THYROID AND BRAIN:

UNDERSTANDING THE ACTIONS OF THYROID HORMONES IN BRAIN DEVELOPMENT AND FUNCTION

Juan Bernal

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Thyroid and Brain: Understanding the Actions of Thyroid Hormones in Brain Development and Function

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FOREWORD

Thyroid hormones, small iodine-containing molecules, play a key role in the growth, development, and metabolism of mammals and the metamorphosis of amphibians. The demand for iodine in hormone synthesis imposed additional stress on creatures transitioning from sea to land. Despite efforts to implement iodine supplementation, 35% of the world population remains iodine deficient today, with 35 million severely affected individuals. A significant proportion exhibits enlarged thyroid glands (goiter) and experiences growth and mental retardation. Furthermore, thyroid hormone deficiency affects one in every 2,500 newborns in iodine-sufficient areas. Timely correction of thyroid hormone deficiency induces catch-up growth, while delays in treatment result in irreversible defects in brain development. This has led to the global implementation of routine newborn screening for early detection of congenital hypothyroidism.

The book titled "Thyroid and Brain: Understanding the Actions of Thyroid Hormones in Brain Development and Function", is authored by a preeminent expert who has devoted his entire career to the study of thyroid hormone action in the brain, thus gaining the respect of his peers for his extensive knowledge of the field. The book covers in-depth brain development, structure, and function as related to the thyroid. It provides timely, up-to-date information that will serve as a valuable resource to both specialists and non-experts. The intricate actions of these hormones are lucidly described, creating a narrative that reads almost like a novel, with each fact leading to new information, expanding the horizons of current knowledge.

The fourteen chapters delve into thyroid hormone transport, metabolism, and action as they pertain to the brain, encompassing the spectrum of thyroid hormone effects in health and disease. The book provides detailed descriptions of molecules regulating thyroid hormone action on the brain, including gene expression, properties, biochemistry, localization, and physiology. Recent advances in genetics and molecular biology are presented. While central regulation of thyroid hormone secretion involving the hypothalamus and pituitary is important, *in situ* thyroid hormone metabolism provides appropriate local hormone bioactivity. It ensures optimal concentrations of the biologically active thyroid hormone, T3, to brain cells as required for the particular period of development. These mechanisms regulate thyroid hormone action with a timeline specific to different brain regions. Although the book focuses on the human brain, it presents animal studies, particularly in rodents, as vital for expanding knowledge on the subject. This research reveals that gene dependence of T3 is age and brain region-dependent, with diverse time window sensitivity. T3 regulates genes involved in nearly all aspects of brain function, from developmental genes to genes involved in metabolic and cell signaling pathways. Finally, the author includes a historical overview of each topic, giving due credit for important key discoveries.

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PREFACE

Thyroid hormones play a pivotal role in orchestrating a myriad of biological and physiological functions. These encompass the regulation of vertebrate growth and development, amphibian metamorphosis, circadian rhythms, photoperiod, metabolism, body temperature, heart rate, and crucially, brain development and function. The mechanism through which these effects are predominantly mediated involves T3 binding to nuclear receptors, serving as ligand-regulated transcription factors.

Among the myriad actions of thyroid hormones, one of paramount importance is their profound influence on brain development and function. Thyroid hormones intricately regulate processes such as neurogenesis and gliogenesis and the migration, differentiation, and synaptogenesis of neuronal and glial cells. Notably, a deficiency in thyroid hormones during development, or disruption of the mechanisms of thyroid hormone action, results in severe neurological impairment and intellectual retardation. In the adult human brain, thyroid disorders may manifest as psychiatric disturbances. The intricate interplay of thyroid hormones in these processes underscores their indispensable role in maintaining optimal neurological function throughout the lifespan.

This book represents a comprehensive endeavor to distill the wealth of information accumulated over the past five decades into a single volume. The field has witnessed a remarkable acceleration, propelled by groundbreaking advances in cellular and molecular neurobiology, facilitated by breakthroughs in molecular genetics and transcriptomics methodologies. Though many questions remain unanswered, the convergence of these advancements has ushered in a new era of understanding, providing a solid foundation for the exploration and elucidation of intricate neurological processes and, in particular, the action of thyroid hormones in the brain.

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ACKNOWLEDGEMENTS

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DEDICATION

To my beloved grandchildren,

Adrian, Emma, and Marcos

May this book inspire you to explore, learn, and chase your dreams. Your curiosity and joy for life are a constant source of wonder and pride. This work is a testament to the power of knowledge, and I hope it encourages you to seek out the mysteries of the world with the same passion and dedication.

With all my love,

Juan Bernal

CHAPTER 1

An Introduction to Thyroid Physiology

Abstract: This chapter is a general introduction to this book and contains basic concepts of thyroid hormone signaling for a better understanding of the book's subject. It begins with an introduction that offers a simplified view of thyroid hormones as iodine-containing compounds and the regulatory function of the hypothalamus-pituitary-thyroid axis, followed by a description of the thyroid gland and thyroid hormone synthesis. Iodide transporters concentrate iodide in the gland and after oxidation, it is incorporated into thyroglobulin tyrosyl residues. The coupling of iodotyrosyl residues forms T4 and T3, which are released after thyroglobulin hydrolysis. Thyroid hormones act *via* nuclear receptors, which are ligand-regulated transcription factors, and T3 is the primary active thyroid hormone that binds to the receptors. T3 is produced primarily in extrathyroidal tissues by the action of deiodinase enzymes catalyzing the removal of an iodine atom from T4. Thyroid hormones are ancient signaling molecules with critical actions on growth and metabolism that regulate many developmental transitions, with evolutionary roots at the base of the chordate species.

Keywords: Amphioxus, Colloid, DUOX2, Dehalogenase, Deiodinases, DIO, Evolution, Endostyle, Follicles, Genomic actions, Iodine, lamprey, Metamorphosis, Nuclear receptors, Non-genomic, NIS, Peroxidase, Selenium, Thyroglobulin.

INTRODUCTION

The thyroid gland is a butterfly-shaped endocrine gland located in the neck that regulates the body's growth and metabolism. One of the thyroid gland's unique features is its large amount of iodine, which is around 15 mg for an adult gland weighing 14-18 g [1]. The German chemist Eugen Bauman was the first to measure iodine in the thyroid at the end of the 19th century and was astounded by the result because such amounts of iodine are highly toxic. He proposed that the gland produced a biologically active iodinated compound and named it iodothyrin.

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The thyroid gland produces two iodinated hormones, thyroxine (T4, 3,5,3',5'-tetra-iodo-L-thyronine) and triiodothyronine (T3, 3,5,3'-triiodo-L-thyronine) (Fig. **1**). Kendall isolated and crystalized thyroxine in 1914 [2], and Gross and Pitt-Rivers [3], and Roche *et al.* [4] discovered triiodothyronine in 1952 [5, 6]. Iodine accounts for 65% of the T4 molecular weight and 59% of that of T3. The non-iodinated nucleus of T4 and T3 is called thyronine and consists of two phenolic rings and an alanine side chain. Most of the actions of thyroid hormones are due to T3 acting through the cell nucleus, and T4 is primarily a T3 precursor but also has some intrinsic actions.

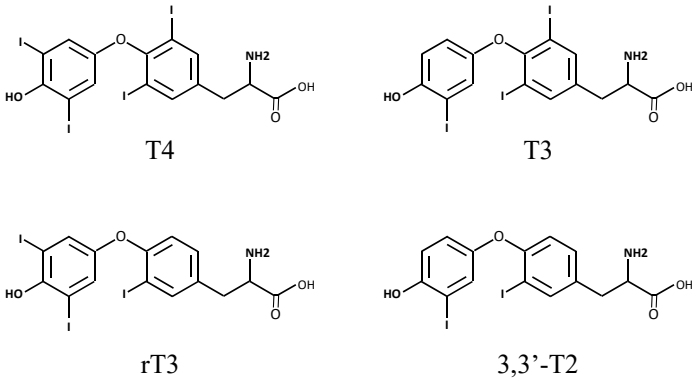

Fig. (1). The thyroid hormones, T4 (thyroxine, 3,5,3'5'-tetraiodo-L-thyronine) and T3 (3,5,3'-triiodo-L-thyronine) and their deiodination products rT3 (reverse T3, 3,5,5'-triiodo-L-thyronine) and 3,3'-T2 (3,3'-diiodo-L-thyronine).

The production and secretion of thyroid hormones are controlled by thyroid-stimulating hormone (TSH), a protein with two subunits, α and β, produced and secreted by the anterior pituitary gland, or adenohypophysis. TSH is under positive control by a neuropeptide, the thyrotropin-releasing hormone (TRH), primarily produced in the paraventricular nucleus (PNV) of the hypothalamus. PNV neurons discharge TRH into the median eminence and reach the TSH-producing pituitary cells by the portal vessels. The TRH-TSH-thyroid axis forms a negative feedback loop whereby thyroid hormones modulate thyroid stimulation by TSH by inhibiting TRH and TSH secretion. When the levels of T4 and T3 in the blood are low, the pituitary gland releases more TSH, which signals the thyroid gland to produce and secrete more hormones. The concept of the homeostatic "set point" pertains to the optimal range in TSH and free T4 concentrations tailored to each individual, a range more narrow than the reference range for the general population [7].

This chapter offers a general overview of the synthesis and secretion, blood transport and metabolism, and mechanism of action of thyroid hormones.

THE UNIQUENESS OF THE THYROID GLAND

Several characteristics make the thyroid gland unique among the body's organs: its embryological origin; its structure, which is organized in follicles; the storage of its secretion outside the producing cells resembling more an exocrine than an endocrine gland; its functional dependence on two micronutrients, iodine and selenium; the specific expression of a large protein, thyroglobulin; and the biochemical mechanisms of thyroid hormone synthesis.

Embryology

Embryologically, the thyroid gland originates from an invagination of the pharyngeal floor of a group of cells expressing specific genes, descending to their final position and adopting several morphologies depending on the specie [8].

Key genes in the evolutionary mechanisms of thyroid specification are fibroblast growth factor 2 (*FGF2*) and bone morphogenetic protein 4 (*BMP4*), probably derived from the cardiac mesoderm [9]. Three key thyroid transcription factors are *NKX2.1* (thyroid transcription factor 1, *TTF1*), *FOXE1* (thyroid transcription factor 2, *TTF2*), and the paired box 8 (*PAX8*) [10, 11] with expression starting by gestation week five (GW5) in humans [12]. Transient expression of these genes is sufficient to differentiate mouse embryonic stem cells into thyroid follicles in the presence of TSH [13, 14].

The Thyroid Follicles

The follicle is a key and unique structure adopted by the thyroid cells, which form a monolayered epithelium around a central cavity filled with the so-called colloid, almost entirely thyroglobulin. In addition to these structures where the thyroid hormones are produced, the gland contains other cells dispersed among the follicles, the parafollicular or C cells producing calcitonin, a hypocalcemic hormone that antagonizes parathyroid hormone.

The follicles first appear in humans by GW10-11 [15]. The follicular cells are highly polarized epithelial cells, with an apical border facing the colloid and a basal border contacting the blood vessels, which form a vascular network around the follicles. This structure evolved in postmetamorphic animals to maximize the efficiency of iodine uptake [16]. The follicles also allow for the extracellular storage of a vast amount of thyroid hormones in the colloid.

Thyroid Hormone Synthesis

In humans, thyroid hormone synthesis begins by GW11-12 [15]. The highly specialized thyroid cells express thyroid-specific and enriched genes involved in

CHAPTER 2

Congenital Hypothyroidism

Abstract: Congenital hypothyroidism is a thyroid hormone deficiency disorder present at birth due to thyroid gland failure. There are two types: primary and central. Primary congenital hypothyroidism is caused by either developmental disorders of the thyroid gland or defects in thyroid hormone synthesis. The central type, which is much less common, is caused by decreased TSH secretion or bioactivity. Thyroid dysgenesis and dyshormonogenesis are the major causes of congenital hypothyroidism. Most cases are multifactorial, involving several genes, and a small percentage is monogenic. Thyroid failure occurs prenatally, but maternal thyroid hormones may prevent fetal hypothyroidism and protect the brain. Untreated congenital hypothyroidism severely affects postnatal development, but neonatal screening allows for early thyroid hormone treatment, effectively preventing hypothyroidism.

Keywords: Athyreosis, Cretinism, Dyshormonogenesis, Goiter, Maternal hormones, Thyroid dysgenesis, TSH resistance.

INTRODUCTION

Congenital hypothyroidism, by definition, refers to a deficiency of thyroid hormone present at birth. Historically, the most common cause was iodine deficiency. The terms “sporadic goiter” and “sporadic cretinism” were coined to describe isolated cases, sometimes occurring within families, that resembled iodine-deficient cretins in populations with sufficient iodine levels. In 1850, Thomas C. Curling documented the first two cases of athyreotic cretins [1], while C. Hilton Fagge described four similar cases of “sporadic cretinism” with athyreosis in 1871 [2]. William Osler identified a distinct form of cretinism characterized by an enlarged gland, which he termed “sporadic goitrous cretinism” in 1897 [3]. It was not until 1950 that John B. Stanbury provided crucial insights into the pathogenesis of thyroid dyshormonogenesis. He described the first two cases of goitrous, severely hypothyroid patients exhibiting a defect in iodine incorporation into thyroglobulin, the iodide organification defect [4].

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Most cases of congenital hypothyroidism are permanent due to an absent or functionally defective thyroid gland, including TSH resistance *i.e.*, primary hypothyroidism [5 - 7]. Conversely, central hypothyroidism is a deficiency in thyroid hormone production due to insufficient stimulation by TSH of an otherwise normal thyroid gland [8]. Cases of transient hypothyroidism at birth include maternal antithyroid therapy, iodine deficiency or excess, maternal anti-TSH receptor antibodies [9], and congenital hepatic hemangiomas highly expressing DIO3 [10]. Certain factors elevate the chances of transient hypothyroidism during infancy, including low birth weight, male gender prevalence, and ethnicity, which is more common among non-white parents [11].

Untreated congenital hypothyroidism causes developmental impairments, stunted growth, deficient bone development, and significant intellectual deficits. While some signs of hypothyroidism, such as prolonged neonatal jaundice and poor feeding, may be visible at birth, newborns often appear outwardly normal. This condition affects approximately 1 in 2500 newborns worldwide. The introduction of universal neonatal screening by Jean Dussault in Quebec in 1975 [12] marked a pivotal moment, enabling the implementation of highly successful hormone replacement therapy following early diagnosis. For clinical practice recommendations, the author suggests consulting recent reviews on the subject [13, 14] and the Endo-European Reference Network Initiative on Guidelines for Diagnosing and Managing Congenital Hypothyroidism [11].

PRIMARY CONGENITAL HYPOTHYROIDISM

Primary congenital hypothyroidism may be due to three causes: developmental defects of the thyroid gland, known as thyroid dysgenesis (OMIM #218700), genetic alterations of the thyroid hormone synthesis pathways or thyroid dyshormonogenesis, and defective TSH binding or signaling (TSH resistance).

Thyroid Dysgenesis

Thyroid dysgenesis is the most frequent cause of permanent congenital hypothyroidism, accounting for about 65% of all cases, with about 2% being familial. Thyroid dysgenesis includes agenesis, athyreosis, thyroid hypoplasia, and ectopias. Agenesis is the lack of initiation of thyroid morphogenesis. Athyreosis is the absence of the gland due to defective development following the specification of the thyroid anlage. Thyroid dysgenesis is multifactorial, and most cases are caused by non-Mendelian mechanisms involving several genes [15 - 17]. Some risk factors, such as sex (more frequent in females), birth weight, and gestational age have been described [18].

Less than 5% have an identified genetic cause, which includes disruption of developmental genes causing syndromic congenital hypothyroidism such as *PAX8*, *NKX2-1*, *NKX2-5*, *FOXE1* and *GLIS3* [19 - 22]. Most genes cause thyroid dysgenesis when one allele is mutated (*PAX8*, *NKX2.1*, *NKX2.5*), whilst others require biallelic mutations (*FOXE1*) to cause hypothyroidism.

PAX8, NKX2-1 (TTF1, thyroid transcription factor 1), and FOXE1 (TTF2) are key transcription factors during early thyroid development [23]. *PAX8* mutations cause thyroid dysgenesis in only a minority of cases [24]. Heterozygous *PAX8* mutations cause TSH resistance (see below) and may cause syndromic congenital hypothyroidism with urogenital malformations [25]. *NKX2-1* mutations cause congenital hypothyroidism, interstitial lung disease, and chorea because it is also involved in the migration of striatal interneurons [26]. *NKX2-5* is another homeobox gene involved in heart development and thyroid organogenesis [20]. FOXE1 is a member of the forkhead family of transcription factors involved in thyroid gland morphogenesis and proper palate development [27, 28]. Loss of function of this protein causes congenital hypothyroidism and cleft palate [29 - 31]. *GLIS3* encodes a zinc-finger nuclear protein involved in pancreatic beta-cell development, and mutations of this protein cause neonatal diabetes associated with congenital hypothyroidism and other malformations [21].

Other candidate genes that may have a role have recently been identified, including *CDCA8*, *JAG1*, *TUBB1*, and *NTN1* [16].

Borealin (CDCA8, Cell Division Cycle-Associated Protein 8) is a component of the chromosomal passenger complex, an essential regulator of mitosis and cell division. It is cell-cycle regulated and involved in microtubule stabilization and spindle formation. It is expressed early in thyroid development, and mutations result in athyreosis and ectopy [16]. *JAG1* encodes the NOTCH1 receptor ligand. *JAG1* mutations cause the Allagile syndrome (OMIM 118450), with intrahepatic biliary duct malformations and hypothyroidism in some patients [32]. *JAG1* may have a role in thyroid hypoplasia. *TUBB1* encodes a member of the β-tubulin family. Several families with ectopy, associated with abnormal platelets, harbor mutations of this gene [33]. *NTN1* encodes netrin, a protein involved in cell migration and axon guidance, linked to thyroid ectopy and cardiovascular defects [34].

Thyroid Dyshormonogenesis

This term includes cases of congenital hypothyroidism with a gland-*in-situ* and defective thyroid hormone synthesis [30, 35]. Its recorded frequency has increased since the early studies on congenital hypothyroidism and now accounts for 30-

CHAPTER 3

Deiodinases in the Brain

Abstract: Deiodinases (DIO) are central to regulating thyroid hormone action in the brain because they control the tissue concentration of the active hormone triiodothyronine (T3). DIO2, the outer ring, 5'-deiodinase expressed in the brain, converts T4 to T3 and is active primarily in two glial cell types: astrocytes and tanycytes. Astrocytes produce all of brain T3 during the fetal period and a significant fraction in adults. T3 from astrocytes reaches other neural cells, mainly neurons, devoid of DIO2. The T3 produced in the tanycytes travels to hypothalamic nuclei to perform neuroendocrine functions. *DIO2* is expressed in the human fetal brain's neural stem cells, known as outer radial glia. The inner ring, 5-deiodinase DIO3, converts T4 and T3 to the inactive compounds reverse T3 (rT3) and 3,3'T2, respectively, a reaction equivalent to suppressing thyroid hormone action. Brain DIO3 is active mainly in neurons. Thyroid hormones regulate the gene expression and enzymatic activity of DIO2 and DIO3. When T4 concentrations rise, DIO2 activity falls, and when T4 goes down, DIO2 increases. T3 stimulates the *DIO3* gene, and DIO3 activity increases when T3 increases. The combined actions of DIO2 and DIO3 exert a "homeostatic-like mechanism" to maintain locally appropriate bioactivity of thyroid hormone by providing individual brain cells with the optimal concentrations of T3 required at different stages of development. These mechanisms regulate thyroid hormone action with a timeline specific to different brain regions.

Keywords: Allan-Herndon-Dudley syndrome, Astrocytes, Brain development, Cerebral cortex, Cerebellum, Choroid plexus, Human fetal brain, MCT8, OATP1C1, Radial glia, Tanycytes.

INTRODUCTION

The deiodinases are essential regulators of thyroid hormone action in the brain. Studies in this field are divided into three periods [1]. The first period began in 1951, with the finding that T4 undergoes *in vivo* deiodination [2]. This time was dominated by the analysis of tissue deiodination activity and the link between T4 deiodination and its biological activity [3]. The second stage began with the discovery that tissue deiodination is due to specific enzymes known as deiodinases. Visser *et al.* [4] discovered the first deiodinase enzymatic activity in the liver. The liver deiodinase removed the iodine atom in position 5' of the T4 phenolic or outer ring. This deiodinase was later designated as type-1 deiodinase (DIO1). A different 5'-deiodinase was described later in the rat cerebral cortex [5]

and became known as type-2 deiodinase (DIO2). A third deiodinase, DIO3, found in monkey hepatocarcinoma cells [6], removes the iodine in position 3 of the tyrosyl rings of T4 and T3. These discoveries opened the way to measuring tissue deiodination activity and physiological regulation. The third period started with the cloning of deiodinases from 1991 to 1994 [for a detailed historical account, see [1]]. The availability of cDNA clones allowed studies on the developmental and specific tissue and cellular expression of the different deiodinases and their regulation at the genomic level.

THYROXINE, A TRIIODOTHYRONINE PRECURSOR

There were two main hypotheses on thyroid hormone action in the 1970s: T3 is the active hormone, and T4 deiodination is a source of T3 in tissues. These hypotheses were formulated early after the discovery of T3 [7] but were extremely difficult to test experimentally until the nuclear receptors for T3 were discovered [8 - 10]. The fact that T4 is a prohormone received experimental support by demonstrating T4 to T3 conversion in humans [11, 12]. The correlation between biological activity and conversion of T4 to T3 supported these hypotheses [13].

The assumption was that T3 secreted by the thyroid gland is in rapid equilibrium with T3 formed in tissues and that the plasma T3 concentrations reflect similar T3 concentrations and action in all tissues. Obregon *et al.* [14] challenged this concept by demonstrating that the T3 formed in tissues is not entirely exchangeable with circulating T3, whether secreted by the thyroid gland or given exogenously. They discovered that the proportion of total T3 originating from T4 is highest in the brain, liver, and kidney and lowest in the heart and muscle. They proposed that thyroid hormone action in some tissues, like the brain, is dependent on the local T4 to T3 conversion rate. Changes in thyroid hormone secretion from the thyroid gland do not necessarily translate into changes in tissue T3, and this concept is crucial for understanding thyroid hormone action in the brain.

Other investigators reached similar conclusions. Crantz and Larsen [15] injected adult rats with a single dose of radioactive T4. Three hours later, they measured the T3 derived from the injected T4 in cell nuclei isolated from the cerebral cortex and the cerebellum. In just three hours, the T3 derived from the injected T4 accounted for about two-thirds of the total T3 present in the cell nuclei, the site of T3 action. T3 derived from T4 in the cerebral cortex accounted for 73% of nuclear receptor occupancy under steady-state conditions, with T3 from the circulation contributing an additional 21% occupancy for a total receptor occupancy of more than 90% [16].

Van Doorn *et al.* [17] confirmed and extended these results by using rats on isotopic equilibrium, which more closely simulates physiological conditions than

single injections of the hormone. They discovered a fundamental difference between the brain and other tissues: the majority of T3 in the cerebral cortex and cerebellum originated locally from T4, while T3 in the kidney, liver, and muscle was derived primarily from circulation. Another important finding was that the fraction of T3 derived from T4 in the brain increased in hypothyroid rats, which was indicative of a compensatory increase in the local T4 to T3 conversion.

Other species exhibit a similar phenomenon. In our laboratory [18], we measured the occupancy of T3 nuclear receptors in tissues dissected from fetal lambs at 100 days of gestational age (sheep have a 150-day gestation period). We discovered that T3 occupies nuclear receptors in the brain at approximately 66% of the maximal capacity, while the occupancy in the liver and lung at this developmental stage was less than 10%. (Fig. **1**).

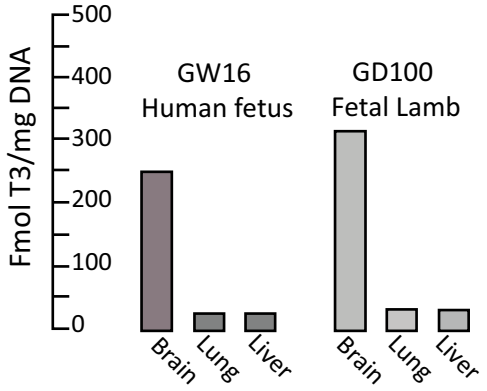

Fig. (1). T3 concentration in brain, lung, and liver of gestation week 16 (GW16) human fetus and gestation day 100 (GD100) fetal lamb. T3 is present in the brain but barely detectable in the lung and liver. Note the similarity between the two species. Data from [18, 19].

We concluded that the brain is an early and preferential target of thyroid hormones during fetal development compared to other tissues. The high saturation of nuclear receptors in the brain compared to low saturation in the lung and liver was most likely due to the T4 5'-deiodinating activity presumably present earlier in the brain than in other tissues. In humans, the brain also shows high occupancy of nuclear receptors at the beginning of the second trimester, when T3 is undetectable in other tissues (Fig. **1**) [19]. These early observations suggested that local T4 to T3 conversion is essential for thyroid hormone action in the brain and that this particular tissue requires high T3 concentrations at early developmental stages.

CHAPTER 4

Unraveling the Role of Maternal Thyroid Hormones on Fetal Development

Abstract: Over the past four decades, a substantial body of evidence has emerged demonstrating the permeability of the placenta to thyroid hormones. Maternal thyroid hormones cross the placental barrier, becoming present in embryonic tissues well before the onset of thyroid gland function in both rodents and humans. This raises a fundamental question regarding the extent to which certain early developmental processes rely on maternal hormonal influence. While this concept is firmly supported by robust experimental data in rodents, the situation in humans is more nuanced. Numerous clinical observations suggest that a reduction in T4 levels in the blood of otherwise euthyroid pregnant women, a condition known as hypothyroxinemia, may have adverse effects on fetal development. However, clinical trials aimed at assessing the impact of treating maternal hypothyroxinemia with T4 have yielded disappointing results thus far, leaving the matter unresolved.

Keywords: Deiodinases, Fetal development, Hypothyroxinemia, Hypothyroidism Iodine, Placenta, Thyroidectomy.

INTRODUCTION

Up to 1984, the prevalent idea was that the placenta was impermeable to thyroid hormones from the mother and that thyroid hormones circulating in the fetus had an exclusive fetal origin. Thus, Roti *et al.* [1] stated that "*there is now general agreement that thyroid function in the fetus is essentially independent of maternal thyroid status, providing indirect evidence that the placenta is relatively impermeable to the thyroid hormones [2]*." Obregon *et al.* questioned this concept [3]. Using a unique and sensitive radioimmunoassay in tissue extracts obtained by a laborious extraction method, they found T4 and T3 in rat embryo-trophoblasts at embryonic (E) days 12-13 and in whole embryos from E13. The onset of fetal thyroid function in the rat fetus is at E17.5, so the hormones found had to be of maternal origin. Other results from the same and different authors [4 - 8] firmly established the notion that thyroid hormones from the mother cross the placenta from early in gestation, providing the fetal tissues with low but significant amounts of T4 and T3 before the onset of fetal thyroid gland function. The critical questions are to what extent maternal thyroid hormones exert biological actions in

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the embryonic tissues and, in the case of humans, whether isolated low levels of T4 during pregnancy affect human fetal brain development.

PLACENTAL PERMEABILITY TO THYROID HORMONES IN RODENTS

One approach involved analyzing the impact of severe maternal hypothyroidism [5]. The absence of the maternal thyroid gland induced by thyroidectomy led to a significant reduction in the concentrations of both T4 and T3 in the trophoblast and fetal organs. These studies demonstrated that in rats, the placenta allows the passage of T4 and T3, which are already present in embryonic tissues before the fetal thyroid gland becomes functional. Subsequently, the fetal pool of thyroid hormones is a combination of maternal and fetal contributions. Maternal T4 accounts for approximately 17.5% of the fetal extrathyroidal T4 pool at the termination of pregnancy [9]. This proportion may be even higher in mice since administering T4 daily to normal pregnant dams resulted in 75% of fetal liver T4 at E18 originating from the maternal compartment [10]. In this same study, brain DIO2 activity maintained brain T3 concentrations stable after wide ranges of maternal and fetal T4 concentrations.

The Relevance of T4 as the Source of T3 in the Fetal Brain

In thyroidectomized pregnant rats, TSH increases in the mother but not in the fetus. Conversely, administering the antithyroid drug MMI (methimazole) results in hypothyroidism in both the mother and the fetus, and TSH increases in both. Administering T4 to MMI-treated dams suppresses TSH levels in both the mother and the fetus. The fetal pituitary is sensitive to maternal T4 but not to maternal T3 since when an equivalent dose of T3 is administered, it mimics the effects of T4 in the mother but has no effect on fetal TSH levels [11, 12].

T4 administration to mothers results in the accumulation of T3 in various maternal and fetal organs, including the brain. When T3 is administered, it reaches most fetal tissues, but its accumulation in the brain is limited (Fig. **1**) [10, 11]. This implies that the fetal brain has a limited permeability to T3, and T4 serves as the primary source of brain T3. These findings underscore the critical role of maternal T4 in fetal brain development. In cases of congenital hypothyroidism, maternal T4 provides a protective shield for the fetal brain until birth, and early T4 treatment proves effective in preventing potential neurological damage

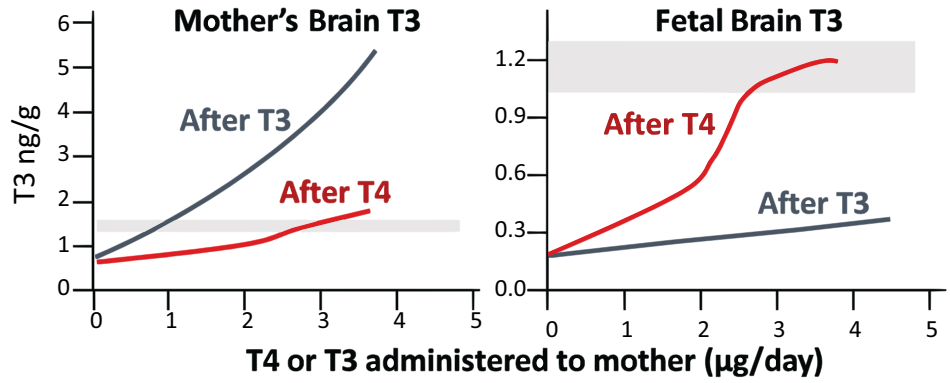

Fig. (1). Accumulation of T3 in the brain after administration of increasing doses of T4 or T3 to pregnant rats treated with MMI, which produces maternal and fetal hypothyroidism. The horizontal gray rectangles indicate the range of normal T3 in either case. In the mother, T3 accumulates in the brain after T4 or T3 treatment. In the fetus, only T4 treatment normalizes the levels of T3. Drawn from data by Calvo *et al.* [11].

THE PERMEABILITY OF THE HUMAN PLACENTA TO THYROID HORMONES

Vulsma *et al.* demonstrated that the human placenta is permeable to thyroid hormones [13]. Children born with a non-functional thyroid had a blood T4 concentration of 35-70 nM, which could only be of maternal origin. During the next 8-19 days, T4 decreased and fell below the detection limit. Maternal T4 in cord blood represented about 30% of normal serum concentrations, but this was in a situation where the fetal thyroid gland was not functional, which poses the question of whether there is significant maternal contribution to an euthyroid fetus.

In human-term placentas perfused *ex vivo* [14], significant transfer of T4 occurred only after DIO3 inhibition, in agreement with the barrier role played by DIO3 [15, 16]. DIO3 activity is present in syncytiotrophoblasts, cytotrophoblasts, decidua, and fetal vessels, *i.e.*, the maternal-fetal interfaces [17], thus forming a barrier to the passage of excessive thyroid hormones from the mother to the fetus [18].

The analysis of T4 transport in perfused human placentas [14] indicates that the rate of T4 transfer from the fetal to the maternal side was faster than from the maternal to the fetal side, indicating asymmetrical transport. The authors suggested that there is negligible transfer of T4 to the fetus across the human placenta at term, and the main role of placental transfer is to protect the fetus from excessive thyroid hormones. These results contrast with observations in euthyroid mice at term described above [10].

CHAPTER 5

Endemic Goiter and Cretinism: Pathophysiology of Iodine Deficiency

Abstract: Iodine is an essential component of thyroid hormones, and its deficiency causes endemic goiter, cretinism, and a constellation of syndromes known as iodine deficiency disorders. Although iodine deficiency still affects most of the world, national or regional salt iodization programs have increased the number of countries with adequate intake. Endemic cretins were classified as either predominantly neurological or myxedematous (hypothyroid). Severe maternal iodine deficiency causes fetal neurological damage during the first half of gestation, which is prevented by administering iodine to mothers before or early in pregnancy. Myxedematous cretins present thyroid atrophy, hypothyroidism, and growth arrest, and no neurological involvement. Physiological adaptations to iodine deficiency include thyroid growth (goiter) and thyroidal autoregulatory mechanisms leading to decreased serum T4 and preserved serum T3. This situation is known as hypothyroxinemia, as described in Chapter **4**. The brain, which depends on the T3 generated locally, shows an increased type 2 deiodinase activity and T3 formation from T4. When iodine intake is severe, these mechanisms cannot maintain T3 concentrations in the brain, leading to brain damage.

Keywords: Cretinism, Dehalogenase, Deiodinases, Goiter, Hypothyroidism, Hypothyroxinemia, Micronutrients, Pregnancy, Selenium.

INTRODUCTION

Iodine is an essential component of thyroid hormones, accounting for 65% of the weight of T4 and 59% of T3. However, the relationship between iodine deficiency and endemic goiter and cretinism was not established until the 19th century [1]. For centuries goiter had been treated empirically with dried seaweed and sponges, but the discovery of iodine in these marine organisms was accidental, prompted by a military need. During the Napoleonic wars, in 1811, Bernard Courtois, a French chemist, used brown kelp as an alternative to wood to obtain nitrates for gunpowder. Brown kelp is exceptionally efficient in concentrating iodine, representing 1% of its dry weight [2]. Courtois observed a violet vapor when he treated kelp ashes with sulfuric acid, and Gay-Lussac and Humphrey Davy recognized this material as a new element, which was named “iode” in French and iodine in English, derived from the Greek word for violet.

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Soon after the discovery of iodine in 1811, the Swiss physician Jean-François Coindet related the beneficial effects of seaweed treatment on goiter with its iodine content and started goiter treatment with iodide solutions in 1820. However, he used high, toxic doses, and iodide treatment was considered harmful. Coindet's theory about iodine deficiency as the cause of goiter was reinforced by Chatin in 1851, who found a correlation between water iodine content and goiter incidence in many regions of France. Von Fellenberg later confirmed and extended Chatin's findings [3].

At the end of the 19th century, the German chemist Eugen Baumann measured iodine in the thyroid and proposed the presence of an iodinated, active substance produced in the gland. In 1831, Boussingault first proposed using iodine-fortified table salt for goiter prevention [3]. Programs for preventing iodine deficiency on a population basis, based on the distribution of iodine-fortified table salt, were first attempted in the early 1920s in the USA and Switzerland in regions with high incidences of goiter and cretinism [1, 3, 4].

Although national or regional salt iodization programs have increased the number of countries with adequate iodine intake from 67 in 1993 to 117 in 2022 [1], iodine deficiency still affects most of the world (for updated information, visit the Iodine Global Network webpage at http://www.ign.org). In particular, moderate iodine deficiency during pregnancy leading to maternal hypothyroxinemia may pose a developmental risk for progeny, even in developed countries with a median sufficient iodine intake [5].

THE IODINE CYCLE

Iodine deficiency is one of the most common micronutrient deficiencies, with iron, folate, vitamin A, and zinc [6], and the leading cause of preventable mental deficiency worldwide. Iodine intake is dependent on soil content and fertilization practices. Iodine is abundant in seawater at a 58 μg/L concentration [7]. It is primarily present as iodate ($IO3^-$) and iodide (I^-) ions in variable proportions. Due to phytoplankton and macrophytic algae metabolism, smaller amounts are present as organic iodine (iodocarbons), mainly methyl iodide (ICH3). Iodine is transferred from seawater to the atmosphere as volatile organic iodocarbons and molecular iodine (I2), which form oxidized iodine (IO) species after photolysis, providing nuclei for the particulate matter of clouds and falling on the soil with the rain [2].

Plants grown in iodine-rich soils, usually coastal areas, have an iodine content that may be 100 times those grown in iodine-poor soils. The latter are frequently located in mountainous regions with iodine-depleted soils due to erosion. Severe iodine deficiency has historically affected regions in the mountain ranges of the

Andes, the Alps, the Atlas, and the Himalayas, as well as regions with frequent floodings [8].

Dietary iodine is absorbed primarily as iodide by the gastrointestinal tract and diffuses rapidly into the blood and tissues [9]. Besides the thyroid gland, other organs concentrate iodide, such as the salivary glands, the mammary glands, the ovaries, the choroid plexus, and the placenta. Most excreted iodine appears in the urine. Individual iodine concentrations in the urine reflect recent intake and vary widely so that an isolated measurement does not reflect individual iodine status. Instead, the median value of spot urine samples (urine iodine content, UIC) is used as a marker of the population's iodine status. A region is iodine-sufficient when the non-pregnant population's median UIC equals or exceeds 100 μg/L.

Iodine Requirements

The daily iodine requirement above 12 years of age, excluding pregnant and lactating women, is 150 μ/day [8]. The recommended intake during pregnancy and lactation is 250 μ/day. During pregnancy, the iodine requirement is higher due to several factors, such as increased thyroidal secretion and renal clearance and the transfer of iodine and thyroid hormones to the fetus. Iodine needs during pregnancy may require supplementation because even in iodine-sufficient countries, food-based iodine consumption may not be enough to ensure an intake above 150 μ/day [10]. During lactation, iodine is secreted in the milk, satisfying the baby's needs, which are around 50 μg/day. Recommendations for children between 1 and 8 years old are 90 μg/day, and for 6 to 12 years old, 120 μg/day.

Historically, iodine deficiency has been associated with goiter and cretinism. However, it causes a broad spectrum of abnormalities, including goiter, hypothyroidism, various degrees of neurological impairment, intellectual deficits, high incidence of abortions and stillbirths, increased perinatal and infant mortalities, and neonatal goiter and hypothyroidism (Table **1**). These alterations are collectively known as iodine deficiency disorders (IDD) following the original proposal by Hetzel [11].

Table 1. Iodine deficiency disorders [Hetzel 1983 [11]], and WHO.

Age Group	Health Consequences
All	Goiter, hypothyroidism, increased susceptibility to nuclear radiation.
Fetus	Spontaneous abortions, stillbirth, congenital anomalies, perinatal mortality.
Neonate	Endemic cretinism (mental deficiency, deaf-mutism, spastic diplegia, squint, hypothyroidism, short stature, infant mortality.

CHAPTER 6

Cellular Transporters for Thyroid Hormones

Abstract: Thyroid hormones require transporter proteins that facilitate their influx and efflux through the cellular plasma membranes. There are many families of thyroid hormone transporter proteins, most of which transport other substrates, including bile acids, amino acids, monocarboxylates, and organic anions. The only transporter specific for thyroid hormones is the monocarboxylate 8 transporter or MCT8. MCT8 is present in the brain barriers and the membranes of neural cells. MCT8 mutations cause the Allan-Herndon-Dudley syndrome, described in the next chapter. Besides MCT8, the amino acid transporters LAT1 and LAT2 might have a physiological role in T4 and T3 transport. The organic anion transporter polypeptide 1C1 or OATP1C1 is a T4 transporter present in the mouse, but not the human, blood-brain barrier, and facilitates T4 transport to astrocytes and radial glia expressing type 2 deiodinase. A neurodegenerative disorder in a patient has been attributed to an OATP1C1 mutation. This chapter describes the physiological aspects of thyroid hormone transport across the different transporter families.

Keywords: Brain barriers, BBB, Cerebrospinal fluid, CSF, Choroid plexus, LAT1, LAT2, MCT8, OATP1C1.

INTRODUCTION

Thyroid hormones are lipophilic molecules with a high oil:water partition coefficient [1] due to the iodinated aromatic rings [2]. For this reason, the traditional thought was that thyroid hormones would cross the cellular membranes by passive diffusion. However, the rate of passive diffusion decreases rapidly for molecular sizes above 100, with an upper limit of 600. T4 and T3, with molecular masses of 777 and 651, respectively, have a low rate of passive diffusion. In addition, thyroid hormones are charged molecules at the physiological pH due to the dissociation of the tyrosyl ring's amino and carboxylic groups and the phenolic ring's hydroxyl group [2]. Without carriers, thyroid hormones remain trapped in the phospholipid bilayer [3].

Hennemann *et al.* [4] reviewed the early studies on the presence of carriers facilitating the cellular uptake of T4 and T3 and proposed that organic anion transporters (OATP) and amino acid transporters (LAT1 and LAT2) might have a physiological role in thyroid hormone transport. The relevance of transporters

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received confirmation with the discovery of a neurological syndrome associated with mutations in the monocarboxylate transporter 8 (MCT8), a specific thyroid hormone transporter [5, 6]. One year before, Friesema *et al.* had described that MCT8 is very active in the transport of T4, T3, rT3, and T2, with Km 2-5 μM and expressed in the liver, kidney, brain, and heart [7]. Following these discoveries, studies on thyroid hormone transporters and their relevance to disease opened a new field in the intersection between thyroidology and neurology [8].

Thyroid hormone cell membrane transporters typically have a Km in the micromolar range, whereas the free hormone in biological fluids is in the picomolar range. This means that transporter concentration is not rate-limiting, and variations in transporter number would have little effect on net transport.

PROTEIN FAMILIES WITH THYROID HORMONE TRANSPORTING ACTIVITY

Bile Acid Transporters

The liver-specific *SLC10A1* gene encodes a seven-transmembrane domain protein, the sodium-taurocholate cotransporter (NTCP), involved in the enterohepatic circulation of bile acids. This protein transports the sulfate derivatives of T4 and T3 and the thyroid hormone analog eprotirome [9, 10]. *SLC10A1* is not expressed in the brain.

Amino Acid Transporters

There are many classes of amino acid transporters [11], but the ones relevant for thyroid hormones are the system L [leucine preferred [12, 13]] and the system T (tryptophan preferred) transporter TAT1, also known as monocarboxylate transporter 10 (MCT10, SLC16A10). The system L transporters are heterodimeric transmembrane proteins that facilitate the transport of large neutral amino acids, including leucine, tyrosine, phenylalanine, and tryptophan, and the small neutral amino acids glycine and alanine. LAT1 (SLC7A5) and LAT2 (SLC7A8) are 12-transmembrane domain proteins that heterodimerize with the one-transmembrane domain glycoprotein 4F2hc (the CD98 antigen, SLC3A2). They are Na+-independent and transport T4 and T3 with the Km in the micromolar range, compared to millimolar for amino acids [14 - 16].

The primary function of LAT1 is the cellular efflux of glutamine in exchange for leucine and other essential amino acids [11]. *SLC7A5* is predominantly expressed in the blood-brain barrier [BBB [17]]. Mutations in *SLC7A5* have been linked to motor dysfunction and autism spectrum disorders in both humans and mice, attributed to altered transport of branched-chain amino acids across the BBB [18].

SLC7A5 is also expressed in the syncytiotrophoblast layer of the human placenta [15, 19, 20], where it may facilitate the transplacental transport of thyroid hormones. In the mouse brain, *Slc7a5* mRNA expression is notably higher in the BBB compared to astrocytes, neurons, and oligodendrocytes [21]. In the human fetal cortex at midgestation, *SLC7A5* mRNA is found in endothelial cells, some projection neurons, and interneurons derived from the medial ganglionic eminence [22]. Expression in interneurons might be particularly relevant given the possible alterations of GABAergic transmission that might underlie LAT1 dysfunction [18].

LAT2 is less effective than LAT1 in transporting iodothyronines [16] and is not present in the BBB [23, 24]. *In situ* hybridization of mouse brain slices during the postnatal period shows neuronal *Slc7a8* expression in the cortex, hippocampus, amygdala, and thalamus, with high expression in the thalamic paraventricular nucleus [25, 26]. *Slc7a8* mRNA is also expressed in microglia [21]. Similarly, in the human fetus, *SLC7A8* expression is observed in microglia and some neurons [22, 27]. Disruption of *Slc7a8* does not significantly impact thyroid economy [25, 28], but it does prevent the transient perinatal hyperthyroidism observed in *Slc16a2* knockout mice [25].

Organic Anion Transporter Polypeptides (OATPs)

The SLCO family of solute carrier proteins, known as organic anion transporter polypeptides (OATPs), consists of 12-transmembrane domain proteins mediating the transport of amphipathic organic compounds, such as steroids, drugs, anionic oligopeptides, and bile salts [29, 30]. Many of these transporters are expressed in the brain [31]. Fourteen members of this family in mice and seven in humans have thyroid hormone-transporting activity [32, 33]. Among them, OATP1C1 (SLCO1C1, formerly known as OATP14) is the most relevant. Other family members, such as OATP1A4 (SLCO1A4), OATP3A1 (SLCO3A1), and OATP2B1 (SLCO2B1), may have a physiological role in thyroid hormone transport, although it has not been conclusively demonstrated. In all cases, the Km for T4 and T3 transport falls within the micromolar range. Recent genome-wide association analyses have identified SLC17A4 as a high-affinity transporter for T4 and T3 in the liver, kidney, and gastrointestinal tract [34].

OATP1A4 transports T4 and T3 *in vitro* and is predominantly expressed in endothelial cells [21, 35]. OATP3A1 transports T4 and is primarily expressed in oligodendrocytes in mice [21]. The rat OATP2B1 transports T4, and the mouse gene, *Slco2b1,* is abundantly expressed in endothelial cells and microglia, with lower levels observed in oligodendrocyte precursors [21]. Human OATP1C1 efficiently transports T4 and T4 sulfate, while T3 transport is negligible [36]. This

CHAPTER 7

The Allan-Herndon-Dudley Syndrome: Pathophysiology and Mouse Models of MCT8 Deficiency

Abstract: Mutations of the thyroid hormone cell-transporter gene, monocarboxylate transporter 8, or MCT8, cause an X-linked syndrome characterized by altered thyroid hormone concentrations in serum, profound neuromotor impairment, and cognitive deficits. This chapter describes the clinical features of the syndrome and analyzes the mechanisms of disease from studies of MCT8 deficiency in mice. The final section of the chapter describes the available treatments and experimental therapies.

Keywords: Blood-brain barrier, Deiodinases, DITPA, Dystonia, Dyskinesia, Fetus, Gene therapy, Hypotonia, Interneurons, Myelin, Psychomotor retardation, Sobetirome, Transport, TRIAC, X-linked syndrome.

INTRODUCTION

In 1944, Allan, Herndon, and Dudley described 24 male patients with severe X-linked psychomotor retardation syndrome [1]. The patients appeared normal during the first postnatal months. Then they developed severe central hypotonia, inability to hold up their head, dystonic movements (involuntary muscle contractions causing repetitive movements and abnormal posturing), and spastic paraplegia. They made clumsy attempts to walk by the time they were three to five years old, had a profound cognitive impairment, and garbled speech. This condition was mapped to chromosome X in 1990 [2, 3].

In 2004, the groups of Samuel Refetoff in Chicago and Theo J. Visser in Rotterdam described patients presenting a similar clinical picture but also altered concentrations of thyroid hormones in the serum, consisting in increased T3 and reduced T4 and rT3, with normal or slightly increased TSH [4, 5]. The hormonal changes directed attention to a defect in thyroid hormone metabolism. After discarding mutations in the deiodinase genes, they found mutations in the *SLC16A2* gene, encoding the thyroid hormone transporter MCT8 (monocarboxylate transporter 8). One year later, *SLC16A2* mutations were found in patients of the original families described by Allan, Herndon, and Dudley [6],

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finally disclosing the genetic cause of the disease sixty years later. This condition is one form of reduced sensitivity to thyroid hormones due to a deficiency in cellular transport [7 - 9].

THE ALLAN-HERNDON-DUDLEY SYNDROME (ORPHANET #300523)

Clinical Aspects

Since the mutated gene is on the X chromosome (Xq13.2), the disorder affects male patients, with one published exception [10]. Some female carriers may exhibit mild abnormalities resulting from skewed X-chromosome inactivation, which leads to the inactivation of the chromosome carrying the wild-type gene. The affected infants present nystagmus, marked truncal hypotonia, and muscle weakness, progressing to spasticity and spastic quadriplegia. They also show dyskinesia, often paroxysmal, athetoid movements (involuntary writhing movements of hands and feet), abnormal positioning of the hands, lack of speech, and profound cognitive impairment with an IQ below 30 [11-16]. The MRI of most patients shows hypomyelination, especially in the early years [17]. Elevated T3 causes a state of peripheral tissue thyrotoxicosis with muscle wasting. Impaired transport of T4 and T3 to the brain causes cerebral hypothyroidism and neurological impairment [18].

The largest cohort of these patients published so far is the one by Groeneberg *et al.* [19], of 151 patients with 70 different *SLC16A2* mutations. Although the median age at presentation was four months, the median age at diagnosis was 24 months. The estimated prevalence rate is 1/70,000 male individuals, and the median overall survival is 35 years, with a median age at death of 10.5 years. The most frequent initial symptoms in this set of patients were global developmental delay and truncal hypotonia with poor head control. Less common initial symptoms were poor weight gain and feeding problems. By the end of the first year, dystonic movements of limbs and neck and abnormal positioning of the hands appear. A high proportion of the patients have seizures and paroxysmal dyskinesias.

A hallmark of MCT8 deficiency is abnormal or delayed myelination on MRI. In clinical settings, this finding has sometimes directed attention to myelin gene pathologies, particularly to Pelizeaus-Merchbacher disease, caused by mutations or duplications of the *PLP* gene (encoding the myelin proteolipid protein). In one study, 11% of patients initially diagnosed with Pelizeaus-Merchbacher disease had MCT8 but not PLP mutations [20]. The patients' MRI improves with age, but not the clinical course. There has been some discussion about classifying MCT8 deficiency within the white matter abnormalities and whether the myelin defect is transient or permanent [21 - 23].

MCT8 deficiency should be appropriately considered a hypomyelination disorder. In an extensive review, Vancamp *et al.* [24] noted that in patients submitted to MRI, myelination abnormalities were present in 80% of patients of two years and younger, 63% of patients between two and six years, and 33% of patients older than six years. Despite the apparent decrease of myelin deficits with age, abnormal myelination is generally not considered transient, and even in patients with normal-appearing white matter, there might be abnormalities in some myelin tracks.

There are around 150 pathogenic MCT8 variants, consisting of large deletions, frameshifts resulting in nonsense variants, and single amino acid changes in any part of the protein. Characterization of the mutated protein includes analysis of its transport capacity using labeled hormones in cultured cells and its intracellular localization by immunofluorescence. There are three types of mutations based on the protein's functional activity, which correlate with the severity of the disease [16, 25 - 28]: i) mutations that alter the transport kinetics but not the membrane localization of the protein, ii) mutations altering the protein stability and its membrane localization, and iii) mutations fully inactivating the protein.

Histopathological Changes in the MCT8-Deficient Brain

There is only one published study on brain histopathology in MCT8 deficiency, which is worth describing in detail. The subjects were a 30-week-old male fetus and an 11-year-old child [29]. This study showed for the first time that the lack of MCT8 causes cerebral hypothyroidism. It supported the view that the neurological impairment is due to impaired thyroid hormone transport and not to any other metabolite that might be an unknown MCT8 substrate. It also demonstrated that brain damage is already present in the gestation week 30 (GW30) fetus, even though signs and symptoms of the disease are not present at birth and appear during the first postnatal months.

The histopathological signs described below resemble the effects that hypothyroidism causes in rodents: delayed myelination and permanent hypomyelination, altered neuronal differentiation, reduced synaptogenesis, and reduced expression of thyroid hormone-regulated proteins such as the myelin proteins, neurofilaments, and calcium-binding proteins, with the virtual absence of parvalbumin interneuron staining. Changes in thyroid hormone concentrations and deiodinase mRNAs also indicated brain hypothyroidism.

CHAPTER 8

Thyroid Hormone Receptors in the Brain: Distribution and Deletion Effects on Brain Structure and Behavior

Abstract: The thyroid hormone receptors, encoded by the *THRA* and *THRB* genes, transduce the actions of T3. Receptor expression analysis gave clues on thyroid hormone and receptor functions in specific brain regions or cell types. This chapter describes the studies performed on rodents on receptor expression by various methodologies, including *in situ* hybridization and the phenotype of *Thra* and *Thrb* knockout mice. Most brain regions express the receptors from fetal stages. Receptor expression studies on rodents indicate that thyroid hormones regulate neuronal migration and differentiation during neocortical and cerebellar development. Given the critical role of thyroid hormones in brain development, it was expected that disruption of the receptor genes would be equivalent to hormone deprivation. However, in many cases, this is not so, raising the question of the role of unliganded receptor activity in hypothyroidism. This chapter ends with the few available data on receptor expression in the human fetal brain.

Keywords: Aporeceptors, Binding affinity, Cerebellum, Cerebral cortex, Development, Granular layers, Hippocampus, Knockout mice, Purkinje cells, Receptor isoforms, Striatum, Thyroid hormone analogs.

INTRODUCTION

Hormone receptors transduce biological responses after binding to cognate ligands with dissociation constants within the physiological free hormone concentration range. Thyroid hormone receptors are nuclear transcription factors whose intrinsic transcriptional activity is modified by ligand binding. The primary action of thyroid hormones is to regulate gene transcription rates, with T3 serving as the primary receptor ligand due to its 10-fold higher receptor-binding affinity compared to T4.

Early studies on the receptors relied on nuclear T3 binding assays [1, 2], which measure the affinity and quantity of receptor protein. These assays did not differentiate between the different isoforms discovered later, as they exhibit similar binding affinities for T3. Given the brain's complexity, understanding the

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role of receptors in the brain necessitates defining their regional and developmental expression patterns. With the cloning of the receptors, it became possible to analyze the expression of individual isoforms across developmental times and in different brain regions with cellular resolution.

For a general introduction to nuclear thyroid hormone receptors, refer to Chapter **1**. Two receptor genes, *THRA* and *THRB*, encode different mRNAs and proteins [3, 4]. Among the various protein products, the authentic receptor proteins are TRα1 from the *THRA* gene and TRβ1 and TRβ2 from the *THRB* gene. TRα2 and the truncated variants are not receptors because they lack the ability to bind T3. TRβ1 and TRβ2 have the same sequence except for the amino terminus, and the term TRβ refers to either isoform.

T3 RECEPTOR EXPRESSION IN THE RODENT BRAIN

The earliest point during development at which the receptor protein is measurable in the rat brain is embryonic day 14.5 (E14.5) [5]. The receptor increases three-fold just before the onset of thyroid gland function at E17.5 and reaches its maximum concentration in the whole brain by postnatal day 6 (P6) [6 - 8]. Brain receptor occupancy by the hormone increases in parallel with plasma and cytosol total and free T3, reaching a maximum of 60% on P15 [9].

All receptor proteins have the same affinity for T3. An indirect method to differentiate between the TRα1 and TRβ subtypes is by using the T3 analog TRIAC as a competitor in T3 binding assays. TRIAC exhibits a higher affinity for TRβ than for TRα1 [10, 11], and T3 competition curves can provide an estimate of the predominant form of receptor present in cells or tissue extracts. According to this assay, the receptor present in the brain during rat fetal development is primarily TRα1 [5].

Binding assays allow for the determination of bulk receptor proteins in the brain with low regional resolution. Immunohistochemical analysis enables regional discrimination but requires very specific antibodies and may not be suitable for expression analysis. An alternative approach is to measure mRNAs using *in situ* hybridization [12]. This technique is specific and sensitive, can be quantitative, and offers cellular resolution, but there may be a limited correlation between mRNA and protein concentration.

Early Expression and Distribution of Receptor mRNAs

Bradley *et al.* [13, 14] and Mellström *et al.* [15] performed the first studies on rats using the *in situ* hybridization technique to analyze the distribution of the different receptor mRNAs in brain sections (Fig. **1**).

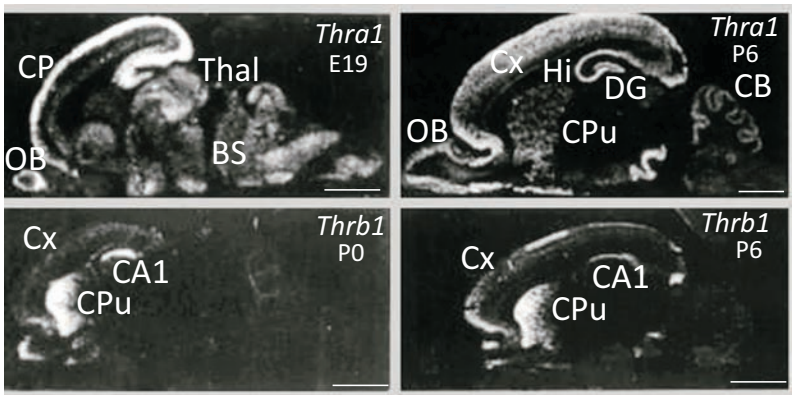

Fig. (1). *Thra1* (upper panels) and *Thrb1* (lower panels) *in situ* hybridization in parasagittal sections of the rat brain at E19 and P0 (left panels) and P6 (right panels). Abbreviations: BS, Brain stem; CA1, hippocampus CA1 field; DG, dentate gyrus; Hi, hippocampus; CP, cortical plate; Cx, cortex; CPu, caudate-putamen; OB, olfactory bulb; Thal, thalamus. *Thra1* expression is broad throughout all developing brain regions at E19. At P6, expression occurs in all cortical layers: the hippocampus, the cerebellum, the olfactory bulb, and the striatum. At P0 and P6, *Thrb1* expression predominates in the hippocampal CA1 field, the rostral caudate, and non-uniformly in the upper cortical layers. Modified from Mellström *et al.* [15].

The macroscopic distribution of *Thra* indicates primary expression in neurons. At the earliest stages of development (E11), coinciding with the onset of cortical neurogenesis, the neural tube exhibits a weak signal for *Thra1* mRNA [13].

Subsequently, the signal strengthens, and significant amounts of *Thra1* are present by E13, aligning with the detection of the protein by E14.5. *Thra1* is widely distributed in all developing regions, while *Thra2* displays the highest and broadest expression. Conversely, *Thrb* exhibits a more restricted expression pattern. It is important to note that these data cannot be used to infer the relative amounts of different protein isoforms, as there is a limited correlation between mRNA and protein abundance [16, 17].

Some specific features of the developing cortex, hippocampus, striatum, and cerebellum are as follows (Fig **2**):

In the developing cerebral cortex, precursor neurons in the ventricular layer generate neurons that, by E13, undergo radial migration to form the cortical plate. Cortical layers form with the arrival of new neurons in a process known as inside-out migration. In rodents, the mature six-layered cortex is completed a few days after birth. At the early stages, the ventricular layer, containing neuron precursors, exhibits *Thra1* expression, while both *Thra1* and *Thra2* are present in the cortical plate. In the adult six-layer cortex, layers 2-3 show greater *Thra1* expression, while layers 3-4 exhibit *Thrb1* expression [14, 15].

CHAPTER 9

Pathophysiology and Mouse Models of Thyroid Hormone Resistance Syndromes: A Focus on the Brain

Abstract: Thyroid hormone receptor mutations cause syndromes of resistance to the action of thyroid hormones (RTH) with autosomal dominant inheritance. Mutations in the *THRA* gene, encoding TRα1 and TRα2, cause RTHα, and those in *THRB*, encoding TRβ1 and TRβ2, cause RTHβ. In RTHα, relatively mild changes in circulating thyroid hormones coexist with signs of congenital hypothyroidism. In contrast, in RTHβ, TSH levels are not suppressed despite elevated thyroid hormone levels. The mutant receptors have low or no T3-induced activation and display dominant negative activity, inhibiting the wild-type receptors' transcriptional activation. This chapter describes the main characteristics of RTH, including a discussion of the mouse models of the disorder, with an emphasis on neural aspects.

Keywords: Anxiety, Attention deficit and hyperactivity disorder (ADHD), Behavior, Congenital hypothyroidism, Development, Deiodinases, Dominant negative activity, Intellectual deficiency, Interneurons, Mouse models, Receptor mutations, Thyroid hormone receptors.

INTRODUCTION

The classical forms of resistance to thyroid hormones (RTH) arise from mutations in the thyroid hormone receptors *THRA* (RTHα) and *THRB* (RTHβ) genes [1]. The initial RTH cases, such as those in family G described by Refetoff, DeWind, and DeGroot in 1967 [2], were affected by a unique form of resistance of recessive inheritance caused by homozygous deletion of the *THRB* locus [3]. The patient exhibited goiter, deaf-mutism, stippled epiphyses, elevated plasma protein-bound iodine, hyperactivity, and dysmorphic features. No additional cases of *THRB* deletion have been reported. Most cases result from dominantly inherited heterozygous *THRB* mutations affecting TRβ1 and TRβ2, with 10% being sporadic. In 10%-15% of cases, no receptor mutations can be demonstrated [4, 5]. Various possible explanations exist, including mosaicism for a receptor variant not expressed in all cells, among other factors [6]. The estimated prevalence of RTHβ is 1 case in 19,000-40,000 newborns, affecting over 900

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families and involving 230 TRβ mutations [5, 6]. There are five individuals who are homozygous/hemizygous for the mutation, exhibiting a severe phenotype [7, 8].

It took 45 years after the description of the first RTHβ patient and 23 years after the identification of the first *THRB* mutation to discover the first RTHα patients by Chatterjee and Visser groups in 2012 [9-11]. Over 20 different *THRA* mutations have been identified [6], many through exome sequencing. Many cases with mild symptoms likely remain undiagnosed because T4 and T3 may stay within the normal range.

GENERAL ASPECTS OF RTH

Several reviews provide a comprehensive coverage of RTHα and RTHβ [5, 6, 12, 13]. *THRA* and *THRB* mutations leading to RTH occur in three similar clusters of TRα and TRβ surrounding the T3 binding pocket in three-dimensional protein models. All *THRB* mutations discovered in RTHβ affect TRβ1 and TRβ2. *THRA* mutations in the carboxy-terminal end affect only TRα1, whereas others affect TRα1 and TRα2 [6]. The patient phenotypes exhibit high heterogeneity, with variations even among individuals with the same mutation within the same family [14].

The hallmark of RTHβ (Fig. **1**) is an increase in circulating total and free T4 and T3, alongside normal or slightly elevated TSH, caused by faulty control of pituitary TSH production by mutant TRβ. Other tissues predominantly expressing TRβ, such as the liver, kidney, and adipose tissue, are less sensitive to T3. Conversely, tissues predominantly expressing TRα, like the heart, bone, intestine, and skeletal muscle, exhibit signs of thyroid hormone excess.

A critical property of the mutant receptors is their dominant negative activity (Fig. **2**). A mutant T3 receptor from one allele interferes with the activity of the wild-type receptor, inhibiting tissue responsiveness to T3 even in heterozygotes [4, 5, 15 - 17].

Finding RTHα patients was challenging because, unlike RTHβ, TSH levels are normal in RTHα as TRα plays a subsidiary role in TSH control. The patients also exhibit moderate changes in serum thyroid hormones. T4 is in the low normal or slightly below; T3 is in the high normal or slightly above; rT3 is low. A high T3/T4 or T3/rT3 ratio serves as a useful biochemical index. It is unclear why T4 tends to be low and T3 high in RTHα. The most likely reason is altered deiodinase expression. T3 positively regulates *DIO3* expression, acting specifically through TRα1 [18]. A mutant TRα1, with dominant negative activity, would repress *DIO3* expression in tissues such as the skin and the brain, resulting in elevated T3

concentrations, which enhances *DIO1* expression in the liver and kidney. As a result, T4 and rT3 will experience a higher 5'-deiodination rate. Signs of thyroid hormone deficiency will manifest in tissues predominantly expressing TRα1.

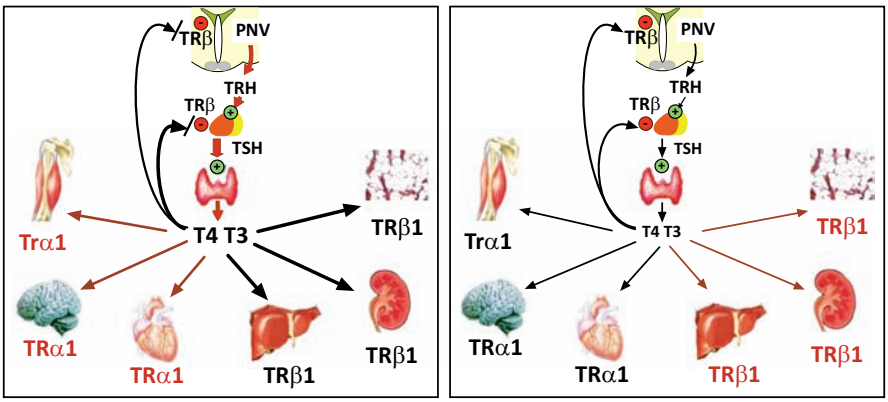

Fig. (1). In RTHβ, left panel, the TRβ mutation causes decreased sensitivity of the hypothalamus and pituitary axis to the negative feedback by thyroid hormones, increasing TSH and thyroid hormone production. Tissues predominantly expressing TRα are in a hyperthyroid state. In RTHα, right panel, the hypothalamus-pituitary-thyroid axis is unaffected, and thyroid hormone secretion is normal, but subsequent metabolism may alter the final concentrations of T4 and T3 in serum, decreasing T4 and increasing T3. Tissues expressing TRα will be hypothyroid, whereas tissues expressing TRβ may have mild hyperthyroidism.

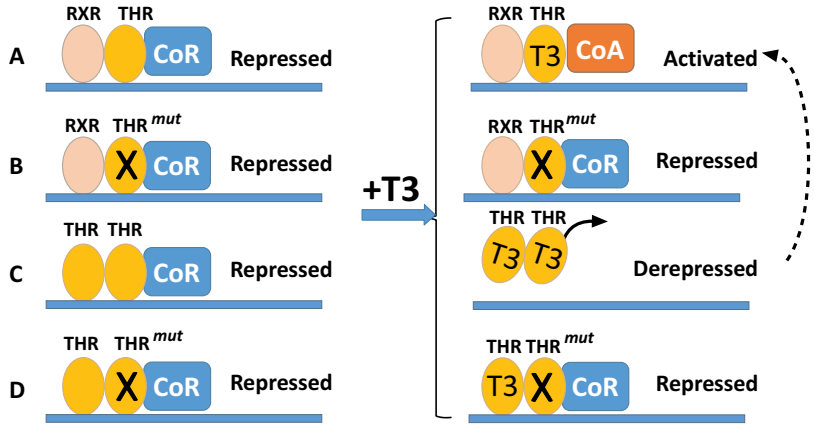

Fig. (2). Dominant negative activity of mutant receptors. A: The thyroid hormone receptor (THR) heterodimerizes with RXR. Without T3, the RXR-THR heterodimer recruits nuclear receptor corepressors (CoR, mainly NCoR) and represses gene expression. In the presence of T3, CoR is released, and coactivators (CoA) are recruited, activating gene expression. B: A mutant THR (THR^{mut}), unable to bind T3, maintains its association with CoR and gene repression in the presence of T3. C: THR homodimers repress gene expression and, upon T3 binding, dissociate from DNA and derepress gene expression. Homodimer dissociation allows the RXR-THR heterodimer to bind to DNA sites, and gene expression is activated upon T3 binding as in A. D: A receptor mutant bound to a wild type THRα or THRβ maintains the association with CoR and gene repression. These mechanisms require the mutant receptors to keep the ability to form homo or heterodimers and bind DNA. The information to construct this figure comes from [17].

CHAPTER 10

Thyroid Hormone-Regulated Genes in the Brain

Abstract: Thyroid hormone exerts its actions by binding to nuclear receptors and regulating gene expression. Gene expression regulation by thyroid hormone in the brain is highly complex, with thousands of genes under the direct or indirect influence of T3. Adding to the complexity, gene dependence of T3 is age- and region-dependent, with diverse time window sensitivity. The maximal gene expression responses to T3 in rodents extend from the last 2-3 days of fetal life to the end of the first month, peaking around postnatal days 15-21. T3 regulates genes involved in almost all aspects of brain function, from developmental genes to genes involved in metabolic and cell signaling pathways. In most cases, the effect of T3 is to fine-tune the relative abundance of selected gene products at the right time and place, promoting maturational processes during developmental transitions.

Keywords: Astrocytes, Cerebrocortical cells, Cell migration, Development, Extracellular matrix, Neurotransmitter receptors, Potassium channels, Retinoic acid, Transcriptional regulation.

INTRODUCTION

The regulation of gene expression is pivotal for understanding thyroid hormone action in the brain. This chapter delineates general aspects of thyroid hormone regulation of gene expression, with subsequent chapters delving into specific gene expression patterns within the framework of developmental processes, including myelination and the development of the cerebral cortex, hippocampus, cerebellum, striatum, and sense organs.

T3 serves as a regulator of receptor activity within the cell nucleus, orchestrating the transcriptional rate of specific genes [1]. Receptors bind to short DNA sequences known as thyroid hormone-responsive elements (TRE) and engage with gene expression regulatory complexes, encompassing corepressors and coactivators. Most commonly, the unliganded receptor or aporeceptor typically interacts with corepressors, whereas the liganded receptor or holoreceptor associates with coactivators. Understanding thyroid hormone action necessitates the identification of regulated genes and their roles in developmental and physiological processes. Describing these actions linearly proves challenging,

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given the pleiotropic results of T3 action, which manifests as a network of intricately interconnected pathways akin to a shape-changing web.

Early studies after discovering the T3 nuclear receptors showed a fundamental difference between the brain and other tissues. While a correlation existed between receptor concentration and the effects of thyroid hormone on oxygen consumption in most tissues, exceptions included the brain, spleen and testis [2, 3]. Responses to thyroid hormone in the brain were elusive until the identification of the first thyroid hormone-dependent mRNAs [4 - 6].

One approach to identifying thyroid hormone-regulated genes involves scrutinizing genes with altered expression in hypothyroidism [5]. Animals, usually rats or mice, rendered hypothyroid through surgical, chemical, or genetic means undergo quantification of gene expression, allowing a comparison with non-treated animals. This method gauges aporeceptor activity, which may or may not align with the physiological role of thyroid hormones. The indirect effects of systemic hypothyroidism tend to overshadow those of direct cellular actions [7].

A more direct approach involves analyzing T3 effects on cultured cells, preferably primary cultured cells [8], which have not been exposed to oncogene action for immortalization. The ultimate goal is to identify T3 target genes, *i.e.*, genes responding directly after T3-receptor interaction, and contextualize gene activity within the physiological response to thyroid hormones. The definition of a particular gene as a direct transcriptional target of T3 necessitates a TRE mediating transcriptional control (type I action).

Directly regulated genes in specific cells *in vivo* can also be pinpointed by expressing a mutated version of the T3 receptor with dominant negative activity using the Cre/loxP technology. Flamant's group has utilized this approach to analyze the response of GABAergic neurons and other cells [9, 10].

How many genes of neural expression are thyroid hormone targets in rodents? Our study using cultured mouse cerebrocortical cells in primary culture estimated that T3 regulates the expression of approximately one thousand genes, 371 at the transcription level, constituting 2% of expressed genes, and at least 106 contain a T3 receptor binding site [8]. A meta-analysis of *in vivo* and *in vitro* studies concluded that T3 regulates 734 genes in the rodent brain, with at least 28 genes that can be considered genuine T3 target genes [11]. A recent resource dataset of thyroid hormone-regulated genes is available [12].

PATTERNS OF BRAIN GENE REGULATION BY THYROID HORMONE *IN VIVO*

Before the advent of high-throughput gene expression techniques, such as microarrays [13] and RNA sequencing [RNA-Seq [14, 15]], it was usual to analyze one gene at a time, focusing on the developmental expression patterns in the absence or presence of thyroid hormones. In rodents, most known thyroid hormone-regulated genes were sensitive to thyroid hormone only during a developmental window, mainly during the postnatal period, with a few exceptions, such as neurogranin (*Nrgn,* previously known as *Rc3* for Rat cortex 3) [16], which was also sensitive in adult animals. The thyroid hormone control of gene expression timing is region-dependent for many genes. The usual effect of early hypothyroidism is a delay in the temporal expression of a gene in some regions, while other regions may not be affected. Following are some examples of these types of controls

Controlling Gene Expression Timing

The maximal sensitivity of the rodent brain to thyroid hormone is during the first month of life. With a few exceptions, the role of thyroid hormone is to control the timing and the rate of gene transcription. The onset of mRNA and protein accumulation is delayed in hypothyroid animals, but their final tissue concentrations eventually reach the expected value even without treatment [17]. *Reln* (encoding the protein reelin) and the myelin genes are two examples of this phenomenon.

Reelin Expression

Reelin [18] is an extracellular matrix protein expressed by several cell types, including Cajal-Retzius cells located in the marginal layer or future layer 1 of the developing cerebral cortex [19]. In rodents, the hippocampus, olfactory bulb, and cerebellar granule cells also express *Reln*. Reelin has a crucial role in the radial migration of neurons during development. In the cortex, reelin halts the migration of neurons originating in the ventricular and subventricular layers, which migrate during the formation of the cortical layers by the process known as “inside-out migration” and is, therefore, crucial for proper cortex layering. Disruption of the *Reln* gene in the *reeler* mouse results in disorganized cortical layers, with cell mispositioning, causing a characteristic neurological phenotype [20]. T3 increases reelin protein concentration *in vivo* and after addition to cultured hippocampal slices from hypothyroid rats (Fig. **1**) and [21].

CHAPTER 11

Actions of Thyroid Hormones on Myelination

Abstract: The control of myelination in the central nervous system is a classical action of thyroid hormones. In rodents, thyroid hormone deficiency during the fetal and postnatal periods delays central myelin deposition and oligodendrocyte gene expression. Oligodendrocytes differentiate from precursor cells (OPC), originating from radial glial cells in the ventricular and subventricular zones after multiple cell fate decisions controlled by developmental genes. The interplay between growth factors acting at the cell membranes and nuclear receptors, such as those for T3 and retinoic acid, regulates OPC differentiation. Growth factors promote OPC proliferation, and the liganded nuclear receptors promote cell cycle exit. Myelination occurs in axons that reach a critical size, and thyroid hormone might also indirectly affect myelination through axonal maturation effects. In the clinical setting, myelination can be analyzed by magnetic resonance imaging in hypothyroid states with variable results.

Keywords: Axons, Allan-Herndon-Dudley syndrome, Cell cycle, Congenital hypothyroidism, Myelin, MBP, Magnetic resonance, Nuclear receptors, OPC, Oligodendrocytes, PDGF, PLP, OLIG2, SHH, White matter.

INTRODUCTION

This chapter focuses on one of the major physiological processes controlled by thyroid hormone: central myelination. Myelination is a precise and highly orchestrated process controlled by many factors, including thyroid hormone. In the central nervous system, myelination is carried out by oligodendrocytes, the myelin-producing cells in white and gray matter. These cells form many individual sheet-like extensions of the cell membrane, undergoing a large increase in surface area. Myelination involves recognizing the axon followed by the spiral wrapping of these membrane extensions around the axon with extrusion of the cytoplasm (compact myelin). Myelin insulates the axons, providing metabolic support and enabling saltatory conduction of electric signals [1].

Mature myelin consists of 75% lipids and 25% proteins. The lipids include galactosphingolipids, cerebrosides, phospholipids, saturated long-chain fatty acids, and cholesterol. Among the proteins of central myelin, four are unique to myelin. The proteolipid protein [PLP, P_0 in the peripheral nervous system [2]] is the most abundant and contributes to binding the myelin wraps by adhesion at the

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outer side of the membrane. Missense *PLP* mutations in mice lead to the *jimpy* mouse [3], and *PLP* mutations in humans to Pelizaeus-Merzbacher disease [4]. The second most abundant is myelin basic protein (MBP), which adhere to each other on adjacent membrane surfaces, acting as a zipper. In mice, *Mbp* gene defects produce the *shiverer* mouse [5]. The 2,3-cyclic nucleotide phosphodiesterase (CNP) interacts with actin filaments. The myelin-associated glycoprotein (MAG) accounts for one percent of the proteins. MAG is a member of the immunoglobulin superfamily localized in the periaxonal side of the oligodendrocyte membrane.

Several factors promote myelination. Axon electric activity is essential for activity-dependent oligodendrocyte generation and myelination throughout life [6]. Axon size is a significant factor in promoting myelination, with a critical size below which axons remain unmyelinated [7 - 9].

THE GENERATION OF OLIGODENDROCYTES

Before delving into the role of thyroid hormone, let us briefly review the earliest steps of neurogenesis and gliogenesis in the CNS. At the beginning of neurogenesis, the neural tube neuroepithelial cells give rise to the radial glial cells, the universal stem cells, from which all cells of the CNS derive, except the microglia [10]. The neural tube undergoes regionalization into ventral and dorsal parts and subsequent cell specification. Pattern formation and positional information in the neural tube are regulated by gradients of ventralizing sonic hedgehog (SHH) and dorsalizing bone morphogenetic proteins (BMPs). Subsequently, specific transcription factors act on cell fate decisions and progeny subtype specializations [11, 12].

One of the genes induced by SHH is *Olig2*, which is essential for developing the oligodendrocyte lineage. BMPs and WNT inhibit OLIG2 and specify astrocytes. OLIG2 defines different paths depending on its phosphorylation state [11]. The phosphorylated OLIG2 promotes neuron specification in rodents at embryonic (E) days E9-E10.5. By E12.5, OLIG2 dephosphorylation causes a neuron-glial switch through sequestration of the proneural factor NGN2, leading to the formation of oligodendrocyte precursor cells (OPCs). The DLX1/2 transcription factors repress OLIG2 and develop interneurons. OLIG1 acts in later stages and is not required for OPC generation. OPCs migrate to different places, and the OLIG2-induced SOX10 and NKX2.2 promote the expression of *Pdgfra* (platelet-derived growth factor receptor α), which is characteristic of OPCs. PDGFRA promotes OPC proliferation and survival.

Another relevant factor involved in cell fate decisions is ASCL1, also known as MASH1. Early in development, ASCL1 acts as a proneural factor, and at later

stages, it becomes involved in the neuron-glial switch promoting OPCs at the expense of astrocytes [11].

OPCs arise in several waves from the ventricular zones of different structures and migrate in different directions [12, 13]. In mice, the first wave of OPC generation occurs at E12.5 in the medial ganglionic eminence and entopeduncular area from *Nkx2.1*-expressing precursors. A second wave takes place at E15.5 in the lateral and caudal ganglionic eminences from *Gsx2* precursors. The third wave occurs at the time of birth from *Emx1* precursors in the cortical ventricular zone. All these different OPC lineages converge to cells with similar transcriptional profiles postnatally [14]. Terminal differentiation of OPCs into oligodendrocytes and myelination occurs after the OPCs exit the cell cycle.

Whereas in the peripheral nervous system, myelination by Schwan cells depends on the presence of neuregulin on the axon surface; in the CNS, myelination is a default pathway of oligodendrocytes [1]. Oligodendrocyte differentiation results from the balance between inhibitors of differentiation and promyelinating factors. Among the former are the NOTCH and WNT pathways [15], the proneural factors SOX5 and SOX6, and the inhibitors of DNA binding 1 and 2 (ID1 and ID2). The promyelinating factors, OLIG1, NKX2.2, and SOX10, are induced during migration. OLIG1 and SOX10 induce *Mbp* expression [11].

Oligodendrogenesis in Humans

Recent studies using single-cell RNA-Seq have provided insights into the origin of human OPCs. These cells arise from the outer radial glia (oRG) in the enlarged outer subventricular zone [16]. They give rise to pre-OPCs expressing the epidermal growth factor receptor (EGFR) and OPCs late in the second trimester. Unlike rodents, these human OPCs are transient amplifying cells undergoing repeated symmetric divisions that greatly enlarge the progenitor pool. This feature is unique to humans and contributes to the great increase in white matter content. White matter accounts for 50% of human brain weight, whereas in rodents, it is 15% [1]. OPCs increase dramatically by gestational week 24 (GW24) at the end of neurogenesis. The dividing OPCs produce daughter cells that repel each other and migrate in different directions. OPC dispersion lowers cell density and facilitates OPC proliferation and white matter expansion. Protocadherin 15 (PCDH15) is involved in cell repulsion [16].

The common thought is that gliogenesis starts after the termination of neurogenesis by GW25. However, van Bruggen *et al*. [17] have identified oligodendrocyte lineage cells as early as GW8. These pre-OPCs originate from radial glia in the ventricular zone of the medial ganglionic eminence and a subpopulation of outer radial glia in the subventricular zone. This early wave of

CHAPTER 12

How Thyroid Hormones Shape the Brain

Abstract: This chapter provides a comprehensive exploration of the role of thyroid hormones in the development of key brain structures: the cerebral cortex, hippocampus, striatum, and cerebellum, as well as the sense organs retina and cochlea. Hypothyroidism is generally associated with impairments in axodendritic development, synaptogenesis, neuron migration and differentiation, and myelination. In the developing cerebral cortex, hypothyroidism delays the appearance of Cajal-Retzius cells, critical for the proper migration of neurons, causing migration defects. The maturation of the transient subplate layer, crucial for establishing thalamocortical connections, is also delayed. The hippocampal formation experiences a reduction in the number of granular cells and mossy fibers. In the cerebellum, hypothyroidism arrests the maturation of the Purkinje cells and delays the migration of the granular cells to the internal granular layer. In the striatum, hypothyroidism delays the accumulation of the medium-spiny GABAergic neurons, the principal cells of the striatum. Parvalbumin interneurons in the cerebral and cerebellar cortices are also affected. Thyroid hormone induces extensive remodeling during cochlear and retinal maturation. Contrary to expectations, receptor-deficient mice often do not exhibit these alterations, while the expression of mutant receptors with impaired T3 binding results in hypothyroid features. In rodents, the effects of thyroid hormones are most prominent during the postnatal period. Conversely, in humans, the second trimester of pregnancy is a crucial period for neural development. The coordinated development of the thyroid hormone signaling system, encompassing brain T3 and the ontogenesis of receptors, deiodinases, and regulated genes, closely aligns with late maturational processes. This intricate interplay underscores the significance of thyroid hormones in shaping the structural and functional aspects of the developing brain.

Keywords: Basal ganglia, Cajal-Retzius cells, Cerebellum, Cerebral cortex, Cochlea, Cones, Cortical layering, Cortical plate, Deiodinases, Granular cells, Hair cells, Hippocampus, Interneurons, Neocortex, Nuclear receptors, Purkinje cells, Pyramidal cells, Radial glial cells, Retina, Rods, Subplate.

INTRODUCTION

Clinical evidence surrounding congenital hypothyroidism underscored the essential role of thyroid hormones in the maturation of the central nervous system. Early initiation of thyroid hormone treatment proved beneficial in preventing growth retardation and intellectual deficits associated with congenital hypothy-

roidism. The changes caused by thyroid hormone deficiency during the perinatal period were irreversible if treatment was delayed by several weeks.

Observations of severe iodine deficiency, particularly in cases of neurological cretinism, further emphasized the irreversible damage to the cerebral cortex and basal ganglia during fetal development. These clinical insights led to the formulation of the concept of thyroid hormone-dependent critical developmental stages. Subsequent investigations, facilitated by the use of rat and, more recently, mouse models, sought to analyze these critical stages.

Most brain growth in humans and rodents occurs after birth, but the newborn rat's thyroid gland is less developed than the human's thyroid at birth. A newborn rat aligns with a second-trimester human fetus, and a newborn human corresponds to a 6-10-day-old rat. As an approximate and helpful reference, we may take a whole trimester of human pregnancy, equivalent to 10 days in the rat. While acknowledging the simplicity of this reference, it is essential to recognize that evolutionary acquisitions in neural structure and function often impede direct species comparisons [1, 2]. Consequently, this chapter will highlight human findings when possible.

In rats, the main critical period for thyroid hormone action spans from embryonic day 17-18 (E17-E18) to weaning on postnatal day 21 (P21), extending to the end of the first month. The thyroid gland initiates hormone secretion by E17.5, and thyroid hormones reach maximal concentrations in the brain by P15, marking the peak of thyroid hormone sensitivity. Notably, the presence of thyroid hormone receptors in the brain by E14.5 suggests that the critical period may commence several days before the onset of thyroid gland function. The critical period of thyroid hormone dependency varies with specific developmental processes, exemplified by myelination. The myelination wave proceeds from a caudal to a rostral fashion. While the critical period for myelin gene expression in the cerebellum concludes by P10, it extends beyond the first month for the cerebral cortex [3].

THYROID HORMONES PROMOTE AXODENDRITIC DEVELOPMENT

Early seminal work by Eyars and Taylor [4] defined the primary impacts of hypothyroidism on the brain. Neonatal hypothyroidism induced by methyl thiouracil thyroid blockade from the first day of life until P15 or P24 resulted in reduced body growth, primarily due to secondary growth hormone deficiency [5], and smaller brains. Animals with hypothyroidism exhibited delayed acquisition of stereotyped behaviors, including the air-righting response and placing reflex. In the cerebral cortex, pyramidal neurons in layer V were smaller and increased in number per surface unit. The heightened cell density was attributed to deficient

accumulation of "interstitial substance" and impaired axodendritic network development (often known as the neuropil), stemming from reduced axon and dendrite growth, leading to diminished neuronal connections.

Subsequent rigorous cortical morphometric studies by Madeira *et al.* [6] validated Eyars' findings on neuropil reduction following hypothyroidism. Studies by Ruiz-Marcos and Morreale de Escobar [7 - 10] emphasized dendritic spines as significant targets of thyroid hormone action. The dendritic spines are small protrusions along the dendrites through which neurons establish connections. Each axon terminal contacts the head of a spine, transmitting the synaptic signal. Dendrites may have thousands of spines, which are dynamic structures in continuous remodeling. The distribution of dendritic spines along the apical shaft of pyramidal cells follows a mathematical model [11]. Postnatal hypothyroidism decreases the number of dendritic spines of pyramidal cells, with the remaining spines deviating from the mathematical distribution model related to the severity of hypothyroidism. Early T4 treatment prevents these changes, which otherwise become irreversible [12]. Adult-onset hypothyroidism also reduced spine numbers without deviating from the mathematical distribution model [13].

Other studies looked at the development of the cholinergic system. Patel *et al.* [14] proposed that the interaction between thyroid hormones and NGF controls subcortical cholinergic neurons. Hypothyroidism arrests the development of the forebrain cholinergic system taking place during the first postnatal weeks, particularly the nucleus basalis of Meynert (NBM, Fig. **1**), providing cholinergic fibers to the cortex, striatum, hippocampus, and amygdala [15, 16]. On the other hand, the brain stem cholinergic system, providing fibers to the thalamus, is not sensitive to thyroid hormones [17].

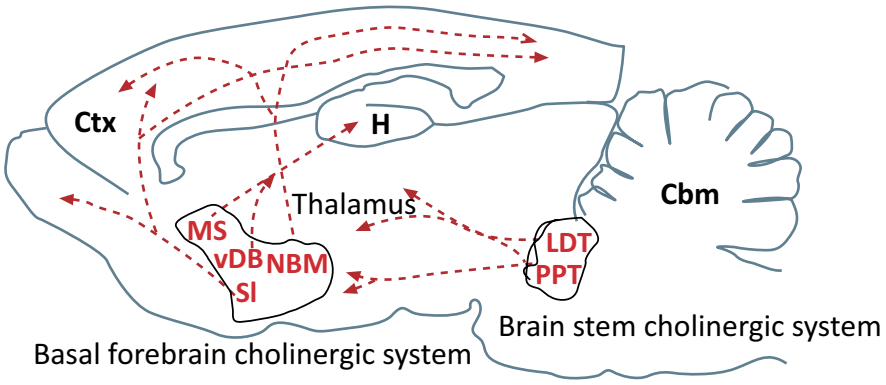

Fig. (1). The rodent cholinergic systems. MS, medial septum; vDB, vertical limbs of Diagonal band of Broca; NBM, nucleus basalis of Meynert; SI, substantia innominata; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nuclei; Cbm, cerebellum; H, hippocampus; Ctx, cerebral cortex.

CHAPTER 13

Mechanisms of Thyroid Hormone Action on Adult Neurogenesis

Abstract: In adult mammals, neurogenesis persists throughout life in two active sites: the ventricular-subventricular zone along the lateral ventricles and the subgranular zone of the hippocampus. In rodents, postnatal neural stem cells with astrocytic properties, originating from embryonic ventricular radial glia, generate a continuous, lifelong supply of neurons for the olfactory bulb and glia for the corpus callosum. Thyroid hormones play a regulatory role in this process. In humans, ventricular neurogenesis is minimal, but hippocampal neurogenesis extensively remodels the dentate gyrus, influencing memory and mood. Hippocampal neurogenesis begins with stem cells in the dentate gyrus subgranular layer, generating a sequential lineage of intermediate precursors and neuroblasts. These neuroblasts migrate to the granular layer, differentiate into granular cells, and integrate into the existing dentate gyrus neuronal pool. Thyroid hormone specifically regulates the late stages of this process, promoting the terminal differentiation of neuroblasts and facilitating their functional integration. Hypothyroidism disrupts hippocampal neurogenesis, impacting learning, memory, and mood. The intricate regulation of adult neurogenesis by thyroid hormone highlights their crucial role in maintaining cognitive and emotional functions.

Keywords: Astrocytes, Calbindin, Calretinin, Dentate gyrus, Differentiation, Hippocampus, Learning, Memory, Neuroblasts, Proliferation, Radial glia, Subgranular zone, Stem cells.

INTRODUCTION

Neurogenesis is a developmental process generating functionally integrated neurons. Studies on rodents have defined two neurogenic niches that supply neurons to the dentate gyrus and the olfactory bulb throughout life; the precursors of these neurons are stem cells located in the dentate gyrus subgranular zone and the lateral ventricle subventricular zone, respectively. Adult neurogenesis induces substantial remodeling of the dentate gyrus in humans. However, unlike mice, where neurogenesis in the subventricular zone of the lateral ventricles is significant, the extent of this process in humans remains a matter of controversy [1, 2]. Environmental enrichment, physical exercise, and learning new tasks can stimulate adult neurogenesis.

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NEUROGENESIS IN THE ADULT RODENT SUBVENTRICULAR ZONE

The adult rodent brain contains neural stem cells along the walls of the lateral ventricles in a zone with characteristics of the embryonic ventricular and subventricular zones (Fig. **1**) [3, 4]. These neural stem cells (B cells) are a particular type of astrocytes that establish contact with vessels and connect to the ventricular space *via* a primary cilium. B cells originate from radial glia at postnatal stages. Between postnatal day 7 (P7) and P12, they acquire neural and gliogenic potential and can be activated from a quiescent state to produce a stable supply of neurons and glia. First, they produce transit-amplifying precursors (C cells), which generate DCX (doublecortin)-expressing neuroblasts (A cells). Neuroblasts then migrate *via* the rostral migratory stream to the olfactory bulb, differentiating into GABAergic interneurons. B cells also generate a small proportion of oligodendrocyte precursor cells, which migrate mainly to the corpus callosum and astrocytes.

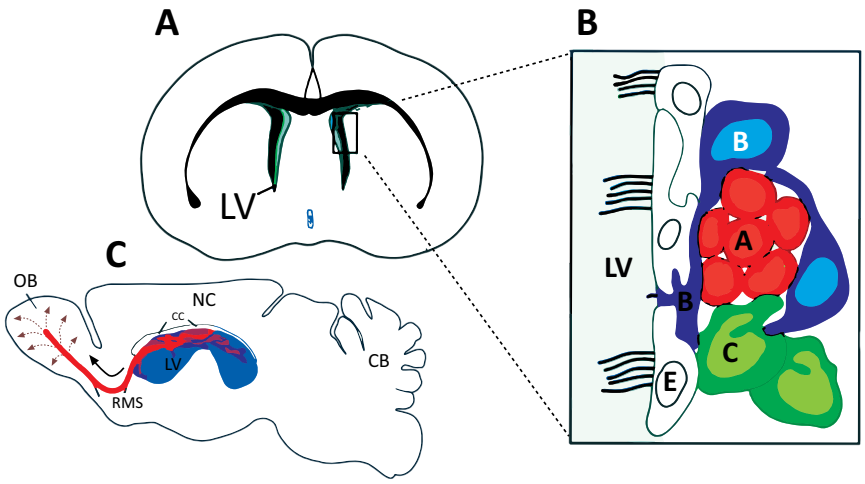

Fig. (1). Adult neurogenesis in the rodent subventricular zone. **A**: Drawing of an anterior coronal section of the adult rodent brain showing the location of the lateral ventricles (LV). **B**: Cellular organization of the subventricular layer adjacent to the ependymal layer of the lateral ventricle. The neural stem cells (B cells, blue), contacting the cerebrospinal fluid through a primary cilium, give rise to transient-amplifying precursors (C cells), which generate the neuroblasts (A cells)). **C**: Drawing of a sagittal section of the adult rodent brain showing the rostral migratory stream (RMS) in red, through which the neuroblasts, or A cells, migrate to the olfactory bulb (OB) where they differentiate into interneurons. Reproduced with permission from Alvarez-Buylla and García-Verdugo [3]. Copyright 2002, Society for Neuroscience.

Vancamp *et al.* [5] hypothesized a particular role of thyroid hormones in this postnatal transition because it coincides with the postnatal surge of, and maximal sensitivity to, thyroid hormones. From P7 to P20, astrocytes increase in the

subventricular zone. These cells express *Dio2* and the T4 transporter *Oatp1c1*, and the TRα1 receptor isoform is widely expressed. Therefore, the subventricular zone contains all the elements necessary for T3 action [5]. Furthermore, hypothyroidism alters the expression of thyroid hormone targets and disrupts neurogenesis by blocking the cell cycle and increasing the number of *Sox2*-expressing progenitors. The view is that in the adult subventricular zone, T3 acts through the TRα1 receptor, repressing the neural stem cell gene *Sox2* and favoring the appearance of DCX-positive migrating neuroblasts [6, 7].

As stated above, adult neurogenesis in the subventricular zone is very active in rodents and is vital for olfaction. Some authors doubt its relevance in humans, in contrast to the importance of hippocampal neurogenesis [1].

ADULT HIPPOCAMPAL NEUROGENESIS

Adult hippocampal neurogenesis (Fig. **2**) is subject to regulation by thyroid hormone [8]. Adults with hypothyroidism exhibit reduced hippocampal volume [9] and memory deficits related to hippocampal deficiency [10], indicating that dysregulation of adult hippocampal neurogenesis may be implicated in these alterations, impacting learning, memory, and mood and contributing to depression and anxiety states.

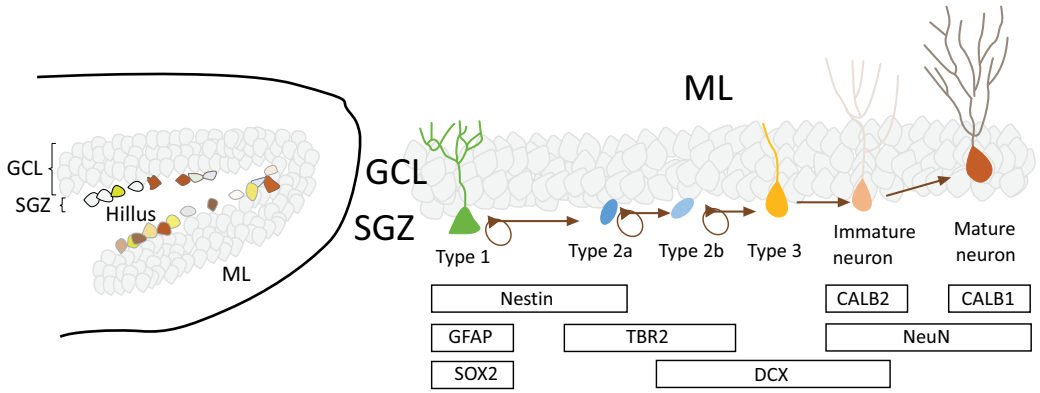

Fig. (2). Adult neurogenesis in the dentate gyrus's subgranular zone (SGZ). Left panel: the dentate gyrus, with the location of the SGZ. GCL, granular cell layer; ML, molecular layer. Right panel: stages of adult neurogenic progenitors progression to differentiated granular cell neurons. Specific markers are expressed at different stages. The arrowed circles indicate precursor self-renewal through symmetric division.

Stem cells in the dentate gyrus subgranular zone (Type 1 precursors) express nestin, GFAP, and SOX2. These cells are in a reversible quiescence state. A minor fraction of these cells undergo asymmetric division, giving rise to a lineage of intermediate precursor cells. Recent studies have identified factors that regulate

CHAPTER 14

Thyroid Hormones and Mood Disorders

Abstract: Thyroid hormone deficiency or excess may cause emotional disturbances and mood disorders, encompassing major depressive syndromes and bipolar disorders, along with various other neuropsychiatric conditions, some of which may have developmental origins. In particular, profound long-term untreated hypothyroidism can culminate in severe psychosis, historically referred to as myxedema madness. Addressing the underlying thyroid condition typically proves highly effective in rectifying the associated brain disorder. Subclinical thyroid diseases have also been implicated in emotional and cognitive disorders, prompting inquiry into the optimal treatment window. Moreover, thyroid hormones have demonstrated potential in expediting or augmenting the effects of standard mood disorder treatments in euthyroid patients, hinting at a baseline state of localized cerebral hypothyroidism with an uncertain pathogenesis, potentially remediable through high doses of thyroid hormones.

Keywords: Alzheimer's disease, Bipolar disorders, Depression, Hypothyroidism, Hyperthyroidism, Mood disorders, Rapid cycling, Subclinical hypothyroidism, Subclinical hyperthyroidism.

INTRODUCTION

In 1898, the Clinical Society of London published the report of a committee that had been established five years prior "to investigate the subject of myxedema". The term "myxedema" was coined by William M. Ord, the committee's Chairman, in 1877, during an era when the disorder was perceived as an independent ailment characterized by widespread mucin deposition. The attribution of its origin to the thyroid gland emerged from observations made by Theodor Kocher in 1883, wherein total thyroidectomy replicated all symptoms associated with myxedema [1].

The report on myxedema concluded that "*a general review of symptoms and pathology leads to the belief that the disease described under the name of myxoedema, as observed in adults, is practically the same disease as that named sporadic cretinism when affecting children; that myxoedema is probably identical with cachexia strumipriva; and that a very close affinity exists between myxoedema and endemic cretinism*." And "*That while these several conditions*

appear, in the main, to depend on, or to be associated with, destruction or loss of the function of the thyroid gland, the ultimate cause of such destruction or loss is at present not evident." [quoted in Doyle, 1991 [1]].

Despite the recognized benefits of thyroid hormone treatment utilizing sheep thyroid extracts [2], initially demonstrated by Bettencourt and Serrano in 1890 [3] and swiftly corroborated by Murray in 1891 [4], a significant number of undiagnosed, longstanding cases of myxedema persisted, leading to admissions in psychiatric wards without the advantage of thyroid intervention. This was underscored in the 1949 report by Asher entitled "Myxedematous Madness" [5], where he detailed fourteen severe myxedema cases characterized by confusion, dementia, disorientation, and paranoid ideation. The majority of cases exhibited notable improvement with thyroid treatment. In his account, Asher pondered on the potential prevalence of undiagnosed patients with similar presentations in mental health institutions, mistakenly categorized as hopeless psychotics who might have greatly benefited from thyroid therapy. He observed that this condition was rarely identified and attributed his awareness of it to his reading of Cronin's novel "The Citadel" [6]. Asher expressed: "A*part from medical textbooks, A. J. Cronin paints a vivid if sensational picture of a mad myxoedema, and it was from this source that I first learned of the condition*." Fortunately, psychotic cases caused by thyroid dysfunction, as those described by Asher and other authors, are now a rarity because hypothyroid patients receive early treatment.

It is a recurrent theme in thyroid research when studying the brain as a target of thyroid hormones to find unexpected phenotypic associations of genetically manipulated experimental animals with mental states and psychiatric conditions. This suggests that, as seen in the historical precedent of "myxedematous madness", certain neurological and psychiatric disorders may be linked to undetected thyroid dysfunction. An illustrative case expounded in Chapter **7** is the severe neurological condition known as Allan-Herndon-Dudley syndrome, described in 1944. In 2004 it was found to result from mutations in a cellular transporter for thyroid hormones [7].

THYROID FUNCTION AND MOOD DISORDERS

The prevalence of mood disorders is much higher in females than in males, with gender differences observed in their initiation, course, and responsiveness to treatments. Specifically, women have a higher incidence of unipolar depression and bipolar II disorder, characterized by recurrent episodes of depression and hypomania [8]. Additionally, women tend to experience more persistent and treatment-resistant rapid cycling. Although most studies on the relationship

between thyroid hormones and cognition and behavior have been conducted in Caucasians, differences with non-Caucasian populations primarily pertain to the frequency of thyroid diseases, with similar associations found between thyroid dysregulation and mood disorders [9].

Hypothyroidism

Adult-onset hypothyroidism causes deficits in attention, concentration, alertness, slowing of thought and speech, recent memory deficits, difficulty with tasks requiring abstract thinking, and dementia in the long term [10, 11]. They also have an increased propensity to anxiety and melancholic depression. In general, T4 treatment is very effective.

Hyperthyroidism

Hyperthyroid patients are often irritable and agitated, and depression and anxiety are frequent [11, 12]. Studies in Denmark using Danish Health registers [13] found a significant association between hyperthyroidism and the use of antipsychotics and antidepressants, and an increased risk for hospitalizations due to psychiatric conditions. Similarly, the recent study by Folkestad *et al.* [14] using two large Danish registers found a significantly increased risk of dementia, whether Alzheimer's or vascular, in Grave's disease and toxic nodular goiter. A recent observational study [15, 16] on more than 65,000 patients over 65 years old found a clear association between hyperthyroidism and the risk of dementia. The study incorporated iatrogenic hyperthyroidism, which was neglected in other studies despite being the major cause of hyperthyroidism.

Subclinical Thyroid Disease

In subclinical hypothyroidism (SHypo), serum T4 and T3 are normal, but TSH is above 4.0-4.5 mIU/L. In subclinical hyperthyroidism (SHyper), thyroid hormones are in the upper reference level, and TSH is low or undetectable [17]. The prevalence of subclinical thyroid disease in the general population is high, up to 15% [18]. Treatment for SHypo is indicated when TSH is above 10 mIU/L [19]. It is important to clarify whether subclinical thyroid diseases have an increased risk of cognitive or mood disorders. In the case of SHypo, this is crucial when TSH is below the established limit for treatment and the subject merits a more extensive discussion.

In some observational studies, SHypo is associated with an increased risk of neuropsychiatric morbidities, such as depression [20], memory deficits, and altered executive function correlating with functional abnormalities of the frontal lobes [21]. However, other studies involving many patients showed no significant

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