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## Thyroid Hormones and the Central Nervous System

### Key Words

Aging  
Brain development  
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### Abstract

Thyroid hormones have a significant influence on the development and maturation of the central nervous system. Among their actions,  $T_3$  and  $T_4$  have effects on the differentiation of various cell types in the rat brain and cerebellum as well as on the process of myelination. Recently, several investigators have shown effects of thyroid hormones on myelin protein gene expression. Thyroid hormones seem to have a regulatory role with regard to life span. Hyperthyroid animals appear to have a shorter life and, at advanced age, show a myelin deficit. This may be due to the damage produced by the oxidative stress generated by an excess of thyroid hormones.

Iodine deficiency is an important health problem. One of the many consequences that iodine deficiency has on the population is endemic cretinism.

For many years, the consequences of iodine deprivation have stimulated the study of the effects of thyroid hormones on the development of the central nervous system (CNS). Results from our laboratory have contributed to our understanding of this problem and are summarized in this review together with original reports published on this topic by others.

The neonatal thyroid status is known to affect the growth, development and maturation of the mammalian

CNS [1, 2]. These processes are dependent on the presence of thyroid hormones during the so-called 'critical period' of CNS development [3-5]. During this period, there is rapid growth and development, suggesting that the action of the hormone is exerted primarily on biosynthetic processes. This critical period of development is marked by the onset of active myelination. Several investigators have studied the effects of neonatal hypothyroidism on the chemical composition of the CNS during development [1-5]. Neonatal thyroid deficiency leads to a significant increase in the DNA content in the cerebral cortex without affecting that in the cerebellum.

This condition also affects the content of various lipids such as cholesterol, cerebroside, sulfatides and phospholipids (PLs) [5]. Early postnatal hypothyroidism also lowers the activity of a number of enzymes [2]. However, neonatal thyroid deficiency affects the cerebellum composition to a lesser degree than that of the cerebral cortex [2, 6]. The different responses of these two structures to the lack of thyroid hormone might be attributed to the ontogenetic heterogeneity of the cerebellum.

Many of the first studies on the action of thyroid hormones upon nervous tissue were carried out in total brain or cerebellum [2]. The effects of thyroid hormones upon the composition of isolated myelin were studied later on [7–10]. In hypothyroid animals, the lipid composition of myelin appeared to be altered relative to normal controls [8–10], and the amount of these components was found to be markedly reduced. Although the lipid composition of myelin showed a decrease in cholesterol, total PLs and total galactolipids, sulfatides and plasmalogens were the most affected lipids in the myelin membrane isolated from hypothyroid rats compared to control animals [8]. This would imply, in coincidence with previous statements [10], that in this pathological situation, the amount of myelin is reduced but its composition is normal. However, in studies on 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in myelin isolated from neonatally thyroidectomized animals, we found that the activity of the enzyme was reduced both when the data were expressed per gram of fresh tissue or per milligram of protein, indicating that the myelin membrane in hypothyroid animals did not have a normal structure [8]. These data are at variance with those of Matthieu et al. [7] who reported no changes in the specific activity of this enzyme in the myelin of hypothyroid animals. However, when we studied the myelin membrane isolated from the brain of hypothyroid rats using the procedure of Waehneldt et al. [11], CNP activity showed no changes in its specific activity [9], in agreement with the results reported by Matthieu et al. [7]. The fact that the activity of CNP in total brain was reduced [8] tends to indicate that the activity of CNP in hypothyroid rats is decreased and that the differences in the results obtained by these two groups could be explained by variations in the type of myelin obtained by different isolation procedures.

### Effects of Thyroid Hormones and Growth Hormones on Brain Biochemistry in Neonatally Thyroidectomized Rats

One of the most striking alterations produced by neonatal thyroidectomy is the marked inhibition of body growth [2]. It has been suggested that the inhibition of body growth in thyroidectomized animals is an indirect effect of thyroid hormones acting on the pituitary gland. It is well known that neonatal thyroid dysfunction in the rat produces striking alterations in the pituitary gland, mainly characterized by degranulation of eosinophil cells [12, 13]. The effects of bovine growth hormone (BGH) are essentially similar to those of *L*-3,5,3'-triiodothyronine ( $T_3$ ); moreover it also shows a critical period of action. This view is supported by the results showing that BGH was more effective than  $T_3$  for correcting, at least partially, the decreased body weight observed in hypothyroid animals [3]. On the other hand the administration of BGH to hypothyroid rats was nearly as effective as that of thyroxine ( $T_4$ ) in restoring to normal the lowered lipid content in the cerebral cortex, except for the cerebroside content [6]. It is clear that in the absence of thyroid hormone, BGH can promote lipid deposition in the brain. Cerebroside, typical myelin constituents, and sphingomyelin are the only lipids showing a marked increase in the cerebral cortex and cerebellum of normal animals from 20 to 30 postnatal days, coinciding with the period of most active myelination [14]. The failure of BGH to restore the cortical content of cerebroside to normal in hypothyroid animals would indicate that BGH is not effective in stimulating myelination. In agreement with these results, King et al. [15], studying the participation of growth hormone and insulin-like growth factor-I (somatomedin C) in mediating the effects of propylthiouracil-induced hypothyroidism on the myelination processes, showed that growth hormone treatment begun at birth had no effect on the different parameters studied. CNP activity, as well as body weight, showed negligible changes in the 2 groups 20 days after injection. Only  $T_4$  was able to restore the activity of the myelin marker enzyme and body weight.

Noguchi et al. [16] indicated that hypomyelination in a congenitally hypothyroid neonatal mouse was restricted to the cerebrum and that it was not related to arrested glial proliferation. The same authors were also able to demonstrate the poor development of neuronal cells in the cerebrum of a dwarf mouse [17] in addition to hypomyelination [17, 18]. All these data suggest the

possibility of multiple actions of growth hormone and  $T_4$  on myelin formation. This notion is consistent with the evidence that  $T_4$  and growth hormone may have independent and complementary effects during the early postnatal period of brain development [3].

### **Effects of Thyroid Hormones on the Differentiation of Cell Types in the Rat Brain**

Thyroid hormones influence the formation and differentiation of neural cells. Virgili et al. [19] published a biochemical study on the effects of hypothyroidism on different brain regions of 14- and 28-day-old rats as well as adult rats. Among the glial cell markers, CNP activity was highly sensitive to thyroid hormone deprivation in agreement with the very well-known impairment of myelination [8]. These results agree well with those obtained by Patel et al. [20], who studied the effects of hypothyroidism on CNP activity in the rat cortex, hippocampus and cerebellum. On the other hand, astrocytes seem to be less sensitive to hypothyroidism as suggested by the slight increase in the hippocampus of the activity of the astrocyte marker enzyme glutamine synthetase [19, 20].

At present there is little controversy about the effect of neonatal hypothyroidism on the differentiation of oligodendrocytes. Hormone deficiency results in a marked retardation in the maturation of these cells and in the developmental activity patterns of the specific enzymes as well as different components of oligodendroglial cells. One of the main functions of the oligodendrocytes is to myelinate axons in the CNS [21]. Because differentiation of these cells is accompanied by major elaborations of their plasma membrane, it is reasonable to suspect that membrane PLs might play an important role in this process. Although the PL composition of isolated oligodendrocytes has been examined by several investigators [22, 23], it has not been studied as a function of age or in pathological states such as neonatal hypo- and/or hyperthyroidism, two conditions known to produce important alterations in myelin during development of the CNS [8, 24]. The data obtained in our laboratories regarding the PL content and composition of oligodendrocytes isolated from adult rat brain are essentially similar to those reported by others [22, 23]. The major developmental changes in the PL composition of isolated oligodendrocytes were found in the content of phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin, which increased

markedly between 18 and 30 days of age [25]. The increase in phosphatidylcholine content was smaller than for the other PLs, in spite of the fact that it is the most abundant PL and that, together with cholesterol, it exerts its impact on glial cell differentiation by lowering membrane fluidity [26]. Thyroid hormones have been found to play an important role in oligodendrocyte differentiation [27]. Previous studies by Walters and Morell [24] and Adamo et al. [28] revealed that thyroid hormones accelerate the formation of CNS myelin, producing early myelination. In oligodendrocytes isolated from hyperthyroid rat brain, the PL content remained constant while the protein content increased markedly. No changes in the amount of PLs per cell were found in the hyperthyroid rats, while PLs per milligram of total oligodendroglial cell protein were markedly decreased [25]. The changes in myelin composition produced by hyperthyroidism that have been described [28] do not closely follow those produced by this experimental condition in oligodendroglial cells, suggesting that the metabolism of myelin might be, to a certain extent, independent of that in the parent cell.

### **Investigations of the Action of Thyroid Hormones on Myelination in vitro**

$T_3$  has been shown to influence the synthesis of myelin lipids in cultures of cells from the brains of embryonic mice [29]. In these studies, when the cells grown in the presence of hypothyroid serum, the rate of synthesis of myelin sulfolipids drastically diminished, returning to normal levels by the addition to the culture medium of exogenous  $T_3$  after 72 h of exposure. The action was not due to a higher entry of sulfate or to 3'-phospho-adenosine-5'-phosphosulfate (PAPS).  $T_3$  seems to act upon the enzyme glycolipid PAPS-sulfotransferase in a dose-response manner. The same results indicating  $T_3$  dependency were observed on the activity of CNP, a myelin marker enzyme.

Almazan et al. [30], using a rotation-mediated aggregating cell culture system of fetal rat brain were also able to demonstrate that both the activity of CNP and the amount of myelin basic protein (MBP) were enhanced by  $T_3$  in a concentration-dependent manner. At variance with the results of Bhat et al. [27], these authors showed that 3 nM  $T_3$  had no effect on CNP activity. Using pure neurone as well as pure astrocyte cultures obtained from embryonic brain, Pascual et al. [31] have shown that thyroid hormone receptors are localized especially in neu-



rones. This group of investigators reported that the oligodendroglial cells do not contain nuclear  $T_3$  receptors or that their levels are low. These results were in apparent contradiction with the effects of thyroid hormones on the myelination processes. Yusta et al. [32] demonstrated the presence of high affinity binding sites for thyroid hormones in oligodendrocytes grown in cultures. These studies showed that nuclear thyroid hormone receptors were present in oligodendroglia and suggested that the effects of  $T_3$  on myelin formation could be due to a direct action of the hormone upon the cells. The effects of hypo- and hyperthyroidism on the development of the rat cerebellum have been studied extensively [2, 3, 5, 14]. In studies of cerebellum development,  $T_4$  has been shown to induce an increase in cell proliferation in the external granular layer. In the absence of hormone on the other hand, the branching of Purkinje cell dendrites diminished [33–35]. In cultures of dissociated Purkinje cells treated with  $T_3$  neither differentiated GABA function nor neuronal proliferation were significantly influenced by low concentrations of the hormone [36].

### Effects of Thyroid Hormones on Myelin Protein Gene Expression

It is very well known that thyroid hormones increase the transcription of different genes and also affect the stabilization of mRNA [37–40]. Several investigators [37–40] have shown that, in aggregating brain cell cultures during the peak of myelin synthesis  $T_3$  did not affect the MBP gene transcription. The turnover of its mRNA, however, was significantly affected. The levels of MBP mRNA decreased by 60% in the cultures treated with  $T_3$  compared to the controls. MBP mRNA was also found to be reduced in young adult rats made hypothyroid at the age of 5–6 weeks. The administration of exogenous  $T_4$  to these animals restored the decreased values to normal [41]. The results of Farsetti et al. [41] demonstrate that all the MBP mRNA isoforms (those encoding polypeptides of 21.5, 18.5, 17 and 14 kD) were altered by thyroid hormones. However, the MBP mRNA coding for the 21.5- and 18.5-kD polypeptides appeared to be more sensitive to hormone deficiency. The significance of isoforms containing exon II in the process of maturation of the oligodendrocyte was also pointed out by these authors. At variance with what was found by Farsetti et al. [41], in myelin isolated from neonatal hypothyroid rats we found that both the 14-kD MBP (SBP) and 18.5-kD MBP (LBP) were diminished, i.e. the

SBP:LBP ratio was smaller in the hypothyroid animals due to a larger relative decrease in the amount of SBP expressed in these animals. In the myelin precursor membrane, SN<sub>4</sub>, the LBP level was normal while the SBP:LBP ratio was markedly decreased [8]. Farsetti et al. [41] also found a specific hormone receptor interaction with the MBP promoter region, consistent with the action of thyroid hormone upon the MBP gene. The level of the mRNAs for the major myelin proteins were carefully studied by Muñoz et al. [42]. All of them, proteolipid protein (PLP), MBP and myelin-associated glycoprotein (MAG) were found to be decreased in hypothyroid brains. Most studies have attributed the decrease in MBP mRNA to a post-transcriptional effect. These data are at odds with the findings of Farsetti et al. [41]. Most effects of thyroid hormone on genes at the transcriptional level are much greater than the effects observed in the MBP gene. Thus, it is still unclear whether the effects of transcription observed *in vitro* by Farsetti et al. [41] are operative *in vivo*.

Hypothyroid rats have been reported to show a delay in the accumulation of MAG. The effect of hypothyroidism on the accumulation of protein coincided with the levels of MAG mRNA. The maximal level of MAG mRNA was reached by day 20. Hypothyroid animals showed a delay of several days in the onset of mRNA expression, increasing thereafter at the same rate and at similar levels as in normal rats [43]. The authors did not find differences in transcriptional activities for MAG in normal, hypothyroid or  $T_4$ -treated rats and the effects of hypothyroidism on MAG mRNA and on protein were found to be probably caused by a decreased mRNA stability.

Among the neuronal genes studied, thyroid hormone influences the expression of RC3, a brain-specific gene encoding a protein kinase-C substrate [44]. Moreover, Iñiguez et al. [45] have been able to demonstrate that adult-onset hypothyroidism leads to a reversible decrease in RC3 mRNA and RC3 levels that were reversed when hypothyroid animals were treated with  $T_4$ .

### Effects of Excess Thyroid Hormone on the Maturation of Rat Brain

Although it has been repeatedly demonstrated that neonatal thyroid deficiency in the rat markedly impairs the postnatal maturation of the brain, [2, 14, 46], considerably less attention has been given to the study of the effects of an excess of thyroid hormone upon this process.

It has been reported that thyroid hormone administration during early postnatal life accelerates the time of appearance of some landmarks of innately organized behavior [46, 47] and the development of metabolic compartmentation of glutamine associated with neuronal structures [48, 49]. In contrast with these findings, it has also been found that an excess of thyroid hormone during early postnatal life depresses the growth of the animals [50] and that of the cerebrum and cerebellum [28, 48]. The effects of  $T_4$  administration to normal rats during early postnatal life have been studied in connection with changes in the lipid content of the cerebral cortex and the cerebellum of the rat by Faryna de Raveglia et al. [5]. On the 10th postnatal day the cerebral cortex of treated rats showed an increased content of gangliosides and cholesterol. The increase in gangliosides was more pronounced in the cerebellum, which also exhibited a significant increase in PLP. According to the same authors, rats receiving  $T_4$  from birth showed an important reduction in all the lipids in the cerebral cortex on the 20th postnatal day. In the cerebellum this condition leads to a decrease in the concentration of cerebroside and total PLs.

Using quantitative histochemistry for the analysis of DNA and myelin components, Pelton et al. [51], have been able to demonstrate that  $T_4$  administered in excess during early postnatal life produced a severe impairment of myelinogenesis due to a disruption in the normal sequence of glial cell mitosis and differentiation, suggesting that an excess of thyroid hormone, administered chronically, results in an abnormal development of cerebrum. However, the known effect of hyperthyroidism in young animals, blocking mitotic activity preceded by an accelerated cell proliferation, has been always quite controversial. In a recent publication Tomic et al. [52] clearly demonstrated that  $T_3$  does not enhance glial cell proliferation but rather stimulates differentiation of oligodendrocytes. Furthermore experiments carried out in cell cultures, using the incorporation of labeled thymidine, showed many years ago that this hormone has no effect upon the incorporation of this precursor [53]. Walters and Morell [24] were able to demonstrate an acceleration in the process of myelination in hyperthyroid animals, which led controls by 1 or 2 days. In this work, however, the maximum effect occurred at 13 days of age, when protein values almost doubled. Because it is well known that an indication of myelin maturation is a relative increase in the amount of low molecular weight proteins, the results of Walters and Morell [24] were considered as an indication of accelerated myelination.

In the rat CNS there are subcellular organelles which have an enzymatic activity and morphology that correspond to microperoxisomes. Studies done by us, using oligodendrocytes isolated by the method developed by Berti-Mattera et al. [54] in our laboratory, showed that microperoxisomes peaked at the period of myelin formation [55]. It has been shown that  $T_4$  treatment increases the number of peroxisomes in the kidney and liver [56]. In order to determine whether there was any correlation between accelerated myelin formation produced by thyroid hormones and the amount of microperoxisomes in the CNS, we studied the chemical composition of isolated myelin as well as the levels of two peroxisomal marker enzymes, catalase and acyl-CoA dihydroxyacetone phosphate acyltransferase, in control and hyperthyroid animals [28].  $T_3$ -treated animals appeared to initiate myelinogenesis earlier than controls. The composition of myelin in 17-old-day hyperthyroid rats was similar to that found in 30-day-old animals. The level of total protein and lipid components increased in the myelin fraction of 17-old-day animals, while the activity of CNP was slightly higher in the  $T_3$ -treated animals, although the increase was not statistically significant.

The increase in PLs and plasmalogens that we found [28] could be related to a proliferation of microperoxisomes produced by  $T_3$ , as a consequence of an increased rate of synthesis of these lipids through the dihydroxyacetone phosphate pathway. Using a double-labeling protocol with ( $^{14}\text{C}$ )-glycerol and ( $^3\text{H}$ )-glycerol as precursors of glycerophospholipids, it was possible to determine the importance of the dihydroxyacetone phosphate pathway in the synthesis of PLs or plasmalogens. We found that a substantial amount of the  $^3\text{H}$  derived from ( $^3\text{H}$ )-glycerol was lost during the synthesis of myelin plasmalogens. The importance of this pathway in the synthesis of ethanolamine plasmalogens in myelin was suggested for the first time by Benjamins et al. [57] and by Miller et al. [58]. Our results also suggested that thyroid hormones promote an increase in the number of microperoxisomes. Since these organelles contain the enzymes of the dihydroxyacetone phosphate pathway, the increase in the synthesis of plasmalogens could be the consequence of peroxisomal proliferation produced by  $T_3$ . Thus there could be a relationship between the acceleration of myelination found in  $T_3$ -treated animals and the effects of this hormone upon microperoxisomes.

**Table 1.** Effects of hyper- and hypothyroidism upon different structures, cells and membranes of the CNS

	Hyperthyroidism	Hypothyroidism
1 Cerebellum	Increased cell proliferation in the external granular layer [36]	Decreased branching of Purkinje cell dendrites [33–35]
2 Oligodendrocytes	Increased CNPase activity [30] Stimulation of differentiation [52] Increased number of microperoxisomes [55] Increased synthesis of myelin lipids [29] Increased glycolipids PAPS-sulfotransferase activity [29] Increased amount of MBP [30]	Decreased CNPase activity [8, 20] Differentiation? Maturation retarded [20] Decreased synthesis of myelin sulfolipids [29]
3 Astrocytes and neurones		Slight increase in glutamine synthetase activity [19, 20] Decrease in RC3 mRNA and RC3 level [45]
4 Myelination	Accelerated [24, 28]	Delayed [7–10, 15]

### Thyroid Hormones, Oxidative Stress and Aging

A number of tissues, such as brain liver, heart and muscle, when isolated from hyperthyroid animals, show an increased oxygen consumption [59] which could lead, through an increased level of steady-state oxygen intermediates, to the establishment of a condition of oxidative stress in the tissue. Lipid peroxidation indexes, such as spontaneous chemiluminescence and malondialdehyde levels were increased in the liver by  $T_3$  treatment [60], suggesting the development of a condition of oxidative stress.

Videla et al. [61] observed that lipid peroxidation found in human hyperthyroidism was suppressed by treatment with propylthiouracil. This effect could be due to a direct scavenging action of the drug upon free radicals. In addition, since the metabolic rate reverted to normal, there was a lower rate of oxygen consumption and as a consequence a decrease in the generation of active oxygen species which produce lipid peroxidation.

Thyroid hormones appear to have a regulatory role concerning life span. Rats made hypothyroid from birth live longer than their controls [62] and the administration of  $T_4$  significantly shortens life span [63]. Hyperthyroid animals show a pronounced myelin deficit at advanced age [64] which may be interpreted as a consequence of the damage produced by the oxidative stress in the hyperthyroid brain. Myelin is a membrane that is highly susceptible to the attack of reactive oxygen species as indicated by the peroxidation of membrane lipids [65]. Oxidative damage to myelin has been proposed as a primary factor in the acceleration of aging in the human brain [66].

The increase in superoxide dismutase, catalase and glutathione peroxidase activities observed by us in the brain of neonatal hyperthyroid rats [67] can be assumed as a transient compensatory mechanism since the sustained hyperthyroid state leads to a marked increase in the spontaneous brain chemiluminescence and to a shortening of life span. The marked increase in the spontaneous chemiluminescence of the hyperthyroid rat brain appeared to indicate an increase in the steady-state level of oxyradicals. It is apparent that this condition in various organs may contribute to the observed shortening of life span in these rats. There is evidence that lipid peroxidation products accumulate in the myelin membrane of aged rats. This could indicate that damage mediated by free radicals might be the cause of the instability of myelin observed during aging [68]. It has also been shown that the changes observed in myelin during aging can be reproduced in *in vitro* oxidation experiments [68]. *In vitro* oxidation of isolated myelin by free radicals produces a decrease in the major myelin proteins [69], leading to the formation of molecular aggregates of these proteins. These investigators proposed that the oxidative damage could produce aggregation of PLP. In adult hyperthyroid animals we found a decrease in the content of myelin PLP in comparison to controls [64]. This decrease could be due to oxidative damage, although it is not possible to disregard that, in parallel to oxidative damage, the decrease in PLP could be the consequence of a decreased rate of synthesis or to an alteration in the assembly of myelin produced by sustained hyperthyroidism since birth.



## Conclusions

The influence of thyroid hormones in the postnatal growth, maturation and development of the mammalian CNS is of primary importance. Some of the actions of these hormones are summarized in table 1. Their effects are mainly exerted on functional organization and differentiation which are characteristics of brain maturation rather than on metabolic rates. It is also interesting that the effects of thyroid hormones are more like those occurring during amphibian metamorphosis than other tissues. In brain, the action of the thyroid hormones is confined to the time when the plasticity of nervous tissue is great and important functional and structural changes

are occurring. Many questions remain unanswered and important hormone actions are not yet clearly understood. However, what is clear is that myelination is a highly regulated event and that it depends on appropriate maturation and differentiation of oligodendrocytes. It is likely that the brain effects of thyroid hormones are caused by the control of the above-mentioned mechanisms and/or the expression of specific brain genes.

Control by thyroid hormones of gene expression is also supported by the effect of hyperthyroidism. In the presence of an excess of thyroid hormone, brain gene expression was accelerated. This was in agreement with other reports on the acceleration of myelination by the hyperthyroid state.

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