REVIEW

DEVELOPMENTAL THYROID HORMONE INSUFFICIENCY AND BRAIN DEVELOPMENT: A ROLE FOR BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)?

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Abstract—Thyroid hormones (TH) are essential for normal brain development. Even modest degrees of TH disruption experienced in utero can result in neuropsychological deficits in children despite normal thyroid status at birth. Neurotrophins have been implicated in a host of brain cellular functions, and in particular, brain-derived neurotrophic factor (BDNF) has a well documented role in development and function of the nervous system. A number of laboratories have reported the effects of TH administration or severe deprivation on neurotrophin expression in brain. This review provides an overview and update of recent developments in the thyroid field as they relate to the nervous system. Secondly, we describe an animal model of low level TH insufficiency that is more relevant for studying the neurological consequences associated with the modest TH perturbations of subclinical hypothyroidism, or that would be anticipated from exposure to environmental contaminants with a mode-of-action that involves the thyroid. Finally, we review the available in vivo literature on TH-mediated alterations in neurotrophins, particularly BDNF, and discuss their possible contribution to brain impairments associated with TH insufficiency. The observations of altered BDNF protein and gene expression have varied as a function of hypothyroid model, age, and brain region assessed. Only a handful of studies have investigated the relationship of neurotrophins and TH using models of TH deprivation that are not severe, and dose–response information is sparse. Differences in the models used, species, doses, regions assessed, age at assessment, and method employed make it difficult to reach a consensus. Based on the available literature, the case for a direct role for BDNF in thyroid-mediated effects in the brain is not compelling. We conclude that delineation of the potential role of neurotrophins in TH-mediated neuronal development may be more fruitful by examining additional neurotrophins (e.g., nerve growth factor), moderate degrees of TH insufficiency, and younger ages. We further suggest that investigation of BDNF invoked by synaptic activation (i.e., plasticity, enrichment, trauma) may serve to elucidate a role of thyroid hormone in BDNF-regulated synaptic function.

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INTRODUCTION

Thyroid hormones (TH) are critical for normal brain development. Severe TH deficiencies in the neonatal...
period are of clinical interest because they can result in diminished mental capacity which can be mitigated with early diagnosis and hormone supplementation. However, a greater recognition of the impact of maternal subclinical hypothyroidism on fetal CNS function is emerging, and the contribution of TH-disrupting environmental contaminants in this etiology remains to be determined. Because of the established roles of neurotrophins in neuronal migration, differentiation, and plasticity, there has been a focus of efforts to understand their role in TH-mediated neurological effects.

In this review we examined the available literature to evaluate the association between TH disruption and neurotrophin gene/protein expression. TH synthesis inhibitors have been used to pharmacologically induce hypothyroidism in animals, and we review data generated by these models. The roles of neurotrophins, with special attention to brain-derived neurotrophic factor (BDNF), are then evaluated by examining their responses to TH supplementation or deprivation in young and adult animals in vivo. This assessment of the literature will allow us to define clearer roles for TH on neurotrophin function, to evaluate the potential for neurotrophins to serve as biomarkers of developmental TH effects, and to elucidate the course of future studies.

SOME BASICS OF THYROID BIOLOGY

TH is essential for a number of physiological processes in mammalian species. In the hypothalamic–pituitary–thyroid axis (HPTA), thyroid releasing hormone (TRH) from the hypothalamus stimulates the pituitary to release thyroid stimulating hormone (TSH). TSH acts on thyroid gland receptors to activate synthesis and release of TH. Thyroxine (T4) is the predominant form of TH released into the bloodstream and is enzymatically deiodinated to 3,5,3′-triiodothyronine (T3), a high-affinity ligand for the nuclear TH receptors TRα and TRβ, whose activation regulates vertebrate development and physiology. In the circulation, these hormones are bound to serum proteins for transport and delivery to the many TH-dependent organs. The developing brain is one organ most vulnerable to TH insufficiency (Oppenheimer and Schwartz, 1997; Santisteban and Bernal, 2005; Williams, 2008). A key feature of TH action in the brain is the temporal sequence of events it supports, a feature that increases the complexity of research studies, while at the same time providing avenues to identify mechanisms that must exist to control TH action.

TH acts through nuclear receptors encoded by two genes, TRα or TRβ, to affect gene transcription. Thyroid receptors bind to DNA sequences (thyroid response elements, TREs) in the unliganded state and for this reason are called “aporeceptors”. In the absence of T3, these aporeceptors typically behave as transcription repressors toward target genes (Bernal and Morte, 2012). Upon binding of hormone, the aporeceptors then function as transcription activators. As such, the absence of T3 exerts a more detrimental effect than the absence of receptors, as shown in various TR knockout models in which the resulting somatic and neurological phenotype is distinct from, and impairments more subtle than, those resulting from chemical or surgical thyroidectomy.

It is the TRE, not the liganded receptor, which directly regulates specific TH-responsive genes. Few genes have been identified that are directly activated by TH, and of that group, most are transcription factors (e.g., hairless, hr) that modulate the expression of other genes (Thompson and Potter, 2000). TH regulation of these downstream genes is therefore indirect as these genes do not themselves possess a TRE and do not bind the T3-receptor complex. In this manner TH can modulate the expression of a multitude of downstream genes, an action that dramatically increases the sphere of their influence on brain development, as well as the difficulty identifying developmentally important TH-responsive genes (Anderson et al., 2003; Flamant and Samarut, 2003; Quignodon et al., 2004). Furthermore, TRs do not regulate the same genes in all neuronal cell types, nor do they regulate the same gene in the same cell over time during development, necessitating the existence of mechanisms that control the cell specificity and timing of TR action (Iniguez et al., 1996; Williams, 2008; Hernandez et al., 2010). Selective expression of TH-specific transporter proteins in the brain and local metabolizing enzymes represent two mechanisms whose relative contribution to the local fidelity of TH signaling is just beginning to be unravelled.

Adding further to this complexity, the degree of change in the expression of TH-responsive genes in the brain is subtle in nature, standing in marked contrast to TH-mediated change in gene expression in many other tissues. Most known neural genes exhibit transient responsiveness to TH and undergo changes in expression of only two to threefold in response to the hormone (Poguet et al., 2003; Quignodon et al., 2004; Royland et al., 2008). Consequently, it has proven challenging to associate the changes in expression of a particular gene or family of genes to the well known effects of TH on brain development (Oppenheimer and Schwartz, 1997; Thompson and Potter, 2000; Bernal, 2002).

Difficulty linking alterations in gene expression and brain function may also derive from the paradigm traditionally used in these studies. Specifically, models of severe hypothyroidism may lead to a plethora of effects on the somatic development of many organ systems that may obscure the more direct actions of TH on brain development. Observations at the molecular, anatomical, physiological, and behavioral level in such models may not only reflect the actions of TH insufficiency but also may include those secondary to the somatic insult resulting from severe TH deprivation.

Thyroid hormone transporters in the brain

Specific transporter proteins from the monocarboxylate transporter (MCT) and organic anion-transporting polypeptide (OATP) families actively take up T3 and T4 and are primarily expressed on blood vessels, astrocytes, and neurons (Williams, 2008; Bernal, 2011). Individuals suffering from mutations of the MCT8 transporter exhibit severe mental retardation, i.e., Allen–Herndon–Dudley

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syndrome (Schweizer and Köhrle, 2012). The OATP family of transporters, also capable of iodothyronine transport, is expressed in hippocampal, cortical, and cerebellar neurons (Schweizer and Köhrle, 2012). Initial findings with mouse models with targeted deletions of these transporters were disappointing as these mutants did not exhibit characteristics of the neurodevelopmental phenotype observed in humans or rodent models of severe hypothyroidism (Heuer and Visser, 2009; Mayer et al., 2012a). However, recent reports of double knockouts of both transporters in mouse models reveal significant neuroanatomical abnormalities and behavioral impairments (Mayer et al., 2012b). Exciting new discoveries using transgenic models hold great promise for the future elucidation of the role played by TH-transporters in the region-specific and time-dependent presentation of T3 to the neuron. From both a clinical and environmental contaminant perspective, it is important to recognize that these mechanisms alter TH availability to the developing brain in a manner that is not accurately reflected in changes in serum T3 or T4.

**Thyroid hormone metabolism in the brain**

Enzymes that convert T4 to T3 (i.e., deiodinase 2, D2) or deactivate T3 to physiologically inactive reverse-T3 or T2 (i.e., deiodinase 3, D3) provide additional means of control over TH-dependent gene regulation in the neuron (Bernal, 2011; Williams and Bassett, 2011). Neuronal T3 is largely derived from D2-dependent metabolism of T4 in glial cells. D2-deficient mice exhibit elevated circulating T4 and TSH levels but normal circulating T3 and as neonates display reduced T3 concentrations in the brain. In contrast to the glial expression of D2, the inactivating D3 enzyme is highly expressed in neurons. As neonates, D3-deficient mice have elevated levels of serum hormones, whereas serum hormone profiles demonstrate hypothyroidism after PN15. Selective deletions of D2 or D3 have modest effects on the expression level of T3-target genes (e.g., RC2, Hr) and offsprings exhibit a mild general neurological phenotype compared with the abnormalities of hypothyroidism. Collectively, these findings with transgenic mouse models suggest that compensatory mechanisms exist to ameliorate the neurological consequences of transporter and deiodinase deficiencies during the organism’s development, and that significant species differences may exist between humans and rodents (i.e., as seen in MCT8 mutations). However, several laboratories have recently described significant neurological impairments and overlap in brain gene expression with chemical hypothyroid models with different combinations of receptor, transporter, and deiodinase deletions (e.g., Hernandez et al., 2010, 2012; Bernal, 2011; Mayer et al., 2012b).

**Non-genomic actions and novel thyroid signaling molecules**

T3-modulated transcription of target genes via activation of TRs is a slow process, the effects of which manifest over hours and days. However, rapidly occurring effects of TH have also been documented (Davis et al., 2008). T4 itself, and its presumed iodothyronamine metabolites, have rapid physiological actions both in vitro and in vivo. These data indicate that actions of TH can also occur at non-nuclear sites to alter developmental processes and function of fetal, neonatal and adult nervous systems. One presumed TH metabolite, 3-iodothyronamine (T1AM), produces a rapid and profound effect on body temperature, heart rate, and metabolism when administered in vivo (Scanlan et al., 2004). Although initially described as an extrathyroidal metabolic derivative of T4, a recent report indicates T1AM represents a distinct hormone entity of the same biosynthetic machinery in the thyroid gland that is necessary for T4 synthesis (Hackenmueller et al., 2012). As such, this newly discovered TH may constitute a part of TH action in target tissues including the brain whose effects are yet to be revealed.

**EFFECTS OF TH INSUFFICIENCY IN HUMANS**

In humans, severe deficiencies in TH during development are associated with irreversible damage to virtually all organ systems, a condition termed cretinism (Glinoer, 2007). The primary causes of severe developmental hypothyroidism in humans are mainly congenital hypothyroidism and iodine deficiency (Glinoer and Delange, 2000; Glinoer, 2007). Each condition can produce a different spectrum of symptoms, the specific nature of which is dependent on the timing, duration, and severity of the deficiency.

Congenital hypothyroidism refers to a condition in which children are born with very low levels of serum hormone, typically resulting from a malformation of the thyroid gland. Children appear normal at birth, their mothers have normal thyroid function, and in the past, the lack of specific symptoms often delayed the diagnosis and treatment, with the prognosis deteriorating with the passage of time. The full clinical picture prior to the era of systematic neonatal screening included stunted growth and severe mental retardation (see reviews by Dussault and Ruel, 1987; Chan et al., 2005; Glinoer, 2007). Neonatal screening programs for identification and early treatment, now widely instituted in North America and abroad, have largely eliminated congenital hypothyroidism as a primary cause of mental retardation. However, lower global IQ scores, language delays and weak verbal skills, motor weakness, attentional deficits and learning impairments were evident in children with delayed or inadequate treatment (Derksen-Lubsen and Verkerk, 1996). Even in cases where the condition is diagnosed early and treated effectively, subtle impairments in mental function remain (Zoeller and Rovet, 2004).

Iodine is an essential element for the biosynthesis of TH and some of the most serious neurologic impairments from environmental causes are in children born in regions of the world where dietary iodine deficiency is prevalent (Zimmermann, 2007). These children are characterized by stunted growth and a high incidence of mental retardation. Neurological
deficiencies seen in endemic iodine deficiency beginning in utero and that continue throughout infancy are more severe than those resulting from congenital hypothyroidism, indicating that early fetal thyroid function is also critical for normal brain development (Glinoer, 2007).

A number of studies have now demonstrated that modest levels of TH insufficiency characteristic of subclinical hypothyroidism in pregnant women can result in neuropsychological deficits in their offspring, despite normal thyroid status of the child at birth (Haddow et al., 1999; Zoeller and Rovet, 2004; Berbel et al., 2009; Henrichs et al., 2010). These observations of neurological impairments following subclinical reductions in maternal T4 have raised the level of concern over the influence of environmental contaminants which decrease thyroid function (Porterfield, 1994; Blount et al., 2006; Ginsberg et al., 2007; Mendez et al., 2012). The animal literature has lagged behind the human data because models of moderate TH disruption have not been available that parallel the human phenotype. As such, animal research is lacking on mechanisms, biomarkers of effect, and efficacy of intervention to reverse subtle perturbations of the thyroid axis.

ENVIRONMENTAL FACTORS AND SUBCLINICAL HYPOTHYROIDISM

The U.S. Environmental Protection Agency must regulate chemicals that have the potential to pose a hazard to human health. A large number of environmental contaminants with diverse structures have been shown to decrease circulating levels of TH (Brucker-Davis, 1998). Animal studies have indicated that this action of xenobiotics on the thyroid system may contribute to alterations in nervous system development and function (see review by Gilbert and Zoeller, 2010). Some of these environmental contaminants have also been evaluated in humans and been found to disrupt the thyroid axis (Koopman-Esseboom et al., 1994; Blount et al., 2006; Chevrier et al., 2007; Turyk et al., 2007; Baccarelli et al., 2008). Moreover, human exposures to some of these same contaminants are associated with neurodevelopmental impairments (Jacobson and Jacobson, 1996; Koopman-Esseboom et al., 1996; Lonky et al., 1996; Patandin et al., 1999), suggesting that environmental contaminants may produce neurotoxic effects on the developing human by interfering with thyroid function or hormone action. Relative to severe iodine deficiency and congenital hypothyroidism, environmental contaminants reduce circulating TH to a modest degree (Gilbert and Zoeller, 2010). What has not been established is the degree to which thyroid status must be perturbed to produce adverse neurological outcomes. Until recently experimental studies have uniformly examined severe TH deficiency with a limited information on the neurological consequences of low-level thyroid dysfunction. Addressing this question has important implications not only for environmental regulation but also for the ongoing debate of the cost, risk and benefits of treatment of subclinical hypothyroidism.

Pharmacological models of hypothyroidism using propylthiouracil and methimazole

There are two prevalent models of developmental hypothyroidism using the synthesis inhibitors methimazole (MMI) or propylthiouracil (PTU). These agents block the action of the thyroperoxidase enzyme in the thyroid gland that is necessary to couple iodine to tyrosine in order to form T3 and T4 (Cooper et al., 1983, 1984). These agents are typically delivered to pregnant dams through the drinking water beginning in early gestation to compromise hormone supplies to the fetus and neonate, or late in gestation to affect the near term fetus and the thyroid status of the nursing pup. Typically, a single high dose of either PTU or MMI, ranging from 0.02% to 0.05% (equivalent to 200–500 ppm) is administered to pregnant dams through the drinking water beginning early (gestational day 6, GD6) or late (GD17) in gestation. These treatments dramatically reduce circulating levels of T3 and T4, and produce exponential increases in pituitary release of TSH that stimulates the thyroid gland to increase hormone synthesis and release. These models generate a serum hormone profile consistent with hypothyroidism in humans. Phenotypically, these animals are characterized by stunted growth, severe developmental delays, hair loss, and motor and sensory deficits (Dussault and Ruel, 1987; Bernal, 2002; Gilbert and Zoeller, 2010). Other models have incorporated thyroidectomy of the dam and the newborn pups to even more dramatically alter thyroid status (e.g., Iniguez et al., 1996; Kim et al., 2007).

These models have provided valuable information on the critical importance of TH to brain development in the fetal and early neonatal period, but their utility in assessing the impact of moderate degrees of TH insufficiency is limited.

Low dose PTU model characterization

To begin to address this knowledge gap, we have developed a model of low level TH disruption using graded levels of PTU delivered through the drinking water to the pregnant rat from early gestation and continuing throughout lactation (e.g., Sui and Gilbert, 2003; Gilbert and Sui, 2006; Gilbert, 2011). In contrast to the standard models of 200–500 ppm, we have examined concentrations of PTU in the drinking water that range from 1 to 10 ppm. Water consumption is not altered at these concentrations, a significant concern when high concentrations of PTU are used, due to its bitter taste. This model leads to dose-dependent reductions in serum TH, with reductions in T4 being the most sensitive, and at the lower doses, the only indicator of hormone disruption. Body weight gain in dams and pups is not altered at 1 and 2 ppm PTU, and only marginally and transiently in pups at 3 ppm in the later neonatal period. At the 10 ppm dose, body weight over the first 10 days of life is not different from controls,
but thereafter animals fail to thrive (Sui and Gilbert, 2003). They remain stunted in growth, exhibit significant developmental delays in eye opening, unstable gait, and hearing loss, and must be kept with dams for an additional week to ensure survival. We characterize this level of hypothyroidism induced by 10 ppm PTU as severe. Very small body weight differences are sometimes but not always seen at the 3 ppm dose level and a slight delay in eye opening has been observed (Sui and Gilbert, 2003; Gilbert, 2011). Serum T4 is significantly reduced at all dose levels in pups on PN14, and slight reductions in serum T3 are sometimes but not always seen with 3 ppm, but T3 is consistently lower at 10 ppm. Dam serum hormones measured when the pups are weaned are also reduced but to a lesser degree than seen in their offspring (Fig. 1).

**Hippocampal synaptic transmission and plasticity**

The hippocampus is dependent on adequate supplies of TH during development and adulthood (Rami et al., 1986; Madeira et al., 1991, 1992; Zoeller and Rovet, 2004). We have studied the neurophysiological properties in the hippocampal area CA1 and dentate gyrus using field potentials ex vivo and in vivo, respectively. Reductions in excitatory and inhibitory synaptic transmission in the dentate gyrus have been shown at PTU doses of 2 ppm and above (Fig. 2; Gilbert and Sui, 2006; Gilbert et al., 2007; Gilbert, 2011). Reductions in paired pulse depression and enhanced paired pulse facilitation were accompanied by reductions in parvalbumin protein expression, the calcium-binding protein selectively expressed in inhibitory GABAergic interneurons (Fig. 3). BDNF is essential for the differentiation of multiple interneuron subtypes, including the fast-spiking parvalbumin-expressing interneurons that control the excitability of cortical networks (Berghuis et al., 2004). It is possible that TH insufficiency mediates its effects on parvalbumin expression through BDNF but this has not been directly tested. Similarly, BDNF has long been implicated in activity-dependent plasticity (Yoshii and Constantine-Paton, 2010). Long-term potentiation (LTP) of the excitatory postsynaptic potential (EPSP) slope is modestly reduced in adult offspring of PTU-treated dams at all dose levels tested (Fig. 2; Gilbert and Paczkowski, 2003; Gilbert and Sui, 2006; Gilbert, 2011). Population spike LTP was less sensitive and even showed increases in magnitude at higher doses of PTU which may be related to reductions in synaptic inhibition. Hippocampal slice recordings of area CA1 in neonates and adults also reflected altered synaptic transmission and plasticity (Sui and Gilbert; 2003; Gilbert, 2004; Sui

Fig. 1. Serum thyroid hormone profile in the low-dose PTU model. Dams were exposed to varying concentrations of PTU in the drinking water beginning on gestational day (GD6) and continuing through lactation. (A) Serum (mean ± SEM) T4 was reduced in a graded fashion as a function of PTU dose in pups and in dams (n = 5–15 litters/group). (B) More modest reductions in circulating T3 were seen at 3 and 10 ppm PTU concentrations in pups and dams. (C) Serum T4 and (D) serum T3 exhibited full recovery by PN90. *p < 0.05 compared to controls (from Lasley and Gilbert, 2011).
et al., 2005) but the magnitude of the change appeared not as robust as that observed in vivo. This may possibly reflect differential sensitivity of these subregions to developmental hypothyroidism, but are more likely attributable to inherent differences between in vivo and in vitro assessments.

**Hippocampal-dependent learning**

Learning and memory are clearly impaired in animals following developmental hypothyroidism (Davenport and Dorcey, 1972; Akaike et al., 1991; Darbra et al., 2004; Gilbert and Sui, 2006; Axelstad et al., 2008). In the low-dose PTU model we have evaluated learning and memory using the Morris water maze and trace fear conditioning in adult offspring. Both paradigms revealed learning deficits at doses of 3 and 10 ppm PTU (Gilbert and Sui, 2006; Gilbert, 2011). In recent studies, lower doses of PTU (1 and 2 ppm) were without effect on water maze acquisition (unpublished observations), but context learning deficits in a modified version of the trace fear conditioning paradigm were evident in offspring from the lowest 1 ppm dose group tested (Fig. 4). Impairments in context learning were also seen only in male offspring at the lower doses, but the more severe hypothyroidism induced by 10 ppm produced impairments in males and females, and in both context and cue learning (Gilbert and Taylor, 2012). Deficits in context fear learning were subtle in nature and could be ameliorated in all but the 10 ppm dose group by increasing the duration of the shock, a manipulation that serves to enhance the robustness of the learning (Fig. 4). These data are important as they reveal significant and persistent declines in cognitive function in response to modest reductions in serum T4 in the dam (<20%) and the neonate (~50%) and in the absence of change in serum T3, TSH, body weight, or latency to eye opening. Generally, it has been quite

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**Fig. 2.** Developmental exposure in the low-dose PTU model produces dose-dependent impairments in excitatory and inhibitory synaptic transmission and synaptic plasticity as measured in field potentials recorded from the dentate gyrus in vivo. (A) Excitatory synaptic transmission as measured via an input/output function of the amplitude of the EPSP slope of adult offspring of PTU-exposed dams is impaired at moderate degrees of TH insufficiency. Input/output curves from 2 and 3 ppm groups were significantly suppressed relative to controls (data taken from Gilbert, 2011). Previous work demonstrated greater reductions at a higher dose of 10 ppm PTU (Gilbert and Sui, 2006, not shown). (B) Paired pulse depression is reduced and paired pulse facilitation is enhanced in PTU-exposed animals (from Gilbert et al., 2007). Effects are most prominent at the lowest stimulus intensity (20% maximum) and highest PTU dose. (C) Theta-burst stimulation of the perforant path induced long-term potentiation of the EPSP slope, the magnitude of which was significantly suppressed in PTU-exposed offspring at all doses levels when collapsed across intensity (ANOVA with mean contrasts); * indicates all dose groups significantly different from 0 ppm, p < 0.05 (modified from Gilbert, 2011).
difficult to demonstrate impairments in these simple tasks of learning and memory when modest reductions in TH are induced by PTU, the environmental contaminant perchlorate (Gilbert and Sui, 2008), or by marginal dietary iodine deficiency (Gilbert et al., 2012). The lack of effect of these modest alterations in thyroid status on neurobehavior may reflect the lack of sensitivity of the test to subtle deficits as much as the absence of subtle cognitive impairments accompanying low-level TH insufficiency.

Cell fate specificity and white matter abnormalities

White matter formation and myelination are important developmental events that are regulated by TH and growth factors including BDNF (Ibarrola and Rodriguez-Pena, 1997; Ferreira et al., 2007; vonDran et al., 2010). Children exposed to low levels of TH during critical periods of brain development have altered myelination patterns (Jagannathan et al., 1998) and reductions in myelin formation is a hallmark of congenital hypothyroid models. In these models, although the total number of axons is not altered, the number of myelinating oligodendrocytes and myelinated axons in major white matter tracts is significantly reduced (Gravel and Hawkes, 1990; Schoonover et al., 2004; Powell et al., 2012). In the low-dose PTU model, Sharlin et al. (2008) identified structural alterations in the white matter of PTU-exposed pups using in situ hybridization techniques. They reported alterations in the cellular composition of the corpus callosum and anterior commissure, the primary fiber pathways that support communication between the two cerebral hemispheres. At low doses, reductions in mRNA of myelin-associated glycoprotein (MAG), a marker of oligodendrocytes, were coupled with increases in mRNA for the astrocyte marker, glial fibrillary acidic protein (GFAP). The reduction in oligodendrocytes was dose-dependent and accompanied by an equivalent parallel increase in the number of GFAP-positive astrocytes — i.e., oligodendrocyte density declined and astrocyte density rose linearly with serum T4 concentration (Fig. 5). Because both oligodendrocytes and astrocytes derive from the same glial precursor cell and the total number of cells of both phenotypes did not change, these data suggest that the lack of TH altered cell fate specificity, i.e., in the absence of sufficient TH, glial precursors were directed more to an astrocyte than to an oligodendrocyte lineage.

Errors in neuronal migration

Smaller brain size and disorganized cytoarchitecture in the cortex and cerebellum have been described in models of severe hypothyroidism (Rami et al., 1986; Madeira et al., 1991, 1992). Bernal and colleagues reported delayed and disordered migration of cortical and hippocampal neurons in response to iodine deficiency, a model of transient maternal hypothyroidism, and the late-gestation thyroidectomy model of preterm birth (Lavado-Autric...
et al., 2003; Auso et al., 2004; Berbel et al., 2010). A pattern of aberrant neuronal migration has also been described in the low-dose PTU model by our laboratory. We have identified the presence of a misplaced cluster of neurons, a heterotopia, positioned in the corpus callosum of developmentally hypothyroid animals (Fig. 6). TH insufficiency in the prenatal period is both necessary and sufficient for its formation. Clearly observable with simple Nissl staining in offspring of 3 and 10 ppm animals (Goodman and Gilbert, 2007; Saegusa et al., 2010; Powell et al., 2012), recent findings indicate that it is discernible using in vivo MRI imaging techniques (Powell et al., 2012). With neuron-specific antibodies and immunohisto-chemistry, we have recently identified the presence of heterotopias in offspring of dams exposed to the lowest 1 ppm dose of PTU, a dose that reduces maternal T4 by less than 20% (unpublished observations). Although the functional significance of the presence of a heterotopia has not yet been fully elucidated, the reliability of its positioning and its detectability at low doses make it attractive as a simple, sensitive, and reliable biomarker of modest prenatal TH disruption.

**IS THERE A ROLE FOR NEUROTROPHINS IN THE DEVELOPMENTAL EFFECTS OF THYROID HORMONE INSUFFICIENCY?**

Neurotrophins are a family of soluble proteins that regulate the initial steps in neuron–target interactions and promote the survival and differentiation of various nerve cells in the developing and adult nervous system. Nerve growth factor (NGF) was the first neurotrophin discovered and is a key player in directing developing fibers toward their target field to regulate the extent of innervation (Lewin and Barde, 1996). Other members of the neurotrophin family include BDNF and neurotrophins-3, -4, and -5 (NT-3, NT-4/5). Neurotrophins are largely produced and released by...
Fig. 5. Astrocytes expressing glial fibrillary acidic protein (GFAP) are increased (A) and oligodendrocytes expressing myelin-associated glycoprotein (MAG) are decreased (B) in a dose-dependent manner in the corpus callosum of rat pups exposed through gestation and lactation to PTU. Decreases in MAG-positive cells were balanced by increases in GFAP-positive cells and these changes were linearly related to serum T4 (C). The latter relationships suggest that TH insufficiency may disrupt cell fate specificity as both MAG- and GFAP-expressing cells are derived from a common glial precursor (from Sharlin et al., 2008).

\[ p < 0.01; \quad *** p < 0.001 \] compared to controls.

Fig. 6. An abnormal cluster of heterotopic neurons is reliably detected in the corpus callosum following prenatal TH reductions. Large bilaterally positioned heterotopias are evident in Nissl-stained material at high doses (10 ppm, arrows, from Goodman and Gilbert, 2007), and even more prominently using immunohistochemistry for the neuron-specific antibody NeuN. Recently heterotopic neurons have been detected at the lowest doses of PTU assessed, the incidence and size of which increase dose-dependently. Occasionally NeuN-positive cells in the same position were seen sporadically in the corpus callosum of some control animals (bottom left). However, a prominent heterotopia is clearly visible in response to 1 ppm PTU, a dose that reduces maternal TH by < 20% (unpublished observations).
target tissues and are taken up by responsive neurons. They direct various cellular mechanisms including cell fate determination, determination and process outgrowth in the developing brain and in response to CNS activity or injury in the adult brain. Neurotrophins have been implicated in a host of brain functions including neuronal cell survival, neurite outgrowth, cell migration, regulation of excitatory-inhibitory balance, and glutamate-dependent dendritic growth, synapse formation, stabilization and plasticity (Yoshii and Constantine-Paton, 2010). BDNF and its associated receptor TrkB have a wide spectrum of biological actions: BDNF plays a role in promoting survival and differentiation of selected neuronal populations, its expression and release are activity-dependent, and its presence can alter the structure of dendrites and spines of neurons in the developing and mature CNS. Consequently, BDNF has a well documented role in brain development and in adult brain function and synaptic plasticity (e.g., Lewin and Barde, 1996; Lu and Figurov, 1997) as well as in the emergence and therapeutic reversal of nervous system disorders including clinical depression and Alzheimer’s disease (Martinovich et al., 2007; Allen et al., 2011).

In view of the importance of BDNF in these various roles, and because TH is clearly required for normal brain development, a number of laboratories have examined the potential relationship between TH and neurotrophin expression in developing brains. It seems reasonable to propose that alterations in BDNF may underlie some of the persistent neurological impairments associated with developmental hypothyroidism. On review of the literature however, the results are generally mixed with effects that are more consistent for other members of the neurotrophin family (e.g., NGF) than BDNF. To investigate the relationship of TH and neurotrophins, animals have been dosed with hormones or had circulating levels of TH dramatically reduced. Exogenous delivery of T3 or T4 to the adult or neonate generally increases neurotrophin expression, but effects are brain region-dependent, and may vary with hormone type, timing of delivery, animal species, and neurotrophin (see below). The impact of TH deprivation on BDNF also appears to be age- and region-specific, and with a few exceptions inquiry has been limited to severe hypothyroid models. Limited data are available for BDNF in animal models of moderate TH insufficiency that are unencumbered by confounds of general developmental delays, concerns for undernutrition, severe growth impairments, and excessive pup mortality. The following discussion is a brief summary of the available literature concerning effects on neurotrophin expression in the brain that accompany either exogenous supplementation or deprivation of TH in adult and developing rodents.

**Increasing thyroid hormones in adults in vivo**

To examine the relationship of TH and neurotrophins, T3 or T4 have been added to brain cells in culture or administered to euthyroid animals as adults or as neonates. TH increases neurite extension and dendritic expansion in neuronal cell cultures in a BDNF-dependent manner (Koibuchi et al., 2003; Fanwell et al., 2005). Although delivery of T3 (1 μg/kg, sc) to the adult rat in vivo also increases spine density in hippocampal neurons (Gould et al., 1990a,b), the involvement of neurotrophins in this action has not been directly demonstrated. Repeated administration of T4 (2 μg/kg/day, i.p. for 12 days) to the adult mouse does increase the expression of NGF mRNA and protein in cortex, cerebellum, and brainstem (Walker et al., 1979, 1981). In contrast, in the rat, higher concentrations of T3 (50–500 μg/kg/day, s.c. for 10 days) did not alter the basal levels of NGF, NT-3 or BDNF mRNA in the hippocampus or pyriform cortex (Kim et al., 1998; Vaidya et al., 2001). Giordano et al. (1992) reported selective increases in NGF and NT-3 mRNA in the hippocampus but not cortex with high doses of T3 (500 μg/kg/day, i.p. for 8 days), but there was no change in BDNF mRNA expression in either site. In contrast to these older reports based on in situ hybridization, Sui et al. (2010) employed quantitative RT-PCR to evaluate BDNF expression. They report that a single high dose of T3 (300 μg/kg) markedly increased BDNF mRNA expression and the transcription of BDNF exons I, II, IV and V in the hippocampus for at least 24 h, the latest time-point assessed. Increased BDNF gene expression was accompanied by an augmentation of protein expression in the hippocampus with a slightly delayed onset (4 h) that also persisted for 24 h (Sui et al., 2010).

Collectively, these findings indicate that increasing serum T3 concentrations to supra-physiological levels by systemic administration of T3 has varied effects on neurotrophin expression in the adult rodent brain. When present, effects of TH administration are regionally specific, duration-dependent, and augmentation of specific neurotrophins have been inconsistent – some reports favoring NGF and NT-3 with no change in BDNF (Giordano et al., 1992), others reporting increases in BDNF mRNA and protein (Sui et al., 2010). 

**Augmenting thyroid hormones in the neonate in vivo**

As in the adult, early work revealed an increase in NGF protein expression in the cortex, cerebellum, and brainstem of mice treated as neonates with a constant volume of T4 for the first 11 days of life (Walker et al., 1982). This dosing regimen approximated dose levels of over 1 mg/kg, i.p. to the newborn and less than half that much in the PN10 pup. Following a similar T4 dosing regimen in rats, Camboni et al. (2003) reported increased BDNF mRNA and protein expression in the septum of the PN10 pups which subsequently dissipated by adulthood. Neonatal T4 treatment also increased BDNF, NGF, NT-3 and NT-4 mRNA in the hippocampus of the PN10 rat (Luessse et al., 1998). These increases in neurotrophin gene expression were not accompanied by alterations in the expression of their respective neurotrophin receptors (p75, TrkA, TrkB, TrkC), leading the authors to conclude that neurotrophin content rather than receptor function was the basis for their morphological effects produced in brain development (Roskoden et al., 1999). Assessments of neurotrophin protein expression, however, were not performed.
High doses of T3 (1 mg/kg/day, i.p., from PN7 to PN28) facilitated the development of choline acetyltransferase-positive fibers in forebrain cholinergic projection areas and a precocious expression of cholinergic markers in cortex, hippocampus, and amygdala (Gould and Butcher, 1988; Oh et al., 1991). Supporting the observations of Roskoden et al. (1999), these findings in forebrain structures were not accompanied by any change in NGF receptor immunohistochemistry in the cortex. Similar doses of T3 in neonatal rats beginning at various ages (PN1–PN28) increased mRNA expression of NT-3 in the granule cells of the cerebellum but not in the hippocampus, and the effects were limited to animals younger than PN21 (Lindholm et al., 1993). In summary, these findings support those observed in the adult rat in which excess TH was found to augment neurotrophin mRNA expression.

Reducing thyroid hormones in the adult

Many fewer studies have examined the effects of adult onset hypothyroidism on BDNF expression. Basal levels of BDNF protein assessed by Western blot, in addition to a number of the signaling molecules implicated in synaptic plasticity (e.g., calcineurin, CREB, MAP kinase) are reduced in rats following surgical removal of the thyroid gland in adulthood (Alzoubi et al., 2007). These changes were associated with impairments in long-term potentiation in vivo in area CA1, but distinct from developmental hypothyroid models, LTP of the dentate gyrus remained intact (Gerges et al., 2004; Alzoubi et al., 2007). In a PTU model of adult onset hypothyroidism, the opposite effect of enhanced expression of BDNF has recently been reported. Cortés et al. (2012) reported that a high dose of PTU in the drinking water (500 ppm for 20 days) of adult rats increased BDNF protein in the telencephalon and mRNA expression in the cortex, and the hippocampal subfields, including the dentate gyrus. BDNF has been implicated in postnatal neurogenesis in the dentate gyrus of the hippocampus and the olfactory bulb of the adult rat, a process whereby neurons continue to be generated throughout life (Babu et al., 2009; Choi et al., 2009; Lazarini and Lledo, 2011). Several reports have demonstrated reduced capacity for proliferation (Lemkine et al., 2005; Montero-Pedrazuela et al., 2006) or survival of newly born neurons in both of these neurogenic niches of the adult brain under conditions of thyroid compromise in adulthood (Ambrogini et al., 2005; DeSouza et al., 2005; Kapoor et al., 2012). A reduction of adult hippocampal neurogenesis under hypothyroid conditions may involve reduced expression of BDNF in these brain regions. However, we found no reports directly assessing the possible relationship between impaired neurogenesis and BDNF in animals with adult onset hypothyroidism.

Reducing thyroid hormones in the neonate

More work has focused on reducing TH in the developing animal to evaluate the effect on neurotrophins in the brain. Animals have been typically exposed in utero and throughout lactation by treating the drinking water of pregnant dams with MMI or PTU. Koibuchi et al. (1999, 2001) reported reductions in BDNF mRNA and/or protein in the cerebellum of neonatal rats and mice (PN15–30) exposed to high doses of PTU (500 ppm) or MMI (250 ppm) coupled with a very high dose of the iodine uptake inhibitor perchlorate (20000 ppm) from mid-gestation to weaning. Reductions in transcription of BDNF exons II, III, IV, and V were reported for hypothryoid animals co-treated with T4 (no untreated control animals were included in the design). No changes in any BDNF transcript were evident in younger (PN1 and PN7) or older (>PN30) offspring or in cortical tissue from the same animals.

Like Koibuchi et al. (2001), Godbole and colleagues (Sinha et al., 2009) also reported reductions in BDNF mRNA and protein in the cerebellum following exposure of rat dams to MMI (250 ppm without perchlorate) beginning earlier in gestation (GD8 vs GD15 for Koibuchi et al., 2001). These authors observed reductions in BDNF but at an earlier age (PN8) than reported by Koibuchi et al. (2001). The study design was somewhat limited as observations were based on offspring from only two litters. Increases in mRNA for NGF and the neurotrophin receptor p75NTR were observed with no change in the BDNF receptor TrkB or the NGF receptor TrkA. Paradoxically, increases in expression of NGF transcript in the cerebellum were coincident with decreases in NGF protein on PN0 and PN8 suggesting a post-translational modification in protein expression and demonstrating a lack of consistency in message and protein levels of neurotrophins (Caldwell et al., 2008). Opposite effects were seen in a microarray study in mice in which p75NTR was downregulated in MMI-treated pups on PN4, and this observation was confirmed by RT-PCR (Takahashi et al., 2008).

The Godbole laboratory also examined neurotrophins in the cortex, using a similar design and dosing regimen. Kumar et al. (2006) administered 250 ppm MMI to dams from GD8 throughout lactation, and the offspring were continued on this dose until sacrifice. Expression of the 53 kDa precursor form of NGF and the p75NTR receptor were markedly elevated from birth to PN16, suggesting enhanced apoptosis, a function of p75NTR in development (Chen et al., 2009). At the same time, however, TrkA mRNA was diminished. These effects had completely reversed by PN90 – i.e., pro-NGF and p75NTR expression were significantly decreased and TrkA mRNA elevated compared to euthyroid animals. These increases in NGF and the p75NTR receptor, were consistent with mRNA results in the cerebellum (Sinha et al., 2009). Unlike the cerebellum, however, TrkA gene expression was decreased in the cortex of the pup, but elevated in the cortex of the adult.

In offspring of dams exposed to 50 mg/kg/day PTU by gavage (~400 ppm drinking water equivalent) beginning in late gestation (GD17), Neveu and Arenas (1996) reported...
reductions in cerebellar BDNF, TrkC, and NT-3 mRNA on PN15 and PN30, whereas NGF and NT-4 expression levels were increased. High-dose PTU (500 ppm) from GD15-PN21 decreased hippocampal BDNF exon II transcripts and BDNF protein concentrations on PN1, 5, 15, and 30, with recovery evident by PN60 after termination of treatment at weaning (Sui and Li, 2010). The profile of BDNF expression in this cohort of animals however showed an increase in expression with age, a pattern in direct contrast with numerous reports on the normal ontogeny of BDNF in early brain development in rodents (e.g., Das et al., 2001; Kim et al., 2007). Possible epigenetic modifications were suggested to underlie these changes with up-regulation of DNA methyltransferase activity and down-regulation of histone acetylation prior to weaning associated with the decreases in BDNF gene and protein expression.

Further support for these observations was obtained by Wang et al. (2012). A complex model of maternal hypothyroidism was developed via thyroidectomies performed in rats prior to mating, and the dams received partial or full replacement doses of T4 during gestation. On PN3, offspring of dams given no or partial replacement doses of T4 had 30–35% decreases in hippocampal BDNF protein compared to controls that were not surgically altered. Full replacement doses of T4 initiated later during gestation also further ameliorated the reductions in BDNF.

In contrast, Alvarez-Dolado et al. (1994) observed no change in BDNF mRNA in the hippocampus, cortex, or cerebellum at PN15 or PN90 in response to severe hypothyroidism induced by a high dose of MMI (200 ppm) given to the dams beginning on GD9, and coupled with thyroidectomy of the pups on PN5. Decreases in NGF mRNA were seen and are consistent with those reported by Neveu and Arenas (1996), but unlike that report, increases rather than decreases in NT-3 mRNA were observed in the adults thyroidectomized as pups. Increases in NT-3 mRNA also stand in contrast to the observations of Lindholm et al. (1993) who also reported a 50% decrease in NT-3 mRNA expression in severely hypothyroid rats that could be reversed with T3 administration.

In more recent studies, Bastian et al. (2010) found no difference in the expression of whole brain transcripts of BDNF exon IV in PN12 rat pups exposed in utero from GD6 to a much lower dose of PTU (10 ppm), but one which still resulted in a rather severe state of postnatal hypothyroidism (see Gilbert and Sui, 2006). In a followup study, a moderate degree of hypothyroidism induced by a 3 ppm concentration of PTU in the drinking water of pregnant dams that reduced T3 and T4 > 50% and 95%, respectively, did not alter BDNF mRNA or transcripts of BDNF exons IV or VI in the hippocampus or cortex of PN10 hypothyroid brains (Bastian et al., 2012). Neither did Royland et al. (2006) observe changes in expression of BDNF transcript in PN14 cortex or hippocampus in a microarray study with varying doses of PTU (0, 1, 2 and 3 ppm) delivered to dams beginning on GD6. Consistent with these observations, no changes in BDNF protein concentrations were seen in the hippocampus, cortex, or cerebellum in the PN14 neonate (Fig. 7, Lasley and Gilbert, 2011). We have since evaluated expression for total BDNF and BDNF exons I and IV in the hippocampus using the low-dose PTU model and found modest reductions in mRNA in similarly aged pups (unpublished observations). In adulthood however, BDNF protein expression was diminished in a U-shaped relationship as a function of PTU dose in both cortex and hippocampus (Fig. 7, Lasley and Gilbert, 2011), changes that were not reflected in mRNA (unpublished observations). The bases for this unusual dose–effect pattern, observed long after PTU administration had ceased and hormone levels had returned to normal, are unknown. The dose–response characteristics of the expression pattern of BDNF do not align well with any functional measures we have observed in the hippocampus of similarly treated animals (see above). What these findings do suggest, however, is that the dosimetry of alterations in BDNF protein expression is complex, the changes are post-translational in nature and are not dependent on an existing state of hypothyroidism, and that these changes in the adult may be mediated by epigenetic mechanisms (Lasley and Gilbert, 2011).

Opazo et al. (2008) did not see significant reductions in BDNF protein in adult offspring of dams exposed transiently to a high dose of MMI (200 ppm) in late gestation, but the data did trend toward a decline in the hippocampus. Although the concentration of MMI may seem relatively high in this study, the duration of exposure was very brief so the level of hypothyroidism in the dam was relatively mild and transient. The persistence of BDNF declines into adulthood following transient exposure limited to early development and following moderate levels of TH insufficiency are consistent with the results of Lasley and Gilbert (2011) described above.

In a different and more complex model of subclinical hypothyroidism, Liu et al. (2010) evaluated pups born to thyroidectomized dams. In one group, severe TH deficiency was evidenced by dam serum hormone concentrations below the level of detection throughout pregnancy as well as in pups at birth, while dam serum TSH was elevated 30- to 60-fold. Under these conditions, hippocampal BDNF protein and mRNA were reduced in offspring on PN3, PN7, and PN21. T4 supplementation throughout pregnancy created a transient state of hyperthyroidism, resulting in elevated serum T4 30–70% above control levels in mid-pregnancy (GD10 and GD13), followed by a modest degree of hypothyroidism in dams during late gestation and in pups at birth (~30% reduction in T4). TSH was elevated > 10-fold in newborn pups and in dams in early gestation, and more modestly in dams on GD17 (~4-fold). In contrast to non-supplemented dams with severe hypothyroidism, significant reductions in hippocampal BDNF mRNA and protein expression under these more modest hypothyroid conditions were limited to pups sacrificed on PN3 and PN7, with no difference from control on PN21 (Liu et al., 2010). Limitation of disrupted neurotrophin expression to very early times in the postnatal period following more modest perinatal hypothyroidism is
consistent with a recent report by Chakraborty et al. (2012) using developmental PTU exposure (4 ppm). Whether declines in BDNF protein concentrations paralleling the effects of Lasley and Gilbert (2011) and Opazo et al. (2008) would re-emerge in adulthood was not established.

**SUMMARY AND CONCLUSIONS**

For the most part investigations of alterations in neurotrophins occurring as a result of developmental hypothyroidism have been conducted under conditions...
of severe maternal hypothyroidism with assessments taken during the preweaning period when offspring were still TH-deficient. With the exception of Liu et al. (2010) and Chakraborty et al. (2012), in the handful of studies in which less than severe hypothyroidism was invoked, little or no change in expression of BDNF or other neurotrophins was detected in exposed neonates. When present, effects of hypothyroidism were to reduce expression of BDNF during the fetal (Pathak et al., 2011) an early postnatal period (Liu et al., 2010; Chakraborty et al., 2012), and effects were regionally specific. Our work with moderate TH insufficiency has revealed modest reductions in BDNF mRNA in neonates at PN14 (unpublished observations) that were not accompanied by alterations in protein expression (Lasley and Gilbert, 2011). A delayed reduction in BDNF protein expression in the adult offspring, in the absence of mRNA alteration, is suggestive of an indirect or epigenetic modification of BDNF following developmental TH insufficiency. However, given the importance of TH in fetal brain development (Bernal, 2011), further exploration of the dose–response characteristics and ontogeny at earlier time points and lower degrees of thyroid disruption may be instructive.

To conclude, on review of the available literature, no clear consensus can be reached on the effects of TH on BDNF expression in the brain. The results using hypothyroid models have varied from declines, to no significant alteration, to BDNF induction. Differences in the models used, doses, regions assessed, age of assessment, endpoint assessed (mRNA vs protein), and method employed to determine differences (microarray, rt-PCR, in situ hybridization, Western blots, ELISA) may all contribute to a lack of consistency across studies and obfuscate any current synthesis of finding. The complexity of this endocrine system exemplified by the time- and region-specific requirements for TH over the developmental period, the diversity of regulatory mechanisms that control presentation of the ligand to the nuclear receptor, and the non-genomic effects described for TH and their metabolites further obscures the emergence of a coherent picture. In the search to define a unifying mechanism of neurodevelopmental deficits induced by TH insufficiency, and in the regulatory context to identify a sensitive biomarker of effect, the current case for BDNF is complicated and less than compelling.

Future work using models of moderate TH insufficiency, examining dose–response relationships, with concurrent gene and protein determinations over a broad developmental time window that encompasses the fetal period may serve to elucidate the relationship between TH and neurotrophins. Furthermore, because BDNF involvement in network circuit formation and synaptic plasticity is rapidly regulated by neuronal activation, the role of BDNF in TH-mediated neuronal development and function may be more evident when studied under conditions of BDNF induction in response to synaptic activation, plasticity, enrichment, or injury (e.g., Bramham et al., 1996; Shulga et al., 2009).

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