

COMMENTARY

Thyroid Hormones and the Brain

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Significant progress has been made over the past 2 decades toward understanding the molecular basis of thyroid hormone action. It is now widely accepted that thyroid hormones play predominantly a nuclear role and function by regulating the transcription of specific target genes. Understanding thyroid hormone action at the tissue and organismic level requires assessment of the thyroid hormone response apparatus and identification of specific target genes. Progress toward uncovering the molecular basis of thyroid hormone action during mammalian brain development is advancing rapidly. This commentary provides a brief overview of the molecular basis of thyroid hormone action followed by three sections detailing thyroid hormone regulation of brain development at the functional, cellular, and molecular levels. Each section is followed by a discussion of unresolved issues and an analysis of our current level of understanding of each topic. **KEY WORDS:** thyroid hormone; brain; development. © 2001 Academic Press

THYROID HORMONE ACTION

Thyroid hormones exert their action primarily at the nuclear level by regulating the transcription of thyroid-hormone-responsive genes. Thyroid hormones enter the cell, proceed to the nucleus, and bind to the thyroid hormone receptor (TR), a transcription factor belonging to the large family of nuclear hormone receptors. There are two isoforms of TR known as TR α and TR β . Of the two predominant thyroid hormones, triiodothyronine (T3) and thyroxine (T4), T3 binds to the TR with greater affinity and consequently is thought to mediate most thyroid hormone action in the nucleus. Therefore, the term “thyroid hormone” is appropriate. TR interacts with specific sequences of DNA designated as thyroid hormone response elements (TREs). It is thought that the TR binds to the TRE as a heterodimer; primarily with the nuclear receptor retinoid X receptor (RXR). TREs are generally found in the proximal promoter regions of T3-responsive genes. The TR can bind to the TRE in the presence or the absence of T3. In the absence of T3 the receptor interacts with a group of nuclear proteins known as corepressors. In the presence of T3 the receptor

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interacts with coactivators. Thus, T3 determines whether coactivators or corepressors are localized near the start site of gene transcription. The cofactors can then direct conformational changes of the chromatin by acetylating or deacetylating histones. The acetylation of histones by coactivators results in a loosening of chromatin structure, whereas deacetylation of histones by corepressors results in a compaction of chromatin structure which renders it less accessible to the transcriptional machinery. Several reviews have recently been written that describe the molecular basis of thyroid hormone action in greater detail (7, 20, 52, 68).

Thyroid hormone receptors are expressed in neurons, oligodendrocytes, and astrocytes, the predominant cell types in the brain (15, 17, 18, 69, 85, 86). There are differences in the expression patterns of individual TR isoforms during ontogeny. In general TR α is expressed at high levels during early brain development, while TR β expression dramatically increases during late brain development. This general expression pattern is exhibited in two well-recognized targets of thyroid hormone in the brain, the cerebellar Purkinje cell and the oligodendrocyte. Both these cell types express TR α early in development followed by increased expression of TR β as the cells become responsive to thyroid hormone. Such marked changes in TR expression patterns led to the hypothesis that individual TR isoforms may play isoform-specific roles during brain development. However, the generation of TR knockout mice that possess deletions of either the α or β isoforms have not yet revealed any brain-specific phenotype (83). This finding apparently contradicts the dramatic effects hypothyroidism imparts on the developing brain as described in detail in the following sections. This contradiction may be explained, however, by considering the molecular basis of thyroid hormone action. Unliganded TR interacts with corepressors, which in turn can repress transcription of T3-regulated genes. It is possible that continuous repression of gene transcription by unliganded TR in the hypothyroid animal is more deleterious to normal brain development than a lack of T3-dependent gene activation by liganded TR. Thus, during brain development the main role of T3 may be to derepress transcription of T3-regulated genes. This question remains central to uncovering the true role of thyroid hormone in brain development.

THYROID HORMONE AND BRAIN DEVELOPMENT—FUNCTIONAL LEVEL

The Developing Brain

Thyroid hormone plays an important role during late brain development (for reviews see 11, 19, 54, 74). A lack of thyroid hormone during this time results in permanent deficits in brain function, including intellectual development. This period of development corresponds from roughly the third trimester of pregnancy to approximately 3 months after birth in humans (2, 46, 49, 51). Administration of thyroid hormone to the newborn infant during this period of time results in recovery of intellectual development. Delay in treatment past 3

months of age, however, results in permanent and severe deficits in intellectual development. These findings demonstrate the presence of a window of time during which thyroid hormone exerts its developmental effects. Interestingly, thyroid hormone administration at any point during this window of time at least partially rescues the brain from severe impairment. This observation suggests that the processes affected by thyroid hormone during brain development occur over a broad but defined period of time. Recently, however, it has become apparent that even a few days delay in high-dose replacement of thyroid hormone to congenitally hypothyroid infants results in a measurable reduction in later intellectual development (13). Thus, although the developing brain can forgive the absence of thyroid hormone during portions of late brain development, even slight delays in achieving maximal levels of hormone are deleterious.

The effects of congenital hypothyroidism on learning have also been assessed in animal models (29). Adult rats thyroidectomized at birth exhibit behavioral impairment as measured by their inability to learn a task involving escape-avoidance (30). These hypothyroid rats commit a greater number of errors than control untreated rats. Similarly, adult rats thyroidectomized at birth commit a greater number of errors in maze learning (31, 32, 42). Interestingly, animal studies have also demonstrated a window of time when the learning impairment is reversible. Using a Hebb-Williams closed-field test Earyrs determined that adult rats thyroidectomized at birth exhibit an impairment in their capacity for adaptive behavior (28). This impairment was reversible if thyroxine replacement therapy was initiated before neonatal day 10. Replacement of hormone after day 10, however, did not reverse the impairment. As in the human studies, these data support the hypothesis that hypothyroidism during a specific window of time in late brain development leads to an irreversible impairment in learning ability. However, thyroid hormone replacement therapy initiated at any time during this window of time can rescue the developing brain from aberrant development.

The Adult Brain

Thyroid hormone exerts most of its effects on the maturation of the developing mammalian brain. Thyroid hormone action in the adult mammalian brain is poorly understood. Few studies have been conducted that assess mental and psychological dysfunction in hypothyroid adults. There are some data, however, associating hypothyroidism with clinical depression (43). For example, Cleare *et al.* have shown that patients exhibiting frank hypothyroidism demonstrate a correlation between thyrotropin (TSH) level and the Beck Depression Inventory score (21). Plasma TSH levels provide a measure of the degree of hypothyroidism. A series of studies have also implicated thyroid hormone administration as an adjuvant to clinical treatment of depression (47). These studies, however, were conducted in euthyroid patients and may represent a pharmacologic rather than physiologic effect of the hormone.

Memory impairment, learning, attentiveness, and psychomotor slowing have also been associated with hypothyroidism in the adult (14, 92). These deficits in cognitive dysfunction appear amenable to treatment with thyroid hormone. Because these symptoms are also associated with clinical depression, it is difficult to separate these neuropsychiatric features from one another. Finally, hyperthyroidism has also been associated with neuropsychiatric changes (9, 93). Difficulty in maintaining concentration, insomnia, anxiety, emotional lability, and intellectual dysfunction have all been associated with the hyperthyroid state. In both hypo- and hyperthyroidism, however, it remains unclear whether the effects of aberrant thyroid hormone secretion directly or indirectly effect brain function. Thus, it is possible that the effects of hypo- and hyperthyroidism on brain function are the indirect result of thyroid hormone action on peripheral systems. Increased knowledge of the molecular targets of thyroid hormone in the brain may help provide an answer to this question.

Functional Effects—Analysis

Although the effects of thyroid hormone on brain function have been recognized for several decades there are many questions that remain unanswered. For instance, how early does the developing human brain require thyroid hormone? Some studies suggest that the developing brain requires maternal thyroid hormone early in pregnancy (50). If so, does the potential requirement for euthyroidism in the mother reflect a need for maternal thyroid hormone transported to, and directly acting on, the developing fetal brain or is the maternal effect indirect and transmitted via another maternally derived thyroid hormone-sensitive target molecule? Another unresolved and important issue is whether complete recovery of normal intellectual development would be attained by administration of thyroid hormone to congenitally hypothyroid fetuses? What is the optimal dose and time course of thyroid hormone administration in hypothyroid infants that would ensure normal brain development? Finally, what role does thyroid hormone play in the adult brain? Again, are the effects observed in hypo- and hyperthyroidism in the adult brain direct, or are they an indirect manifestation of thyroid hormone effects on peripheral tissues? Is thyroid hormone efficacious in the treatment of clinical depression? That these questions remain unresolved underscores the need for further study of physiologic effects of thyroid hormone on brain function.

THYROID HORMONE AND BRAIN DEVELOPMENT—CELLULAR LEVEL

Late brain development is characterized by maturation of the organ. The processes of axonal and dendritic growth, synapse formation, myelination, cell migration, and proliferation of specific populations of cells, such as the glial cells and certain late arising neurons, all occur late in brain development and are regulated by thyroid hormone (for reviews see 11, 26, 37, 54, 64, 74, 77, 79).

The behavioral and learning deficits associated with thyroid hormone deprivation likely result from the dysregulation of these processes.

Oligodendrocyte Development

The oligodendrocyte is a well-recognized target of thyroid hormone in the developing brain. Thyroid hormone regulates oligodendrocyte production of myelin, as will be discussed in a following section. Thyroid hormone also plays an additional role in oligodendrocyte development by controlling proliferation of the oligodendrocyte precursor cell; the oligodendrocyte type II astrocyte (O-2A) (26). In the absence of thyroid hormone the O-2A cell can proliferate indefinitely in response to specific growth factors. However, in the presence of thyroid hormone the O-2A cell will proliferate for a maximum of eight cell divisions but will then cease cell division and differentiate into a maturing oligodendrocyte. Recent studies further suggest that the proliferating O-2A cell may count time by a mechanism other than counting cell divisions. This conclusion was drawn as slowing down O-2A cell cycling time results in cells leaving the cell cycle in response to T3 after fewer than eight cell divisions (35). Thyroid hormone is not absolutely required for oligodendrocyte maturation, as removal of growth factors will initiate oligodendrocyte maturation regardless of thyroid hormone presence or absence. Thus, an intrinsic timing mechanism exists in the proliferating O-2A cell which serves to limit the duration of proliferation and is responsive to thyroid hormone. The effector component that stops O-2A cell division and is presumably activated by thyroid hormone has not yet been identified. The cyclin-dependent protein kinase (Cdk) inhibitor p27/kip1, however, appears to be a component of the timing mechanism (25). p27/kip1 inhibits the cell cycle by blocking cell cycle progression in G1. Presumably, thyroid hormone regulates expression of the effector molecule that, acting through p27/kip1, stops cell division and initiates differentiation. In keeping with this hypothesis, optic nerves from hypothyroid rats and mice contain fewer mature oligodendrocytes when compared to optic nerves from euthyroid animals (1, 44).

Astrocyte Development

Astrocytes play a myriad of roles in normal brain function. *In vitro*, thyroid hormone has been demonstrated to regulate actin polymerization and the extracellular organization of laminin in astrocytes (34, 84). These effects are postulated to play roles in neural migration. Thyroid hormone has also been demonstrated to induce the proliferation of astrocytes *in vitro* (91). *In vivo*, it has been shown that early thyroid hormone deficiency leads to impaired maturation of radial glial cells in the CA1 region of the hippocampus (67) and the Bergmann astrocytes of the cerebellum (76). An elegant series of studies have also implicated the astrocyte in the 5'-deiodination of T4 to T3 in the

brain. The enzyme responsible for this conversion in the brain, type II iodothyronine 5'-deiodinase (D2), is upregulated in astrocytes in the hypothyroid brain (40, 41). It has been postulated that the majority of free T3 found in the brain is locally produced by astrocytes and that D2 activity provides a level of thyroid hormone action control by increasing its activity when T4 levels are low. This mechanism may thus ensure stable levels of brain T3 concentrations in the face of fluctuations in thyroxine production (reviewed in 57, 58). In contrast, type I iodothyronine 5'-deiodinase (D1), the primary peripheral deiodinase, is downregulated in peripheral tissues during hypothyroidism, perhaps evolving as a mechanism to protect peripheral tissues from T4 depletion during hypothyroidism. Astrocytes may also play a role in T4 transport across the blood-brain barrier, although the mechanism of T4 transport into the brain is not yet clear (10, 24).

Cerebellar Development

Another well-recognized target for thyroid hormone is the developing cerebellum (48, 54, 64). Thyroid hormone affects many aspects of cerebellar development, including proliferation and migration of cerebellar granule cells and the morphologic development of Purkinje cells. In the hypothyroid rat cerebellum the number of granule cells present in the proliferative zone, known as the external granular layer (EGL), is markedly increased 3–4 weeks after birth (62, 75). Migration of the granule cells from the EGL to the internal granule cell layer (IGL) is delayed in the hypothyroid cerebellum and cell death is increased. In the hyperthyroid rat, cell division is initially increased in the external granule cell layer but then is prematurely terminated (59, 73). Dendritic arborization of the cerebellar Purkinje cells is markedly reduced in the hypothyroid rat (63). Similarly, outgrowth of the granule cell axon, the parallel fiber, is retarded in the hypothyroid cerebellum (60, 61). Lack of synapse formation between Purkinje cell dendrites and the granule cell's parallel fiber is thought to result in deletion of the granule cell by apoptosis (94). Thus, in the hypothyroid rat cerebellum one observes a reduction in Purkinje cell dendritic arborization, a delay in granule cell migration, a reduction in parallel fiber outgrowth, and a consequent reduction in the ultimate number of granule cells. Further, the delay in cell acquisition and development results in abnormal proportions of all cell types found in the cerebellum, including basket cells, stellate cells, and astrocytes. Interestingly, as was observed for attempting to reverse the effects of hypothyroidism on learning (28), development of the cerebellum can be normalized only if thyroid hormone is administered during a discrete window of time. Specifically, Legrand and colleagues found that thyroxine replacement therapy can reverse the reduction in the dendritic spread observed in the Purkinje cells of a congenitally hypothyroid rat, if thyroxine is administered before the second postnatal week (63). Administration of thyroid hormone after this period of time does not reverse the effects of hypothyroidism. Thus, it is again apparent that thyroid hormone plays a role

in brain development only during a discrete window of time and that the deleterious effects of congenital hypothyroidism can be mostly reversed if the hormone is administered at any point during this period.

Hippocampal Development

The hippocampal formation is another site of significant neurogenesis during late brain development. Thyroid hormone deprivation during this period of time also affects normal development of this brain region (37). This finding may be of importance with respect to the functional effects of thyroid hormone deprivation, as the hippocampus is involved in learning and memory. Thyroid hormone deprivation during late brain development results in a decrease in the number of dentate gyrus granule cells in the hippocampal formation (78). Dentate gyrus granule axons (mossy fibers) project to the hippocampal CA3 region pyramidal cells. The volume of the mossy fiber system and the number of mossy fiber-CA3 pyramidal synapses are reduced in the hypothyroid state (65). No reduction in number of CA3 hippocampal pyramidal cells is observed in hypothyroid brains (66). However, these cells exhibit stunted development of the dendritic tree (78) and a corresponding reduction in volume of the CA3 layer (66). Neonatal hypothyroidism results in a decrease in CA1 pyramidal cells (66) but appears not to affect the morphology of the remaining cells (37). The dramatic effects of thyroid hormone deprivation on the hippocampal formation make it likely that aberrant hippocampal development contributes to the behavioral effects observed in the hypothyroid animal.

Cerebral Cortex Development

Thyroid hormone deprivation during late brain development results in reduction of dendritic growth and synaptogenesis in the cerebrum (27). The cells in the hypothyroid cerebrum are consequently packed closer together, thus leading to a decrease in the overall size of the brain (8). The number of spines found along the apical shaft of pyramidal cells of the visual cortex is markedly reduced in rats thyroidectomized at 10 days of age (82). The reduction in spine formation was reversible, however, if thyroid hormone was administered to the thyroidectomized animals (81). As for other reversible effects of thyroid hormone, delay in administration results in decreased recovery of the affected neurons. Studies of layer IV of the primary somatic sensory cortex (S1) have revealed thyroid hormone-dependent regulation of posteromedial barrel subfield (PMBSF) development (16). The PMBSF is a cortical region studied as a model for cortical development, organization, plasticity, and sensory integration. Congenital hypothyroidism results in a delay in barrel formation and a reduction in S1 barrel dimensions. Thus, thyroid hormone does not participate in the establishment of the S1 barrel but rather influences the timing of its formation and growth. Finally, maturation of the corpus callosum is abnormal

in the hypothyroid rat (38, 39). Hypothyroid animals demonstrate normal numbers of callosally projecting axons; however, the topography of the projection fields is abnormal. The abnormal topography observed in the hypothyroid corpus callosum axons resembles immaturity in the callosal connections. In summary, the effects of thyroid hormone deprivation on formation of the neocortex suggest that these effects also contribute to the behavioral effects attributed to the congenitally hypothyroid brain.

Cellular Effects—Analysis

Although the effects of thyroid hormone at the cellular level have been studied for many years, certain questions remain unanswered. For instance, are alterations in brain cell number the direct or indirect result of thyroid hormone deprivation? While there is some evidence that thyroid hormones may directly regulate the survival of developing brain cells, it is also quite possible that thyroid hormone influences other processes, such as axonal and dendritic growth, that indirectly affect the survival of specific cell types. What regions of the brain are responsible for the functional deficits in learning and memory observed in the hypothyroid state? Morphologic analyses have identified the hippocampus, cerebellum, and cortex as specific regions of the brain that are affected by thyroid hormone deprivation. Interestingly, each of these regions contribute to memory and learning. Thus, it is conceivable that each region contributes to the overall deficit observed in the hypothyroid animal. In addition, it is likely that the effects of thyroid hormone deprivation on individual regions may impart region-specific functional effects. The specific functional contributions of each of these regions to the hypothyroid brain phenotype remain to be deciphered. Equally unclear are the molecular mechanisms by which thyroid hormone contributes to the functional deficits attributed to these regions of the brain. Is the contribution of thyroid hormone to brain development limited to assisting in the timely establishment of neural connections or are there other less easily observed effects of thyroid hormone on brain function? This question is not readily answered, as deficits in neuronal circuitry will result in additional, secondary effects on brain function. For example, if thyroid hormone is only directly involved in dendritic and axonal growth, thyroid hormone deprivation may also indirectly affect synapse formation, proper cell migration, cell survival, intra- and extracellular signaling processes, etc. Thus, are the morphologic effects described in the preceding section direct or indirect effects of thyroid hormone? The role of thyroid hormone on astrocyte development and function is also not clear at the present. An unanswered question is whether the thyroid hormone-dependent changes in astrocyte morphology observed *in vitro* and *in vivo* have functional significance. The hypothesis that astrocytes control local production of T3 in the brain is very provocative. Questions raised by these findings include whether the production of brain T3 by astrocytes is regulated in a complex fashion by adjacent neurons and glia. Does this local production of T3 result in regions of the brain that are

relatively protected from hypothyroidism due to increased, localized concentrations of T3? Hypothyroidism downregulates D1 in the periphery. Does this pattern of regulation, which differs from the pattern of D2 regulation in the brain, protect peripheral tissues from the effects of low T3 levels? Finally, what is the mechanism by which T4 enters the central nervous system? Is this process regulated during brain development?

THYROID HORMONE AND BRAIN DEVELOPMENT—MOLECULAR LEVEL

The processes regulated by thyroid hormone during brain development, such as axonal and dendritic growth, synapse formation, myelination, cell migration, and proliferation of specific populations of cells, are the result of organized regulation of gene expression. Thyroid hormone exerts most of its action at the nuclear level. Thus, the behavioral and learning deficits associated with thyroid hormone deprivation likely result from the dysregulation of specific gene expression patterns. To fully understand the molecular basis of thyroid hormone action in the brain it is necessary to identify the target genes regulated by thyroid hormone in the developing and mature brain. Many target genes have been identified in the past decade. For the purposes of this commentary, only selected T3-dependent target genes postulated to play a role in either cerebellar or oligodendrocyte development will be discussed.

Genes Involved in Cerebellar Development

The developing cerebellum is a well recognized target for thyroid hormone. Several thyroid hormone-responsive target genes expressed in the cerebellum have been identified. Oppenheimer and colleagues have identified three genes expressed in the cerebellar Purkinje cell (the genes for Purkinje cell protein-2 (*Pcp-2*), calbindin, and myo-inositoltriphosphate (IP-3) receptor) that are regulated by thyroidal status during late rat brain development (87). It is not certain what effect T3-dependent regulation of these genes has on Purkinje cell physiology. Interestingly, these model genes are only T3-responsive during a discrete period of late brain development. In the immature and mature Purkinje cell these genes are refractory to thyroid hormone. In the immature Purkinje cell, expression of the orphan nuclear receptor chicken ovalbumin upstream promoter–transcription factor (COUP-TF) is thought to block the ability of T3 to prematurely activate *Pcp-2* gene expression by competing with TR for *Pcp-2* TRE binding (5, 6). Thyroid hormone has also been shown to regulate cerebellar expression of the transcription factor $ROR\alpha$ (53). The *staggerer* mouse mutant exhibits a derangement of Purkinje cell development, including a reduction in *Pcp-2* expression. $ROR\alpha$ also modulates the transcriptional activity of TR (56). Thus, like COUP-TF, $ROR\alpha$ may play a role in modulating the effects of thyroid hormone during discrete phases of Purkinje cell development. The mechanism whereby the mature Purkinje cell is ren-

dered refractory to T3 is unknown. In total, these data suggest a molecular mechanism whereby thyroid hormone action is confined to a discrete window of developmental time. Expression of specific nuclear proteins renders T3-dependent target genes nonresponsive to T3. Disappearance of these repressor proteins allows T3 to transcriptionally activate gene expression at a precise time during development. Finally, the target genes quickly become refractory to T3 again and turn over control of gene transcription to another set of transcription factors. Thus, the transient nature of thyroid hormone-regulated brain development has been documented at the functional, cellular, and molecular levels.

Studies from other laboratories suggest that thyroid hormone regulation of cerebellar development might be the result of T3-dependent regulation of growth factor expression (reviewed in 54). The expression of the neurotrophs neurotrophin-3 (NT-3), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 4/5 has been shown to be regulated by thyroid hormone in the brain (36, 55, 72). Replacement of NT-3 or BDNF results in some rescue of cerebellar development in hypothyroid animals (72). Thus, thyroid hormone may regulate the development of cerebellar neurons by modulating the transcription of specific growth factors required by the developing cells during discrete stages of development. Additional genes regulated by thyroid hormone in the cerebellum include reelin, neural cell adhesion molecule (NCAM), tenascin-C, *Srg1*, and hairless (3, 4, 45, 88). Reelin and N-CAM are involved in the process of neuronal migration and are attractive candidates for mediating the thyroid hormone-dependent effects on cerebellar organization. Tenascin-C is an extracellular matrix protein involved in neurite outgrowth and cell migration. *Srg1* is a novel protein related to synaptotagmin which is itself a protein involved in neurotransmitter release. Hairless is a zinc finger protein that inhibits TR action by directly interacting with the receptor (89). Finally, thyroid hormone has been shown to promote *BCL-2* expression in newly formed cerebellar granule cell neurons (70). Thyroid hormone regulation of this anti-apoptotic molecule may explain the increase in apoptosis observed in the internal granule cell layer of hypothyroid animals (94).

Genes Involved in Oligodendrocyte Development

The developing oligodendrocyte is a well-recognized glial cell target of thyroid hormone (79). As covered earlier, thyroid hormone acts as an effector molecule to cause the cessation of oligodendrocyte precursor cell proliferation. The identity of the target molecule that mediates this effect is not yet known. In contrast, the effects of thyroid hormone on myelin gene expression in the developing oligodendrocyte have been well described. Early experiments demonstrated that lack of thyroid hormone during late brain development leads to diminished production of the components of myelin. The enzymes involved in the synthesis of myelin lipid are reduced in the hypothyroid state (12). When mRNA levels of the major myelin proteins (myelin basic protein (MBP), pro-

teolipid protein (PLP), and myelin-associated glycoprotein (MAG)) were assessed, it was demonstrated that each of these species was reduced during neonatal hypothyroidism (71, 87, 90). MAG mRNA levels are probably controlled by thyroid hormone at the posttranscriptional level by an unknown mechanism (80). In contrast, it is likely that the MBP gene is directly regulated by thyroid hormone, as this gene possesses a TRE in the 5' proximal promoter region (33). As is observed for the *Pcp-2* gene, MBP expression appears to be transiently regulated by thyroid hormone (85, 87). These data suggest the existence of a molecular mechanism whereby thyroid hormone action is confined to a discrete window of developmental time during oligodendrocyte development. As neuronal maturation must coincide with oligodendrocyte development it is not surprising that T3-regulated neuronal and glial genes are regulated by thyroid hormone in the same transient fashion.

Molecular Effects—Analysis

Thyroid hormone exerts most of its' effects at the nuclear level. Identification of thyroid hormone-regulated brain genes will allow us to determine how thyroid hormone regulates the development and physiology of the brain. Identification of the complete complement of thyroid hormone-regulated brain genes, however, remains a daunting task. There are many approaches that can be taken to identify known and unknown genes expressed in the brain that are transcriptionally regulated by thyroid hormone (22, 23). As was presented in the preceding section, these approaches have begun to yield a number of interesting candidate T3-responsive genes. New approaches, such as DNA microarray analysis, should allow identification of many more new candidate target genes in the near future. Difficulties remain, however, after these candidate genes have been identified. One of the first questions that needs to be asked after target genes are identified is whether these T3-regulated genes are directly or indirectly controlled by thyroid hormone. Does the regulated gene contain a functional TRE in the promoter region? The presence of a TRE supports the hypothesis that the gene's expression is directly regulated by thyroid hormone. It is possible, however, for a target gene to be regulated by thyroid hormone in the absence of a functional TRE. For example, thyroid hormone could directly regulate the transcription of a gene that controls the initiation of a developmental program, such as oligodendrocyte development. The specific genes expressed and/or the level of their expression is likely different between an immature and a mature oligodendrocyte. Thus, by initiating a developmental program through regulating the transcription of a specific gene or genes responsible for triggering initiation of development, thyroid hormone could indirectly regulate the transcription of all genes downstream of the initiation event. Determining whether changes in gene transcription are the direct or indirect result of thyroid hormone action remains one of the difficult challenges in uncovering the molecular role of thyroid hormone in brain physiology.

Once target genes have been identified another difficult task is determining how thyroid hormone regulation of these genes affects brain physiology. This task is not so difficult when the function of the target gene is well established (e.g., the myelin genes). The task becomes much more difficult when the function is not known (e.g., the Purkinje cell protein-2 gene). Testing the hypothesis that a T3-regulated target gene mediates the physiologic effect of thyroid hormone *in vivo* can also be difficult but is ultimately necessary. Many interesting questions concerning the control of thyroid hormone action also remain unanswered. For instance, why do certain genes that are thyroid hormone-responsive during development become refractory to thyroid hormone in the mature brain and how is this achieved? Most of the T3-dependent brain genes identified thus far are regulated during development. Less studied are the genes regulated by thyroid hormone in the adult.

Conclusions

In conclusion, the functional effects of thyroid hormone can be explained by identified deficits in cellular organization observed in the hypothyroid brain. In an attempt to understand the molecular basis of thyroid hormone action during brain development several genes regulated by thyroid hormone during late brain development have been identified. The T3-dependent expression pattern of these genes suggests a molecular mechanism for the observed transient effects of thyroid hormone on late brain development. That functions of some of these genes are consistent with the processes regulated by thyroid hormone during late brain development, such as neurite outgrowth, cell migration, and survival, is supportive of their role in mediating the effects of thyroid hormone at the molecular level. These identified genes probably reflect only a small sample of thyroid hormone-regulated genes in the developing brain. The genes identified to date offer insight into the molecular mechanisms of thyroid hormone action during brain development. Much work remains, however, before a unifying hypothesis encompassing all aspects of thyroid hormone-dependent effects on brain development can be formulated.

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