatially restricted manner. More information has to be obtained as to the contribution of non-genomic action of thyroid hormone and its newly discovered derivatives. Finally, the contribution of different thyroid hormone transporters for thyroid hormone function in the CNS has to be assessed in greater detail by developing conditional knockout models. As a consequence, much work remains before a unifying hypothesis addressing all aspects of thyroid hormone-dependent effects on brain development can be formulated.

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