REVIEW

Thyroid hormone regulators in human cerebral cortex development

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Abstract

Brain development is critically dependent on the timely supply of thyroid hormones. The thyroid hormone transporters are central to the action of thyroid hormones in the brain, facilitating their passage through the blood–brain barrier. Mutations of the monocarboxylate transporter 8 (MCT8) cause the Allan–Herndon–Dudley syndrome, with altered thyroid hormone concentrations in the blood and profound neurological impairment and intellectual deficit. Mouse disease models have revealed interplay between transport, deiodination, and availability of T3 to receptors in specific cells. However, the mouse models are not satisfactory, given the fundamental differences between the mouse and human brains. The goal of the present work is to review human neocortex development in the context of thyroid pathophysiology. Recent developments in single-cell transcriptomic approaches aimed at the human brain make it possible to profile the expression of thyroid hormone regulators in single-cell RNA-Seq datasets of the developing human neocortex. The data provide novel insights into the specific cellular expression of thyroid hormone transporters, deiodinases, and receptors.

Introduction

Thyroid hormones are crucial for brain development, acting through nuclear receptors for T3 to control gene expression (Brent 2012, Mendoza & Hollenberg 2017). The amount of T3 reaching the nucleus of target cells depends primarily on cell membrane transporters and T4 deiodination in tissues. The transporters facilitate the cellular influx and efflux of T4 and T3 (Bernal et al. 2015). The iodothyronine deiodinases type 1 (DIO1) and 2 (DIO2) produce T3 from the precursor T4, whereas DIO3 inactivates T4 and T3 (Hernandez et al. 2021). These pathways are developmentally regulated (Gereben et al. 2008), and the timing and cell type-specific expression are essential clues to understanding the action of thyroid hormones during development. In this paper, we will analyze the development of the human neocortex in the context of thyroid pathophysiology and review recent work on the expression of thyroid hormone transporters, deiodinases, and receptors through transcriptomic profiling using single-cell RNA-Seq datasets (Diez et al. 2021). This paper summarizes a presentation given at the 24th European Congress of Endocrinology, Milan, May 22–24, 2022.

The interest of the study

Although rodents have been very useful to study cerebral development, profound differences exist in cerebral architecture between rodents and primates, including
humans (Bystron et al. 2008, Defelipe 2011). In many cases, rodent models cannot offer a satisfactory explanation to understand the mechanisms of disease in humans. A relevant example is the Allan–Herndon–Dudley syndrome (Krude et al. 2020). This syndrome consists of profound neurological impairment, intellectual deficit, and altered blood thyroid hormone profile. It is caused by mutations of the monocarboxylate transporter 8 (MCT8), a specific membrane transporter for thyroid hormones. Similar mutations in mice replicate the abnormal thyroid hormone profile but do not cause neurological alterations (Wirth et al. 2009). Therefore, it is essential to understand the mechanisms involved in thyroid hormone action in the human brain, especially those that might be unique to humans.

A simplified overview of human cerebral cortex neurogenesis

The production of neurons, or neurogenesis, in the developing human brain runs at an average of 250,000 new cells per minute to achieve the more than 100 billion neurons of the newborn baby (Ackerman 1992). Neurogenesis in the developing cerebral cortex extends from about gestational week 5 (GW5) to GW25 (Fig. 1). During this period, the neurons are formed from precursors and then migrate to specific locations to build a six-layered structure (Bystron et al. 2008, Rakic 2009, Lui et al. 2011).

At the beginning of neurogenesis, the neuroepithelial cells of the neural tube give rise to the radial glial cells, which express glial markers (Pollen et al. 2015). The radial glial cells are highly polarized and extend to two processes: an apical process, anchored to the ventricular surface, and a basal process, reaching the pial surface. These cells are also known as ventricular radial glia. The radial glial cells are the universal stem cells of the cortex (Kriegstein & Alvarez-Buylla 2009). They first undergo rounds of symmetric divisions, expanding the progenitor pool. This is followed by rounds of asymmetric divisions, generating another radial glial cell and one neuron (direct neurogenesis).

The first neurons generated from the radial glial cells are the Cajal–Retzius cells and the subplate cells (Toma & Hanashima 2015). The accumulation of these and other cells forms a temporary structure known as the preplate. New rounds of asymmetric division, involving the generation of intermediate progenitors (indirect neurogenesis), produce the excitatory projection neurons. These neurons migrate along the shaft of the basal process of the radial glia, pass the subplate layer, and are positioned between the Cajal–Retzius cells and the subplate cells (Faux et al. 2012). This process is called preplate splitting and marks the origin of the cortical plate by GW8. The new neurons arriving in the cortical plate migrate to the pial surface displacing back the neurons that arrived earlier, until they reach the proximity of the Cajal–Retzius cells. These cells secrete a thyroid hormone-regulated extracellular matrix glycoprotein (Alvarez-Dolado et al. 1999), reelin (RELN), a stop signal for neuron migration (Jossin 2020). The whole process is known as ‘inside-out radial migration’, by which the neurons are sequentially positioned in layers depending on their date of birth, such that the early-born neurons occupy the deep layers of the cortex (Bystron et al. 2008).

By about GW15, some radial glia loses the apical process and accumulate in the outer part of the subventricular zone. These cells are called basal, or outer radial glia (oRG), and differentiate into neurons of the upper layers. The number of oRG in primates, unlike rodents, is very high, accounting for an enlarged outer subventricular zone (Namba et al. 2021). The radial glia remaining attached to the ventricular surface lacking the basal processes are called truncated radial glia and contribute to gliogenesis.

The inhibitory, GABAergic interneurons are formed in the ganglionic eminences, also from radial glial precursors, and migrate to the cortical plate first by tangential migration through the intermediate zone and the marginal zone, and then by radial migration following the path of radial glial processes (Lim et al. 2018). Among the first interneurons
arriving in the developing cortex are the calretinin (CR), or CALB2-expressing interneurons.

Neurogenesis ends by about GW25 and is followed by gliogenesis. The radial glia is also the precursor of astrocytes and oligodendrocytes. The analysis of gliogenesis is out of the scope of the present review.

**Correlation between thyroid function and cortical development**

Figure 2 shows how the major steps of the developing cortex correlate with relevant parameters of thyroid function. The first important question is the age at which the fetal thyroid gland becomes functional. Embryonic development of the human fetal thyroid gland is complete by GW11 (Shepard & Stapp 1967), and some thyroid-specific genes, notably the TSH receptor, are expressed before GW11 (Szinnai et al. 2007). Around GW11 colloid formation starts, NIS, the sodium–iodide symporter, is strongly upregulated, and iodide concentration and synthesis of thyroglobulin and T4 take place (Szinnai et al. 2007). Serum total and free T4 increase with time, reaching maternal concentrations by GW36 (Thorpe-Beeston et al. 1991). T3 is mostly undetectable until midgestation and below adult levels at birth. T4 from the mother is detectable in the coelomic fluid from GW5–6 and the amniotic fluid contains T4 and T3 from GW11–12 (Contempré et al. 1993).

Taken together, the data show that the thyroid gland is already functional by GW11–12 (Dom et al. 2021), shortly after the preplate splitting and the onset of cortical plate formation.

T4 and T3 increase in the developing cortex at least from GW13 (Kester et al. 2004). In the choroid plexus, there is also a steep increase in T4, but, in contrast to the cortex, T3 remains low, in agreement with its very low serum levels. Similar to what happens in serum, T3 is also undetectable in other organs (Bernal & Pekonen 1984). This indicates that all of the T3 found in the cortex is formed locally from T4 deiodination. In support of this,
DIO2 activity was detected at GW13, the earliest time measured (Kester et al. 2004). The T3 receptor protein, measured by T3-binding assays, is present in the brain at low concentrations already by GW10 (Bernal & Pekonen 1984), and the receptor-encoding mRNA can be detected earlier (Iskaros et al. 2000).

Taken together, these data indicate that thyroid hormones may influence brain development in general during an early period of cortical development, at least shortly after the preplate splitting and the formation of the cortical plate, at the end of the first trimester of gestation.

Pathophysiological correlates

Pathophysiological correlates indicate that the first half of gestation, and especially the second trimester of pregnancy, is critical for the effects of thyroid hormones on brain development. Maternal thyroid hormones, especially T4, cross the placenta and may be critical in the presence of fetal thyroid failure (Vulsma et al. 1989, Morreale de Escobar et al. 2000). As it is well known, maternal thyroid hormones protect the developing brain in cases of congenital hypothyroidism, preventing the neurological impairment observed in neurological cretinism. Years ago, the neurological damage of endemic cretinism was defined as a striatopallidal syndrome due to brain damage during the second trimester, in agreement with the events described above (DeLong et al. 1985).

The Allan–Herndon–Dudley syndrome is caused by the deficient transport of thyroid hormones to the brain (Bernal et al. 2015, Groeneweg et al. 2020, Krude et al. 2020). It is due to mutations of the main thyroid hormone transporter, the MCT8, encoded by the SLC16A2 gene. MCT8 mutations cause cerebral hypothyroidism due to deficient transfer of thyroid hormones through the blood–brain barrier. This is accompanied by peripheral hyperthyroidism due to elevated T3 in serum. The patients usually suffer severe neuromotor impairment and cognitive disabilities. Although the syndrome may be clinically detected as hypotonia with a lack of head control during the first months of postnatal life, the median age at diagnosis is 24 months (Groeneweg et al. 2020). Pathology studies show that the brain lesions that might be responsible, at least in part of these symptoms, are compatible with cerebral hypothyroidism (Lopez-Espindola et al. 2014). These include delayed development of the cerebral cortex and cerebellum, cortical atrophy, reduced number of Cajal–Retzius cells and PV interneurons, altered synaptogenesis, abnormal Purkinje cell differentiation, and delayed myelination. Some of these lesions are already present at GW30. It is likely that these, or other unidentified lesions, are present much before GW30 since MCT8 is present in the brain already by GW14 (Lopez-Espindola et al. 2019).

The role of thyroid hormone regulators: a model of thyroid hormone action in the brain

Understanding how thyroid hormones influence neurodevelopment requires the previous identification of the specific cells expressing the regulators and mediators of thyroid hormone action, namely the
thyroid hormone transporters, the iodothyronine deiodinases, and the thyroid hormone nuclear receptors. An integrated model of thyroid hormone transport, metabolism, and action is described in Fig. 3, which applies primarily to the rodent brain.

The MCT8 protein facilitates the transmembrane influx and efflux of T3 and T4 and is present in the blood–brain barrier and the membranes of neural cells. MCT8 has a prominent role in the blood–brain barrier (Ceballos et al. 2009, Vatine et al. 2017, Lopez-Espindola et al. 2019). Its role in the neural cell membranes is still under debate (Mayerl et al. 2020, 2022). T3 in the brain derives in part from the circulation and part from local T4 deiodination by DIO2, which in rodents is expressed in glial cells such as astrocytes and third ventricle tanycytes (Guadañho-Ferraz et al. 1997, Tu et al. 1997). Strikingly, in the rat fetus, practically all of the T3 in the brain derives from T4, but the reason why circulating T3 does not reach the brain parenchyma in the fetus is unknown (Grijota-Martínez et al. 2021). In human fetuses, MCT8 is present in the astrocyte end-feet in close contact with the endothelial cells (Lopez-Espindola et al. 2019). It is possible, but not demonstrated so far, that MCT8 facilitates the direct entry of T4 into the astrocytes.

As pointed out above, MCT8 mutations cause a syndrome of neuromotor impairment and profound cognitive deficits with altered thyroid hormone concentrations in the blood, known as the Allan–Herndon–Dudley syndrome. Despite its critical role in the blood–brain barrier (BBB), disruption of the Mct8 gene in mice does not result in neurological impairment. However, it produces similar changes in thyroid hormone concentrations in the blood as in the patients. The Mct8 knockout mouse have also minimal changes in brain gene expression (Morte et al. 2010). The reason for the differences between humans and mice in this regard is that mice express a T4 transporter in the BBB, which is not present in the human BBB (Roberts et al. 2008, Ito et al. 2011). This T4 transporter is the organic anion transporter polypeptide 1c1, or OATP1C1 (mouse gene Slco1c1), and transports T4 but not T3 (Pizzagalli et al. 2002). In MCT8-deficient mice, T4 entry into the brain still takes place and results in the production of endogenous T3, preventing cerebral hypothyroidism (Ceballos et al. 2009, Morte et al. 2010). This process is facilitated by the increased DIO2 activity that occurs in Mct8-deficient mice (Dumitrescu et al. 2006, Trajkovic et al. 2007). A situation similar to human MCT8 deficiency is achieved in mice by combined MCT8 and OATP1C1 deficiency, in Slc16a2/Slco1c1 double KO mice (Mayerl et al. 2014). Therefore, the lack of OATP1C1 in the BBB is a major difference in thyroid hormone metabolism between humans and mice. Consequently, the current model of thyroid hormone transport and metabolism, based on studies in rodents, does not entirely apply to humans.

### Thyroid hormone regulators in the developing human cerebral cortex

Very little information exists regarding the specific expression of the thyroid hormone regulators in the human brain during development. Previously, our laboratory analyzed the expression of the thyroid hormone transporters MCT8 and OATP1C1 and DIO2 and DIO3 in slices of human fetal brains using immunohistochemistry (Lopez-Espindola et al. 2019). These regulators were present in radial glia, Cajal–Retzius cells, and cerebrospinal fluid–brain barriers. No information exists on the cellular localization of the receptors.

Thanks to recent advances in transcriptomic analysis using single-cell RNA-Seq approaches (Ziegenhain et al. 2017) to the developing human brain, it is possible to search for the expression of genes of interest in the datasets generated by these procedures. We analyzed five single-cell RNA-Seq datasets from the human fetal cerebral cortex to look for specific expressions of thyroid regulators (Pollen et al. 2015, Nowakowski et al. 2017, Zhong et al. 2018, Polioudakis et al. 2019, Shi et al. 2021). Results from these different datasets gave similar results (Diez et al. 2021). For this reason, only two of them will be reviewed here (Pollen et al. 2015, Polioudakis et al. 2019).

In all cases, we used the original raw data and followed similar pipelines (Diez et al. 2021). The first step was the normalization and identification of the topmost highly variable genes. Then, the expression of these genes was scaled to perform principal component analysis. The top principal components were then used to calculate a uniform manifold approximation and projection (UMAP) map, in which the cells are projected into a 2D scatterplot useful for visualization. We used the same components to cluster the cells into subpopulations. Marker genes in each cluster and the original publication labels were used to determine the cell identities. The UMAP plot was used to visualize the expression of specific genes.

The first dataset was derived from 393 cells microdissected from the ventricular and subventricular zones of fetuses of gestational weeks 16–18 (Pollen et al. 2015). We obtained four clusters of cells: excitatory neurons, interneurons, glia, and proliferating cells (Fig. 4). We then explored the expression of thyroid regulators among these clusters, as shown in the violin plots in Fig..
Cluster 2 corresponds to radial glial cells. DIO2 and SLCO1C1 (OATP1C1) were expressed mainly in this cluster. Sixty percent of deiodinase-expressing cells were identified as outer radial glia and coexpressed the T4 transporter SLCO1C1. The thyroid hormone receptor alpha (THRA) and the MCT8 transporter (SLC16A2) were expressed in cluster 1, identified as excitatory neurons. In contrast, and unexpectedly, the thyroid hormone receptor beta (THRB), was present in cluster 3, corresponding to interneurons.

The second dataset contained information from 40,000 cells isolated from the whole cortex by the drop-seq technique at gestational weeks 17–18 (Polioudakis et al. 2019). In agreement with the previous dataset, DIO2 and SLCO1C1 were expressed in the cluster corresponding to the outer radial glia (Fig. 5). THRA was widely expressed, whereas THRB was specific to a cluster that in the UMAP showed the presence of interneurons from the caudal ganglionic eminence (CGE) and medial ganglionic eminence (MGE). The CGE and MGE can be differentiated by the expression of specific neuropeptides. The MGE expresses somatostatin (SST), whereas the CGE expresses CR (CR/CALB2). THRB was present mainly in the CGE interneurons, expressing CALB2, although it was also present in cells from the MGE. Correlation plots between THRB and CALB2 confirmed the cellular coexpression of both genes in 252 interneurons from the CGE. THRB and SST were coexpressed in 80 interneurons from the MGE.

Since THRA was also expressed in the ganglionic eminences, we also performed correlation plots between THRB and THRA to quantify the proportion of cells expressing both receptors, which accounted for less than 10% of the total number of receptor-expressing cells. This result indicated that THRB performs specific functions in CALB2 interneurons probably different from the more general actions that might be performed by THRA in most neuronal types (Wallis et al. 2010).

CALB2/CR interneurons are among the first interneurons arriving in the cortex, around the time of preplate splitting (Yu et al. 2021). An expansion of GABAergic interneurons occurs during cortical evolution. In rodents, GABAergic interneurons are about 15% of the total neuron population, increasing in proportion to more than 20% in primates. This increase is mainly due to the CALB2/CR interneurons, which in primates...
Figure 5
Profiling of thyroid hormone regulators in the single-cell RNA-Seq dataset by Polioudakis et al. (2019). Upper panel: uniform manifold approximation and projection (UMAP) showing the colored clusters of cells projected in a 2D scatterplot. Only clusters relevant for this discussion are identified: Div, mitotic cells; Endo, endothelial cells; IP, intermediate progenitors; InCGE, interneurons from the caudal ganglionic eminence; InMGE, interneurons from the medial ganglionic eminence; Mg, microglia; Neu, excitatory neurons; OPC, oligodendrocyte precursor cells; oRG, outer radial glia; Per, pericytes; vRG, ventricular radial glia. Blue dots in the rest of the scatterplots represent individual cells expressing the gene of interest: SLC16A2 (MCT8), SLCO1C1 (OATP1C1), THRB (thyroid hormone receptor beta), THRA (thyroid hormone receptor alpha), DIO2 (type-2 deiodinase), CALB2 (calretinin), SST (somatostatin). Lower panel: Violin plots of interneuron markers (DLX5 and CALB2), THRB, and SCGN (secretagogin). DLX5 is a marker of interneurons from the InCGE and InMGE, and CALB2 and SCGN are specific for the CGE. THRB is expressed mainly in interneurons from the CGE but also from the MGE.
account for 35–40% of all GABAergic interneurons (Dzaja et al. 2014). A recent study in humans showed that CALB2 interneurons often express secretagogin (SCGN), whereas, in rodents, very few cells express both genes (Shi et al. 2021). Accordingly, we also found that about 20% of CGE interneurons coexpress THRB, CALB2, and SCGN (Fig. 5, lower panel).

Conclusions

The profiling of thyroid hormone regulators and receptors in several transcriptomic datasets of the developing human cerebral cortex led us to two main novel observations. First, we observed that DIO2 and SLCO1C1, the genes encoding DIO2 and the T4 transporter OATP1C1, respectively, are coexpressed in a subset of the outer radial glial cells. We propose that the outer radial glia is the main site of T3 formation in the cerebral cortex at midgestation. The DIO2 substrate, T4, may reach these cells through the blood vessels and/or through the choroid plexuses since, as shown in Fig. 2, this structure presents a rapid accumulation of T4 from the 13th to the 18th week of gestation (Kester et al. 2004). In results obtained from another dataset not described here (Nowakowski et al. 2017, Diez et al. 2021), the astrocytes also coexpressed DIO2 and SLCO1C1 after GW25, as observed earlier in the postnatal rat cortex (Guadano-Ferraz et al. 1997, Guadano-Ferraz et al. 1999).

The significance of the possible T3 generation in the outer radial glia cannot be appreciated. As pointed out earlier, the outer radial glia is the universal stem cell of the cortex. T3 may be secreted from a subset of these cells and act in a paracrine fashion on nearby cells in a different metabolic state. At present, we can only speculate on possible actions in the human brain from the effects of thyroid hormones in rodents. For example, T3 may act on neuronal precursors derived from a different population of outer radial glia to promote full neuronal differentiation just before migration. Alternatively, T3 may facilitate neuronal migration. Unfortunately, the scarcity of data from the developing human brain prevents answering these questions. THRA, encoding the THRA1 receptor, is expressed in neurons shortly after the last mitosis of neuronal precursors (Wallis et al. 2010), suggesting that T3 might act from this very initial stage of neuronal life. The significance of the outer radial glia for the expansion of the neocortex in primates (Namba et al. 2021) suggests that one evolutionary function of the thyroid hormones is to facilitate the expansion of the neocortex through these mechanisms.

Secondly, we observed that CGE- and MGE-derived CALB2/CR, and SST interneurons, respectively, express in a very selective fashion the THRB gene, encoding the thyroid hormone receptor beta isoforms. We do not know what of the two isoforms encoded by THRB, THRB1 or THRB2, is expressed in interneurons. The interneurons comprise an extremely diverse population of different cells, which may be classified using criteria based on morphology, expression of certain markers, electrophysiological properties, and transcriptomic profile (Tremblay et al. 2016, Lim et al. 2018). A classical classification is based on the expression of Ca2+-binding proteins and distinguished into three main types: the parvalbumin (PV) interneurons, mainly basket and chandelier cells present in layers IV and V; the calbindin (CB) interneurons, consisting of CB-bipolar, CB-multipolar, and CB-chandelier cells, present in layers II–IV; and the CR interneurons, the CR-double bouquet cells, and the CR-bipolar cells (Yanez et al. 2005). These cells are present in associative layers II and III. In particular, the CR-double bouquet cells present vertically bundled axons resembling ‘horse tails’, are present in the human cortex, and are absent from the rodent cortex (Yanez et al. 2005). At present, we do not know whether THRB is selectively present in any of these two classes of cells. One important goal of future studies is the precise identification of the THRB-expressing interneurons and their thyroid hormone-dependent role.

CALB2 interneurons in rodents account for about 16–18% of all GABAergic interneurons, but they increase to nearly 40% of all GABAergic interneurons in primates, in addition to a general increase in all classes of GABAergic interneurons (Dzaja et al. 2014). It is thought that the increased interneuron population is related to the increased associative functions and connectivity of the primate cortex. We further found that 20% of CALB2-expressing, CGE interneurons coexpress THRB and SCGN/secretagogin, a gene that in rodents is expressed only in a few CALB2 interneurons (Shi et al. 2021). The selective expression of THRB in CALB2 and SCGN interneurons suggests unique actions of thyroid hormones in this subset of interneurons, with possible evolutionary implications.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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