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Perspectives in the Study of Thyroid Hormone Action on Brain Development and Function

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The purpose of this review is to provide an up-to-date report on the molecular and physiologic processes involved in the role of thyroid hormone as an epigenetic factor in brain maturation. We summarize the available data on the control of brain gene expression by thyroid hormone, the correlation between gene expression and physiologic effects, and the likely mechanisms of action of thyroid hormone on brain gene expression. In addition we propose a role for unliganded thyroid hormone receptors in the pathogenesis of hypothyroidism. Finally, we review recent data indicating that thyroid hormone receptors have an impact on behavior.

Introduction

BRAIN DEVELOPMENT proceeds through precisely coordinated events in time and space. Induction of neural tissue, differentiation and migration of specific cell types, regional specialization or synapse formation are major milestones in a myriad of intricately associated events. Most of these events are largely determined by genetic factors (1). However, epigenetic factors, such as the thyroid hormones, are also important because they act to control the timing and coordination of mechanistically unrelated processes. For many years thyroid hormone has been considered essential for brain maturation in humans and other mammals. Deficiency of thyroid hormones during critical periods of development leads to profound and potentially irreversible defects of brain maturation (2,3), and clinical syndromes arising from thyroid hormone deficiency during fetal and postnatal periods have been extensively reviewed elsewhere (3,4).

The brain as a target of thyroid hormone

Most of the available evidence supports the view that thyroid hormone does not influence the major early developmental processes such as neural induction, neurulation, and establishment of polarity and segmentation. Thyroid hormone is involved in regulation of later events, such as cell migration and the formation of cortical layers, and in neuronal and glial cell differentiation. Thus, as a consequence of thyroid hormone deficiency during specific fetal stages, altered cell migration in the neocortex results in less defined cortical layering and altered distribution of callosal connections (5). Deficiencies of cell migration are also observed in the hippocampus, resulting in lower number of granule cells in the dentate gyrus. A defining feature of neonatal hypothyroidism is the delayed migration of granule cells in the cerebellum, which results in abnormal persistence of the external germinal layer.

Thyroid hormone also controls differentiation of not only neurons and oligodendrocytes (6), but also astrocytes, and microglia (7,8). Specific neuronal cell types are characteristically affected by hypothyroidism, such as pyramidal cells of the neocortex and Purkinje cells of the cerebellum. The latter show a strong dependence of thyroid hormone, because in its absence they do not fully develop their characteristic highly elaborated dendritic tree. On the other hand, lack of proper differentiation of oligodendrocytes results in myelination deficits.

Deiodinases and the control of triiodothyronine concentrations in the brain

The main active thyroid hormone is triiodothyronine (T3), which in the brain derives in large part from 5′ deiodination of thyroxine (T_4). This pathway is closely regulated by developmental and physiologic factors (9). Concentrations of T_3 in the brain are the result of transfer to the brain through the blood-brain barrier, by a poorly defined mechanism, and the local activities of deiodinases 2 (D2) and 3 (D3). D2 generates T_3 from T_4 by phenolic ring deiodination, whereas D3 inactivates T_4 and T_3 by tyrosil ring deiodination with generation of the inactive products reverse triiodothyronine (rT_3) and 3,3′ triiodothyronine (T_2), respectively. Expression of D2 and D3 in the brain is developmentally regulated. In the rat, D2 activity is low during the fetal period, and increases postnatally, whereas D3 follows the opposite pattern (10). D3 activity is high in placenta and in fetal tissues and high activity has been detected in the uterine implantation site (11). This pattern of expression suggests a protective role during development. In agreement with this, recent data

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have shown that inactivation of the D3 gene leads to partial lethality at birth and severe growth impairment (12). It may also be that D3 expression and the regulation of T_3 generation and its subsequent availability to target cells is a way to control the timing of thyroid hormone signaling in a fashion similar to the control of amphibian metamorphosis (13). In adult animals D3 is diffusely expressed in neurons throughout the brain (14). In contrast, during the late fetal and early postnatal period, D3 mRNA is highly concentrated in discrete nuclei such as the bed nucleus of stria terminalis, central amygdala, and the preoptic area (15). These nuclei are involved in sexual differentiation of the brain during the early postnatal period, but the functional significance for the restricted expression of D3 in these nuclei is not known.

D2 activity increases during the postnatal period and is sensitive to thyroid hormone concentrations. In hypothyroidism, its increased activity tends to normalize T_3 concentrations even with greatly reduced T_4 concentrations. In contrast with D3, which is expressed in neurons, D2 is expressed in two types of glial cells (16,17). The highest expression is found in the tanycytes, a specialized type of glial cells that line the lower third of the walls of the third ventricle. These cells send processes to the adjacent hypothalamus where they frequently end in blood vessels, and to the median eminence, ending in portal vessels. Thus, D2 in these cells could be involved in providing T_3 to the cerebrospinal fluid (CSF) from which it would reach nearby structures by diffusion, and/or the portal blood, influencing pituitary function. Stimulation of D2 in the tanycytes by cytokines with subsequent local production of T_3 and inhibition of thyrotropin (TSH) secretion has been recently suggested to play a role in nonthyroidal illness (18). D2 is also expressed throughout the brain in astrocytes and in some interneurons (19). Astrocytes appear therefore to have an active role not only in the uptake of T_4 from the blood through the blood-brain barrier, but also in generating T_3 and delivering it to nearby neurons.

Molecular basis of thyroid hormone action on brain development

Many of the effects of thyroid hormone on developmental processes in the brain can be correlated with the controlled expression of specific molecules. The most obvious is myelination, which is secondary to the effects of thyroid hormone on oligodendrocyte differentiation and the expression of oligodendrocyte specific genes (20). Lack of thyroid hormone during the postnatal period in rats, at the time of onset of myelination, strongly delays the expression of many oligodendrocyte genes, specifically the genes encoding proteins of myelin such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin-associated glycoprotein (MAG) (21). As a result, the number of myelinated axons in hypothyroid rats is strongly reduced (22).

How thyroid hormone influences differentiation of neural cells is poorly understood. It has been suggested that T_3 is an instructive factor in the early steps of oligodendrocyte generation from stem cells, and that it controls the timing of oligodendrocyte precursor cell differentiation (23,24). It has also recently been shown that T_3 promotes neuronal differentiation of embryonic stem cells in culture (25). The intimate molecular mechanisms by which thyroid hormone promotes differentiation is unknown. Molecular suspects include proteins regulating the cell cycle, such as E2F-1 (see Billon et al. [24]), p53, cyclins and cyclin-dependent kinase inhibitors (26). Thyroid hormone control of neurotrophin expression, especially NGF, BDN, and NT-3, may provide also an explanation for its effects on differentiation of particular cells, such as cholinergic neurons and Purkinje cells (27). In many cases the effects of thyroid hormone on differentiation are subtle but with potentially important functional consequences. For example, in hypothyroidism there is a decreased number of dendritic spines in pyramidal cells of the neocortex and hippocampus, which is reversible and also observed after adult-onset hypothyroidism (28). Although the gross morphology of the neurons is not appreciably modified, changes in spine density have strong consequences on synaptic plasticity. The mechanisms by which dendritic spines are affected by thyroid hormone are unknown. RC3/neurogranin (29), a dendritic spine protein is under thyroid hormone control, both in developing and in adult animals (30), but it is not known whether expression of this protein is related to spine formation.

Proper migration of neurons depends on the interaction of cell surface receptors with proteins present in the extracellular matrix. One of these proteins, reelin (31), is regulated by thyroid hormone during the late prenatal and early postnatal life in the rat (32). Reelin expression is essential for the orderly migration of neurons to their specific destinations in the cerebral and cerebellar cortices, and determines the normal pattern of cortical layers. This protein is produced by Cajal-Retzius cells, which express another thyroid hormone-regulated gene, prostaglandin D2 synthase (33). Other molecules reported to be involved in cell migration, such as laminin, tenascin C, and L1, are also under thyroid hormone control (34,35).

Most of the actions of thyroid hormone are exerted via nuclear receptors, which are ligand-modulated transcription factors. The physiologic ligand is T_3, and therefore it is considered to be the active form of thyroid hormone. Because T_4 has low affinity for the nuclear receptors, it might be considered as a prohormone, whose role would be to deliver T_3 intracellularly through 5′ deodination. There are, however, data suggesting that T_4 may have actions of its own. It regulates D2 activity in astrocytes and has a direct action on F-actin polymerization (36,37). Also in astrocytes, T_4 has been shown to influence integrin-laminin interactions (38), an important process in cell migration.

Whether these nongenomic actions of T_4 actually contribute *in vivo* to the effects of thyroid hormone on neural cell migration is however unknown. Work by Davis et al. (39) also suggests that T_4 has biologic activity *per se*. In this case, the action of T_4 is mediated via the microtubule-associated protein (MAP) kinase pathway and phosphorylation of the T_3 receptor and other targets (39). So far it is not known how this activity of T_4 may contribute to the overall physiologic activities of the thyroid hormones. With respect to the nongenomic actions, mice deficient in all forms of nuclear T_3 receptors have no signs of hyperthyroidism in the face of highly elevated circulating T_4 and T_3, suggesting that most effects of thyroid hormone are mediated through the nuclear receptors (40). Therefore, the contribution of nongenomic actions to the overall effects of thyroid hormone is not yet clear. Davis and Davis (41) have suggested that at least for the

heart, nongenomic actions of thyroid hormone determine the basal activity of transporters and ion channels. If this concept is extrapolated to the brain, the nongenomic actions of thyroid hormone could be important in neurotransmission.

Another long-standing controversial topic is the role of the mitochondria in the overall effects of thyroid hormone in brain and other tissues (4,42). It is clear that thyroid hormone influences mitochondrial function indirectly through the control of nuclear-encoded mitochondrial genes. In addition, the expression of mitochondrial-encoded genes are also influenced by the thyroidal status, and T_3 has direct actions on isolated mitochondria *in vitro* (43). Therefore, direct effects of T_3 on the mitochondria in vivo appear likely. Truncated forms of TRα_1 and RXRα have been described in the mitochondria (44), and it is proposed that T_3 may affect mitochondrial transcription in a way similar to the action on the nucleus. These topics have been recently reviewed in detail (45).

Patterns and mechanisms of gene regulation by thyroid hormone in the brain

During the past 10 years, we and others have identified a number of genes under thyroid hormone control in the brain (for a review, see Bernal [4]). This list of genes is likely to be greatly enlarged when global analysis of gene expression using cDNA arrays technologies are used to identify thyroid hormone target genes. Most of the genes identified so far are expressed and regulated by thyroid hormone during the postnatal period (Fig. 1), and the role of thyroid hormone is to accelerate the normal physiologic process of upregulation or downregulation that these genes experience after birth. A good example is provided by the myelin genes, which are induced a few days after birth, in parallel with the timing of oligodendrocyte differentiation and the myelination wave. In the absence of thyroid hormone, accumulation of myelin gene products, mRNA, and protein proceeds at a slower rate, and final normal concentrations are attained but later in development than in normal animals. Other genes show a region-specific dependence of T_3. One example is RC3. This gene is expressed in subsets of neurons of the cerebrum, and thyroid hormone is needed to achieve normal expression in discrete regions such as layer VI of the neocortex and retrosplenial region, caudate nucleus, and dentate gyrus. The gene is expressed in other regions such as the upper layers of neocortex, and pyramidal cells of hippocampus, but it is not sensitive to thyroid hormone in these locations despite

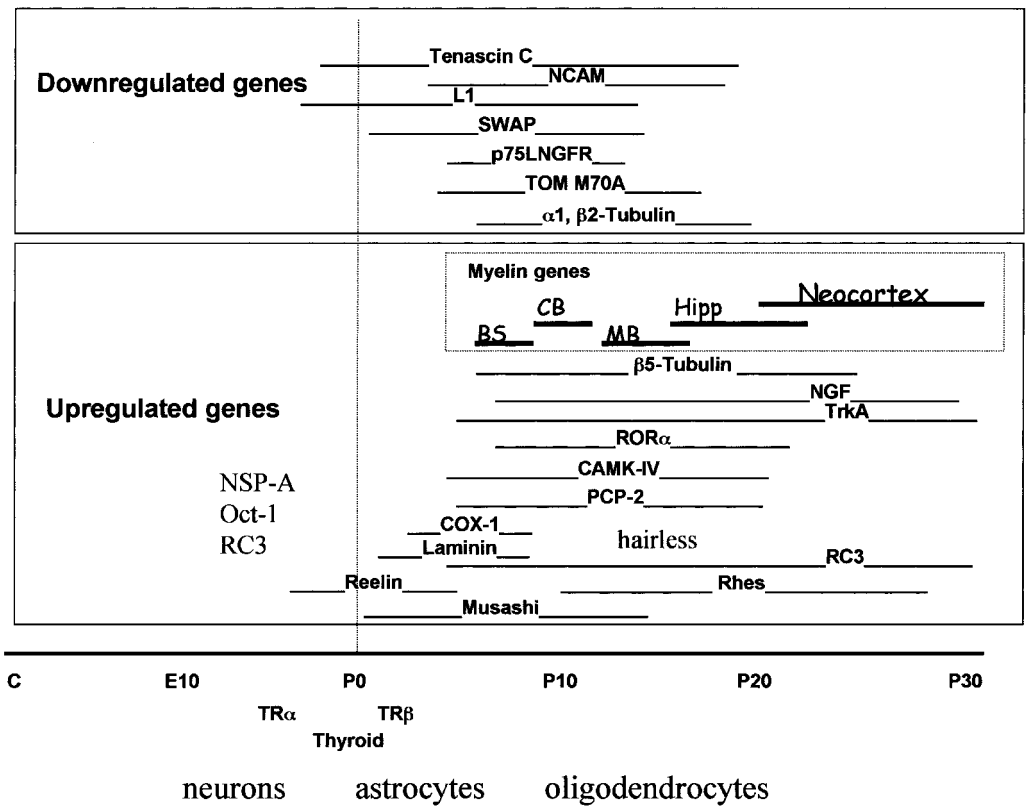

FIG. 1. Thyroid hormone-regulated genes in rat brain. The dependency of gene expression on thyroid hormone supply is represented schematically along a time line from conception (C) through embryonic (E) and postnatal (P) development. Birth is indicated by P0. Most genes are affected by the thyroidal status only during a limited period of development, which is indicated by the horizontal bars. NGF, TrkA, and RC3 are also thyroid hormone-dependent during adult life. A few genes, such as those encoding NSP-A, Oct-1, and RC3 have also been shown to be regulated during embryonic development. The inset delimited by the discontinuous lines illustrates the regional and temporal pattern of thyroid hormone regulation of myelin genes which follows the myelination wave, from the caudal to rostral regions. BS, brain stem; CB, cerebellum; MB, midbrain; Hipp, hippocampus. At the bottom of the figure, the approximate time of first detection of TRα and of TRβ, the appearance of the thyroid gland, and the peak generation of neurons, astrocytes and oligodendrocytes. Modified from Bernal (42) with permission from Elsevier.

the presence of T_3 receptors. Because RC3 is regulated by T_3 directly at the transcriptional level (46,47), the most likely explanation for such a region-specific control is that regulation of this gene is based on a combinatorial distribution of transcription factors, including T_3 receptors. Therefore, in sensitive regions, T_3 receptors might complement the pool of transcription factors needed for target gene expression.

Another example of region-specific control is ZAKI-4. This gene encodes a calcineurin inhibitor expressed throughout the brain (48), but sensitive to T_3 only in layer VI of neocortex. Regulation of this gene by T_3 is indirect because it requires new protein synthesis. In this case, it is likely that in layer VI, T_3 regulates expression of a protein required for ZAKI-4 expression. Recent evidence suggests that the mechanism of ZAKI-4 induction by thyroid hormone involves activation of phosphoinositide-3 kinase (49).

The temporal patterns of thyroid hormone-dependent gene expression in the brain suggest that the critical period of thyroid hormone sensitivity is limited to the first 2–3 postnatal weeks in the rat. In humans the sensitive period would correspondingly start after midpregnancy. However, there may be a bias in this concept, derived from the fact that most searches for thyroid hormone-dependent genes in the brain have been made during the postnatal period, at the peak of T_3 receptor expression and occupancy. From the data illustrated in Figure 1, it can be seen that the period of thyroid hormone sensitivity for some genes extends to the adult period (RC3, TrkA, and NGF) whereas for others it starts during the fetal period (tenascin C, L1, reelin). Earlier ages were not analyzed. In other studies (50,51) it was found that maternal hormones influenced fetal brain expression of RC3, NSP-A, and Oct-1. Maternal hormones have recently been demonstrated to play an important role in cell migration in the fetal neocortex (52). The application of global analysis of gene expression using suitable models of fetal hypothyroidism may help to identify T_3-regulated genes during fetal brain development.

The primary action of thyroid hormone on gene expression is mediated through interaction of the T_3 receptors with responsive elements located in gene regulatory regions (53). Some of the genes known to be responsive to thyroid hormone in the brain contain triiodothyronine response elements (TREs) and in some cases the action of T_3 has been shown to be at the transcriptional level *in vitro*. Genes containing TREs in their promoter or intronic regions include those encoding myeline basic protein (54), the Purkinje cell-specific gene (PCP2) (55), which encodes a G protein nucleotide exchange factor (56), the calmodulin binding and protein kinase C (PKC) substrate RC3 (47), prostaglandin D2 synthetase (57,58) the transcription factor hairless (59), the neuronal cell adhesion molecule (NCAM) (60), and the early response gene NGFI-A (61). Expression of other genes are regulated at the levels of mRNA stability (acetyl cholinester-ase), protein translation (MAP2), or mRNA splicing (tau). Regulation of splicing might be indirect, and subsequent to a primary action on the transcription of splicing regulators (62).

Role of thyroid hormone receptors

In mammals, T_3 receptors are the products of two genes known as TRα and TRβ that encode nine protein products that arise by alternative splicing and differential promoter usage. The TRα gene encodes five protein products (TRα_1, TRα_2, TRα_3, and the truncated products ΔTRα_1 and ΔTRα_2) from which only TRα_1 binds T_3. The TRβ gene encodes four T_3 binding proteins, of which TRβ_1, TRβ_2, and TRβ_3 also bind to responsive elements in DNA. In addition, a truncated protein, ΔTRβ_3 binds T_3 but not DNA. It may be said, therefore, that there are two types of receptors, α and β, and four different receptor isoforms. The physiologic role of the nonreceptor proteins is at present still unclear.

Concerning whether different receptor isoforms subserve different physiologic functions by selectively regulating specific genes, the current view is that the receptor isoforms are mostly equivalent in their biologic activity *in vivo*, including

Table 1. Thyroid Hormone-Dependent Brain Genes Containing T_3-Responsive Elements

Gene product	*Expression in brain*	*Biochemical function*	*Location of TRE*
Myelin basic protein (MBP)	Oligodendrocytes	Structural protein of myelin sheaths	Promoter region
Purkinje cell protein 2 (PCP-2)	Cerebellar Purkinje cells	G protein nucleotide exchange factor	Promoter region and intron
Neurogranin (RC3)	Neurons	Calmodulin-binding and protein kinase C-specific substrate	First intron
Lipocalin-type Prostaglandin D2 synthase (β-trace)	Leptomeninges, schoroid plexus, some neurons and oligodendrocytes	Generates prostaglandin D2 from prostaglandin H. Transport of lipophilic substances	Upstream promoter region
Hairless	Neurons	Transcription factor	Promoter region
Neuronal cell adhesion molecule (NCAM)	Neurons	Adhesion molecule	Intron D
NGFI-A (egr-1, zif268)	Neurons	Early response transcription factor	Promoter region

T3, triiodothyronine; TRE, triiodothyronine response elements.

binding affinity for T_3 (63), and that the different physiologic roles of each receptor depends on their particular patterns of expression. Thus, TRβ is involved in regulation of pituitary, liver, and cochlear function, whereas TRα regulates cardiac function, body temperature, gut maturation, and lymphocyte development. These specific effects of each receptor type are mainly due to their tissue distribution. In the cerebellum, TRα is expressed in the granular cells, whereas TRβ is expressed in the Purkinje cells. Therefore, it is not surprising that the effects of T_3 on migration if granular cells is mediated by TRα, whereas those on differentiation of Purkinje cells are mediated by TRβ (64). Another recent example (see below) is the finding of quantitative differences in TRα_1 and TRβ_1 expression among subsets of GABAergic interneurons of the cerebral cortex and hippocampus, which is correlated with effects on behavior (65). A different case has been made on the control of thermogenesis in brown adipose tissue, where the use of the TRβ-selective ligand GC-1 has allowed to define specific functions for TRα_1 and TRβ_1 (66).

A prominent role of TRα1 in brain development and function may be deduced from its relative expression in cerebrum and cerebellum, accounting for approximately 70%–80% of total T_3 receptor binding (67). Given the widespread distribution of T_3 receptors in the brain it is noteworthy that mutant mice lacking TRα1, TRβ, or both, do not display obvious signs of developmental abnormalities. One possible explanation for this paradox is that in the absence of ligand, transcriptional repression or abnormal regulation of transcription by the unliganded receptor is responsible for the effects of profound hypothyroidism. This is supported by two lines of evidence. One was provided by Samarut et al. (68), which showed that congenitally hypothyroid, Pax 8-deficient mice, which die during the first weeks of life, can be rescued by TRα1 gene deletion. Other evidence was provided by our group in studies on cerebellar development in TRα1-deficient mice (64). In these studies we showed that contrary to what happens in wild-type mice, the mutant mice do not show delayed granular cell migration and arrested Purkinje cell differentiation after induction of neonatal hypothyroidism. It appears, therefore, that these cerebellar alterations which constitute one of the hallmarks of neonatal hypothyroidism in rodents are the result of negative influences of the unliganded TRα_1 cerebellar expression of a mutated form of TRβ_1 with dominant negative activity strongly arrested Purkinje cell differentiation (69).

Role of thyroid hormone receptors in behavior

As pointed out above, absence of thyroid hormone receptors is not associated with obvious developmental alterations. The possibility exists, however, that absence of receptor during development may lead to subtle alterations derived from disturbances in correct maturation of specific neurons. A role of TRα_1 in early neuronal differentiation has been suggested from studies using stem cells in culture (25,70). It is likely that receptor deficiency is therefore associated with alterations in differentiation or migration of selected neuronal groups leading to subtle anomalies of brain function, not easily related to receptor function. Behavioral analysis of receptor-deficient mice may disclose alterations not previously expected on the basis of the known classical

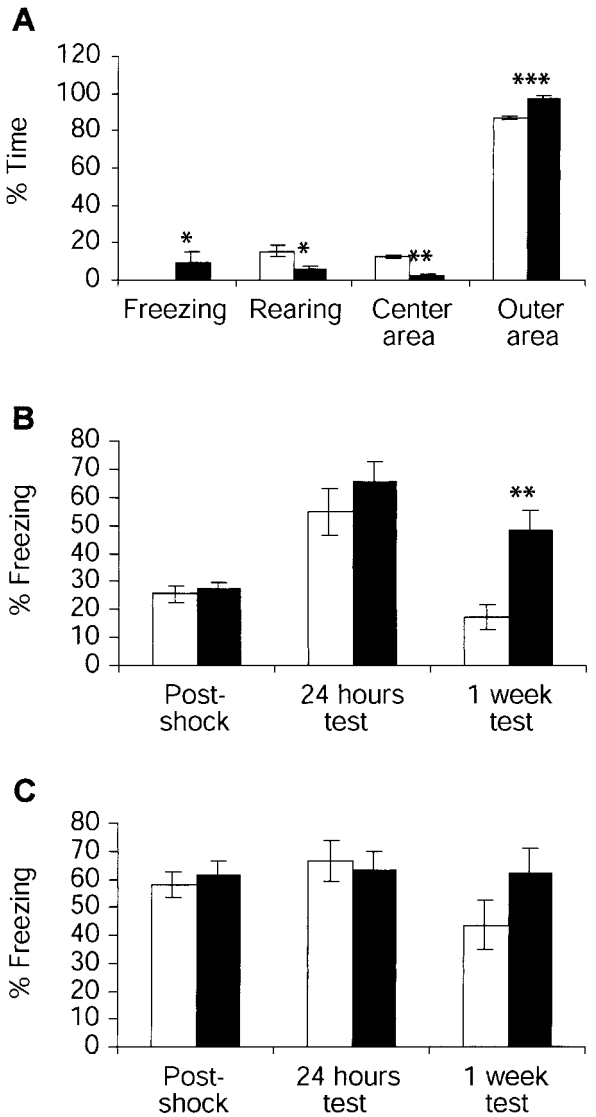

FIG. 2. Behavior of adult TRα_1-deficient mice (solid boxes) in the open field (**A**) and the contextual (**B**) and cued (**C**) fear conditioning tests. The behavior of control wild-type mice is represented in open boxes. In the open field test the animals were placed in the center of a circular open field of 140 cm diameter and 32 cm height, and the behavior and locomotor activity were videotaped. A significant number of TRα_1-deficient mice stayed motionless (freezing), displayed less rearing, and spent less time in the center area of the field than the wild-type mice. The fear conditioning tests took place in Pavlovian conditioning cages. In this context, fear conditioning, which depends on the integrity of both amygdala and hippocampus, the mice learn to associate the context with an aversive stimulus (i.e., an electric shock). Extinction of the response took much more time for the TRα_1 knockout mice. In cued-fear conditioning, a test that depends on the integrity of the amygdala, the mice learn to associate the aversive stimulus with a cue, usually a sound. In this test, both learning (24-hour test) and the extinction of the response (1 week) were similar in knockout and wild-type mice. The results suggested a lesion at the level of the hippocampus, and were correlated with a decreased number of GABAergic terminals in the CA1 field. Reproduced from Guadaño-Ferraz et al. (65) with permission.

effects of thyroid hormone. TRβ-deficient mice did not present any alterations in several tests including the open field test, the Morris water maze test, or contextual fear conditioning tests (71). However, female mice with deletions of either TRα_1 or TRβ_1 show opposite response to estrogens on sex behavior (72): absence of TRβ_1 led to an increase sexual behavior, whereas absence of TRα_1 reduced sexual behavior. In other terms, ligand-activated TRβ_1 would be an inhibitor of sexual response, whereas liganded TRα_1 would have the opposite function. The data for TRβ_1 fit with results from Pfaff and coworkers (73) that showed that thyroid hormone may interfere with the actions of estrogens on female sex responses. At the molecular level it was shown that TR may interfere with estrogen induction of responsive genes by interactions at the level of the estrogen responsive elements of target genes (73). The fact that TRα_1 behaved differently than TRβ_1 in these behavioral paradigms may be because of additional roles of TRα_1 in other forms of behavior.

We have analyzed whether deletion of TRα_1 would lead to behavioral alterations in adult mice using several behavioral tests (65), the first of which was the open-field test to assess locomotor behavior in a new environment. In this test the animals were more prone to display "freezing," (i.e., to stay motionless) (Fig. 2). This indicated that the mice had high emotionality. In a second battery of tests, the animals learn to associate an innocuous stimulus (the conditional stimulus), which might be either the context (as in contextual fear conditioning) or a cue, for example a sound (as is cued fear conditioning), with an aversive stimulus (unconditional stimulus) that is usually an electric shock delivered through metal rods in the cage floor. As shown in Figure 2, there were no differences in behavior at 24 hours of training. However, the extinction of the response was surprisingly delayed in the context fear conditioning test. In other words, TRα_1-deficient mice have a reduced capacity to forget negative memories. The results of these experiments, combined with data on the microstructure of the hippocampus and cerebral cortex, as well as the cellular distribution of thyroid hormone receptors have led us to hypothesize that TRα_1 is involved in the specification of hippocampal neuronal circuits involved in behavior (65).

Conclusions

From the data accumulated during the past 10 years, we are starting to understand the general features of thyroid hormone action in the brain in molecular terms. Knowledge in this field has, however, had a very strong limitation because of the complexity of the target organ under study, which requires the convergence of several disciplines. In addition to a more precise understanding of the classic effects of thyroid hormone deficiency or excess, the analysis of mice with genetically altered thyroid hormone receptors are providing surprising and unexpected results. In particular, the notion that some features of the hypothyroid phenotype may be the result of altered transcriptional regulation by the unliganded receptors more than to the lack of hormone *per se*. In addition, the indication that thyroid hormone receptors may have a role in behavior opens new fields of inquiry and raises the intriguing possibility that receptor mutations may underlie psychiatric forms of behavior in humans.

Acknowledgments

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References

1. Lumsden A, Kintner C, 2003 Neural induction and pattern formation. In: Squire LR, Bloom FE, McConnel SK, Roberts IL, Spitzer NC, Zigmond MJ (eds) Fundamental Neuroscience. Academic Press, San Diego, pp. 363–390.
2. Legrand J 1984 Effects of thyroid hormones on Central Nervous System. In: Yanai J (ed) Neurobehavioral Teratology. Elsevier Science Publishers, Amsterdam, pp. 331–363.
3. Morreale de Escobar G, Obregón MJ, Escobar de Rey F 2000 Is neuropsychological development related to maternal hypothyroidism, or to maternal hypothyroxinemia? J Clin Endocrinol Metab **85:**3975–3987.
4. Bernal J 2002 Action of thyroid hormone in brain. J Endocrinol Invest **25:**268–288.
5. Berbel P, Auso E, Garcia-Velasco JV, Molina ML, Camacho M 2001 Role of thyroid hormones in the maturation and organization of rat barrel cortex. Neuroscience **107:**383–394.
6. Muñoz A, Bernal J 1997 Biological activities of thyroid hormone receptors. Eur J Endocrinol **137:**433–445.
7. Gharami K, Das S 2000 Thyroid hormone-induced morphological differentiation and maturation of astrocytes are mediated through the beta-adrenergic receptor. J Neurochem **75:**1962–1969.
8. Lima FR, Gervais A, Colin C, Izembart M, Neto VM, Mallat M 2001. Regulation of microglial development: A novel role for thyroid hormone. J Neurosci **21:**2028–2038.
9. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev **23:**38–89.
10. Kaplan MM, Yaskoski KA 1981 Maturational patterns of iodothyronine phenolic and tyrosyl ring deiodinase activities in rat cerebrum, cerebellum, and hypothalamus. J Clin Invest **67:**1208–1214.
11. Galton VA, Martínez E, Hernández A, St. Germain EA, Bates JM, St. Germain DL 1999 Pregnant rat uterus expresses high levels of the type 3 iodothyronine deiodinase. J Clin Invest **103:**979–987.
12. Hernandez A, Schneider MJ, Galton VA, St. Germain DL 2003 Physiological consequences of deficiencies of the type 2 (D2) and type 3 (D3) deiodinases [Abstracts S14-1]. 85th Annual Meeting of the Endocrine Society. Philadelphia, PA, 2003, p. 22.
13. Marsh-Armstrong N, Huang H, Remo BF, Liu TT, Brown DD 1999 Asymmetric growth and development of the xenopus laevis retina during metamorphosis is controlled by type III deiodinase. Neuron **24:**871–878.
14. Tu HM, Legradi G, Bartha T, Salvatore D, Lechan RM, Larsen PR 1999 Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. Endocrinology **140:**784–790.
15. Escámez MJ, Guadaño-Ferraz A, Cuadrado A, Bernal J 1999 Type 3 iodothyronine deiodinase is selectively expressed in areas related to sexual differentiation in the newborn rat brain. Endocrinology **140:**5443–5446.
16. Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen

PR, Lechan RM 1997 Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology **138:**3359–3368.
17. Guadaño-Ferraz A, Obregón MJ, St-Germain D, Bernal J 1997 The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. Proc Natl Acad Sci USA **94:**10391–10396.
18. Fekete C, Harney JW, Dora JM, Gereben B, Bianco A, Sarkar S, Rand W, Emerson C, Lechan RM 2003 Type 2 iodothyronine deiodinase (D2) in the mediobasal hypothalamus may contribute to development of the nonthyroidal illness syndrome induced by bacterial lipopolysaccharide (LPS). 85th Annual Meeting of the Endocrine Society, Philadelphia, PA.
19. Guadaño-Ferraz A, Escámez MJ, Rausell E, Bernal J 1999 Expression of type 2 iodothyronine deiodinase in hypothyroid rat brain indicates an important role of thyroid hormone in the development of specific primary sensory systems. J Neurosci **19:**3430–3439.
20. Ibarrola N, Mayer-Proschel M, Rodriguez-Pena A, Noble M 1996 Evidence for the existence of at least two timing mechanisms that contribute to oligodendrocyte generation in vitro. Dev Biol **180:**1–21.
21. Rodriguez-Pena A, Ibarrola N, Iniguez MA, Munoz A, Bernal J 1993 Neonatal hypothyroidism affects the timely expression of myelin-associated glycoprotein in the rat brain. J Clin Invest **91:**812–818.
22. Guadaño-Ferraz A, Escobar dRF, Morreale dEG, Innocenti GM, Berbel P 1994 The development of the anterior commissure in normal and hypothyroid rats. Brain Res Dev Brain Res **81:**293–308.
23. Ben-Hur T, Rogister B, Murray K, Rougon G, Dubois-Dalcq M 1998 Growth and fate of PSA-NCAM+ precursors of the postnatal brain. J Neurosci **18:**5777–5788.
24. Billon N, Jolicoeur C, Tokumoto Y, Vennstrom B, Raff M 2002 Normal timing of oligodendrocyte development depends on thyroid hormone receptor alpha 1 (TRalpha1). Embo J **21:**6452–6460.
25. Liu YY, Tachiki KH, Brent GA 2002 A targeted thyroid hormone receptor alpha gene dominant-negative mutation (P398H) selectively impairs gene expression in differentiated embryonic stem cells. Endocrinology **143:**2664–2672.
26. Perez-Juste G, Aranda A 1999 The cyclin-dependent kinase inhibitor p27(Kip1) is involved in thyroid hormone-mediated neuronal differentiation. J Biol Chem **274:**5026–5031.
27. Lindholm D, Castren E, Tsoulfas P, Kolbeck R, Penha Berzaghi M, Leingartner A, Heisenberg CP, Tesarollo L, Parada LF, Thoenen H 1993 Neurotrophin-3 induced by tri-iodothyronine in cerebellar granullar cells promotes Purkinje cell differentiation. J Cell Biol **122:**443–450.
28. Ruiz-Marcos A, Cartagena P, García A, Escobar del Rey F, Morreale de Escobar G 1988 Rapid effects of adult-onset hypothyroidism on dendritic spines of pyramidal cells of the rat cerebral cortex. Exp Brain Res **73:**583–588.
29. Gerendasy DD, Sutcliffe JG 1997 RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. Mol Neurobiol **15:**131–163.
30. Iñiguez MA, Rodriguez-Pena A, Ibarrola N, Morreale de Escobar G, Bernal J 1992 Adult rat brain is sensitive to thyroid hormone. Regulation of RC3/neurogranin mRNA. J Clin Invest **90:**554–558.
31. D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T 1995 A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature **374:**719–723.
32. Alvarez-Dolado M, Ruiz M, del Rio JA, Alcantara S, Burgaya F, Sheldon M, Nakajima K, Bernal J, Howell BW, Curran T, Soriano E, Muñoz A 1999 Thyroid hormone regulates reelin and dab1 expression during brain development. J Neurosci **19:**6979–6993.
33. GarciaFernandez LF, Rausell E, Urade Y, Hayaishi O, Bernal J, Munoz A 1997 Hypothyroidism alters the expression of prostaglandin D-2 synthase beta trace in specific areas of the developing rat brain. Eur J Neurosc **9:**1566–1573.
34. Alvarez-Dolado M, Gonzalez-Sancho JM, Bernal J, Muñoz A 1998 Developmental expression of tenascin-C is altered by hypothyroidism in the rat brain. Neurosci **84:**309–322.
35. Alvarez-Dolado M, Cuadrado A, Navarro-Yubero C, Sonderegger P, Furley AJ, Bernal J, Muñoz A 2000 Regulation of the L1 cell adhesion molecule by thyroid hormone in the developing brain. Mol Cell Neurobiol **16:**499–514.
36. Farwell AP, Lynch RM, Okulicz WC, Comi AM, Leonard JL 1990 The actin cytoskeleton mediates the hormonally regulated translocation of type II iodothyronine 5′-deiodinase in astrocytes. J Biol Chem **265:**18546–18553.
37. Siegrist-Kaiser CA, Juge-Aubry C, Tranter MP, Ekenbarger DM, Leonard JL 1990 Thyroxine-dependent modulation of actin polymerization in cultured astrocytes. A novel, extranuclear action of thyroid hormone. J Biol Chem **265:**5296–5302.
38. Farwell AP, Tranter MP, Leonard JL 1995 Thyroxine-dependent regulation of integrin-laminin interactions in astrocytes. Endocrinology **136:**3909–3915.
39. Davis PJ, Shih A, Lin HY, Martino LJ, Davis FB 2000 Thyroxine promotes association of mitogen-activated protein kinase and nuclear thyroid hormone receptor (TR) and causes serine phosphorylation of TR. J Biol Chem **275:**38032–38039.
40. Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B, Forrest D 1999 Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes Dev **13:**1329–1341.
41. Davis PJ, Davis FB 2002 Nongenomic actions of thyroid hormone on the heart. Thyroid **12:**459–466.
42. Bernal J 2002 Thyroid hormones and brain development. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) Hormones, Brain and Behavior. Academic Press, San Diego, CA, pp. 543–587.
43. Enriquez JA, Fernandez-Silva P, Garrido-Perez N, Lopez-Perez MJ, Perez-Martos A, Montoya J 1999 Direct regulation of mitochondrial RNA synthesis by thyroid hormone. Mol Cell Biol **19:**657–670.
44. Casas F, Daury L, Grandemange S, Busson M, Seyer P, Hatier R, Carazo A, Cabello G, Wrutniak-Cabello C 2003 Endocrine regulation of mitochondrial activity: Involvement of truncated RXRalpha and c-Erb Aalpha 1 proteins. FASEB J **17:**426–436.
45. Wrutniak-Cabello C, Casas F, Cabello G 2001 Thyroid hormone action in mitochondria. J Mol Endocrinol **26:**67–77.
46. Morte B, Iniguez MA, Lorenzo PI, Bernal J 1997 Thyroid hormone-regulated expression of RC3/neurogranin in the immortalized hypothalamic cell line GT1-7. J Neurochem **69:**902–909.
47. Martinez de Arrieta C, Morte B, Coloma A, Bernal J 1999 The human RC3 gene homolog, NRGN contains a thyroid hormone-responsive element located in the first intron. Endocrinology **140:**335–343.

48. Siddiq A, Miyazaki T, Takagishi Y, Kanou Y, Hayasaka S, Inouye M, Seo H, Murata Y 2001 Expression of ZAKI-4 messenger ribonucleic acid in the brain during rat development and the effect of hypothyroidism. Endocrinology **142:**1752–1759.
49. Cao X, Jong-Kook L, Moeller L, Kambe F, Hayashi Y, Refetoff S, Seo H 2003 Thyroid hormone receptor-mediated regulation of ZAKI-4α expression involves the activation of PI3K and mammalian target of rapamycin (mTOR). 85th Annual Meeting of the Endocrine Society, Philadelphia, PA.
50. Dowling AL, Zoeller RT 2000 Thyroid hormone of maternal origin regulates the expression of RC3/neurogranin mRNA in the fetal rat brain. Brain Res Mol Brain Res **82:**126–132.
51. Dowling AL, Martz GU, Leonard JL, Zoeller RT 2000 Acute changes in maternal thyroid hormone induce rapid and transient changes in gene expression in fetal rat brain. J Neurosci **20:**2255–2265.
52. Lavado-Autric R, Auso E, Garcia-Velasco JV, Arufe Mdel C, Escobar del Rey F, Berbel P, Morreale de Escobar G 2003 Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest **111:**1073–1082.
53. Harvey CB, Williams GR 2002 Mechanism of thyroid hormone action. Thyroid **12:**441–446.
54. Farsetti A, Desvergne B, Hallenbeck P, Robbins J, Nikodem V 1992 Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoter. J Biol Chem **267:**15784–15788.
55. Zou L, Hagen SC, Strait KA, Oppenheimer JH 1994 Identification of thyroid hormone response elements in rodent Pcp-2, a developmentally regulated gene of cerebellar Purkinje cells. J Biol Chem **269:**13346–13352.
56. Luo Y, Denker BM 1999 Interaction of heterotrimeric G protein Galphao with Purkinje cell protein-2. Evidence for a novel nucleotide exchange factor. J Biol Chem **274:**10685–10688.
57. White DM, Takeda T, DeGroot LJ, Stefansson K, Arnason BG 1997 Beta-trace gene expression is regulated by a core promoter and a distal thyroid hormone response element. J Biol Chem **272:**14387–14393.
58. García-Fernández LF, Urade Y, Hayaishi O, Bernal J, Muñoz A 1998 Identification of a thyroid hormone response element in the promoter region of the rat lipocalin-type prostaglandin D synthase (beta-trace) gene. Mol Brain Res **55:**321–330.
59. Thompson CC 1996 Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a hairless homolog. J Neurosci **16:**7832–7840.
60. Iglesias T, Caubin J, Stunnenberg HG, Zaballos A, Bernal J, Miñoz A 1996 Thyroid hormone-dependent transcriptional repression of neural cell adhesion molecule during brain maturation EMBO J **15:**4307–4316.
61. Ghorbel MT, Seugnet I, Hadj-Sahraoui N, Topilko P, Levi G, Demeneix B 1999 Thyroid hormone effects on Krox-24 transcription in the post-natal mouse brain are developmentally regulated but are not correlated with mitosis. Oncogene **18:**917–924.
62. Cuadrado A, Garcia-Fernandez LF, Imai T, Okano H, Munoz A 2002 Regulation of tau RNA maturation by thyroid hormone is mediated by the neural RNA-binding protein musashi-1. Mol Cel Neurosci **20:**198–210.
63. Schwartz HL, Strait KA, Ling NC, Oppenheimer JH 1992 Quantitation of rat tissue thyroid hormone binding receptor isoforms by immunoprecipitation of nuclear triiodothyronine binding capacity. J Biol Chem **267:**11794–11799.
64. Morte B, Manzano J, Scanlan T, Vennstrom B, Bernal J 2002 Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci USA **99:**3985–3989.
65. Guadaño-Ferraz A, Benavides-Piccione R, Venero C, Lancha C, Vennström B, Sandi C, DeFelipe J, Bernal J 2003 Lack of thyroid hormone receptor α1 is associated with selective alterations in behavior and hippocampal circuits. Mol Psychiatry **8:**30–38.
66. Ribeiro MO, Carvalho SD, Schultz JJ, Chiellini G, Scanlan TS, Bianco AC, Brent GA 2001 Thyroid hormone-sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform-specific. J Clin Invest **108:**97–105.
67. Ercan-Fang S, Schwartz HL, Oppenheimer JH 1996 Isoform specific 3,5,3′-triiodothyronine receptor binding capacity and messenger ribonucleic acid content in rat adenohypophysis: Effect of thyroidal state and comparison with extrapituitary tissues. Endocrinology **137:**3228–3233.
68. Flamant F, Poguet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A, Samarut J 2002 Congenital hypothyroid Pax8(-/-) mutant mice can be rescued by inactivating the TRalpha gene. Mol Endocrinol **16:**24–32.
69. Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, Cohen RN, Wondisford FE 2001 An unliganded thyroid hormone receptor causes severe neurological dysfunction. Proc Natl Acad Sci USA **98:**3998–4003.
70. Forrest D 2002 Twists in the tail-change-of-function mutations in thyroid hormone receptors. Endocrinology **143:**2466–2468.
71. Forrest D, Hanebuth E, Smeyne RJ, Everds N, Stewart CL, Wehner JM, Curran T 1996 Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: Evidence for tissue-specific modulation of receptor function. EMBO J **15:**3006–3015.
72. Dellovade TL, Chan J, Vennstrom B, Forrest D, Pfaff DW 2000 The two thyroid hormone receptor genes have opposite effects on estrogen-stimulated sex behaviors. Nat Neurosci **3:**472–475.
73. Vasudevan N, Ogawa S, Pfaff D 2002 Estrogen and thyroid hormone receptor interactions: Physiological flexibility by molecular specificity. Physiol Rev **82:**923–944.

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