Review Article

Contribution of Brain Tissue Oxidative Damage in Hypothyroidism-associated Learning and Memory Impairments

Abstract

The brain is a critical target organ for thyroid hormones, and modifications in memory and cognition happen with thyroid dysfunction. The exact mechanisms underlying learning and memory impairments due to hypothyroidism have not been understood yet. Therefore, this review was aimed to compress the results of previous studies which have examined the contribution of brain tissues oxidative damage in hypothyroidism-associated learning and memory impairments.

Keywords: *Brain tissue oxidative damage, hypothyroidism, learning, memory*

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Introduction

Hypothyroidism is regularly associated with mood disturbances and cognitive impairment, suggesting that thyroid hormones (THs) are critical for normal brain working.[1-4] In particular, hypothyroidism has been associated with a few intellectual deficiencies, including general intelligence, psychomotor speed, visuospatial skills, and memory.[5] Interestingly, motor skills, language, inhibitory efficiency, set-shifting, and sustained auditory attention appear to be less influenced by hypothyroidism.[1,5,6]

Cellular processes and genes regulating the effects of THs in the brain have been recently reviewed elsewhere.[2,7,8] In general, THs accelerate the myelinating process, influence cell migration, and regulate cell differentiation and maturation of specific neuronal populations.[9-11] In addition, THs control the expression of several genes, including neuromodulin/growth associated protein 43 (GAP-43), neurogranin/RC3, and Ca^{2+}/calmodulin-dependent protein kinase II, which regulate different forms of synaptic plasticity and memory formation.[9-11]

Most of the negative effects of hypothyroidism on cognition have been associated with biochemical and biophysical changes in the hippocampus using different tasks that require the hippocampal integration of spatial and contextual information, e.g., Morris water maze, contextual fear conditioning, radial arm maze, and object recognition tasks.[12,13]

Experimental models of hypothyroidism indicate that the detrimental effect of TH insufficiency depends on the magnitude, duration, and timing in which the insufficiency occurs.[8,14] Even mild TH insufficiency during brain development in rats is associated with irreversible neurological defects.[15,16] In adult rats, TH deficiency has been related to hippocampal alterations that could account for the associated cognitive and behavioral deficits.[17-19] In humans, hypothyroxinemia during pregnancy has been related to cognitive and neurological impairment in children;[20,21] furthermore, adult-onset hypothyroidism has been linked to other neurological alterations, cognitive dysfunction, and impaired learning and memory.[22-24]

The mechanisms controlling such effects are not completely understood, but the genomic and nongenomic effects have been recommended.[25] THs affect gene expression, either positively or negatively, by binding to nuclear TH receptors that are connected with important biological functions. Among the processes activated by nuclear hormone receptor, protein expression during the development of the central nervous system (CNS),[26] expression of sarcoplasmic reticulum Ca^{2+}-ATPase1 in the heart,[27] and Bcl2 family genes[28] are

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attractive. The nongenomic effects of THs are connected with the activity of proteins, such as Ca^{2+}-ATPase in red blood cells[29] and de-polymerization of actin filaments.[30] Furthermore, the nongenomic effects of THs involve the modification of cell layer composition.[31,32]

An extreme impairment in the structure and function of the developing CNS induced by hypothyroidism has been thoroughly examined.[1,3] Besides, less consideration has been focused on the impact of TH deficiency on the memory and mechanisms responsible for the effects of hypothyroidism on the memory. Because THs have profound effects on the CNS, it is coherent to ask how hypothyroidism influences cognition. In this way, this review will compress the past studies about the conceivable mechanism(s) of impairing effects of hypothyroidism on memory and cognition.

Hypothyroidism, Memory, and Cognitive Impairment

Clinical observations

THs are essential for brain maturation from early stages of development. THs of maternal origin are the only source for the fetus during the first trimester of gestation until the fetal thyroid becomes gradually functional and continue to be important until birth.[33] Recent studies have shown that even a modest, subclinical maternal hypothyroxinemia, mainly during the first half of gestation, may lead to irreversible neurological damage.[21] Children exposed to maternal hypothyroxinemia present reduced intelligence quotient (IQ) score, subtle deficits in cognition, memory, visuospatial ability,[20] and delayed mental and motor functions.[21,34] Severe reduction of THs during gestation is commonly associated with a low iodine intake, which is essential to synthesize THs.[34] This reduction in the TH supply to the fetus may cause several developmental and neurological defects such as cretinism, mental retardation, deaf-mutism, motor dysfunction, and cognitive impairment, which are irreversible even with early TH treatment.[1,5] Developmental hypothyroidism is the most frequent, preventive cause of mental retardation. When TH insufficiency occurs after birth, as in congenital hypothyroidism, language and memory skills are most predominantly affected and can be prevented by adequate TH replacement early after birth.[8,35]

Adult-onset hypothyroidism is additionally proposed to be connected with clinically relevant cognitive dysfunctions such as psychotic behavior, hallucinations, confusion, as well as learning and memory impairment.[2] Although controversy exits about whether TH replacement can completely restore the learning and memory impairment observed in hypothyroid cases,[22] this treatment has been shown to improve the cognitive decline at least in subclinical hypothyroid cases.[23] In addition, in healthy, euthyroid elderly cases, a positive correlation has been observed between serum thyroxine (T4) levels, but not triiodothyronine (T3) levels, and cognition.[24]

Models of hypothyroidism and hypothyroxinemia

Maternal models

In rats, THs are mainly required for proper brain maturation from the late fetal (E14) to early postnatal stages (3–4 weeks), affecting the maturation of neuronal structures such as the cerebral cortex, basal forebrain, cerebellum, and hippocampus.[36] Different models of experimental hypothyroidism in rodents, mainly in rats, have been used to study the basic pathogenesis of developmental neurotoxicity of inappropriate TH supply during this period. Models of maternal hypothyroxinemia without overt hypothyroidism are generated by feeding rat dams on a low iodine diet in dam rats 3 months before mating and during lactation or by administering goitrogen 2-mercapto-1-methyl-imidazole (methimazole) from E12 to E15. In both cases, low T4 levels between E14 and E16, a period of active neurogenesis, resulted in the alterations of cell migration and cytoarchitecture of the cortex and hippocampus in the 40-day-old progeny.[15,16] The effect of developmental exposure to hypothyroidism on the offspring is usually analyzed by the administration of the reversible, antithyroid goitrogen 6-n-propyl-2-thiouracil (PTU) to pregnant and lactating rat dams. Perinatal reduction in circulating levels of TH by PTU administration from gestation day 18 (GD18) to postnatal day 21 (PN21) has been accompanied by growth retardation, neurological deficits, as well as alterations in auditory and motor functions in pups and adult rats.[37] PTU administration from PN0 to PN19 also causes learning and memory impairment that persists throughout life.[38] Moreover, behavioral abnormalities which are typical of attention-deficit hyperactive disorders are observed in 15-week-old rats when PTU is given from GD3 to PN20.[39]

Recent studies have shown that even moderate perinatal TH insufficiency induced by low doses of PTU from GD6 to PN30 produces perturbation in area CA1 and dentate gyrus (DG) areas in the adult offspring which is in correlation with the impairment of hippocampal-dependent tasks; however, such impairments depend on the PTU dosage and persist in euthyroid adult rats.[40,41] Perinatal administration of PTU from GD21 to PN21 has also been shown to alter the expression levels of genes implicated in axonal growth and learning such as GAP-43 in the cerebral cortex and the muscarinic acethylcholine receptor M1 in the hippocampus at PN22. Although the expression pattern of these genes returns to the normal levels in 9-week-old rats, the impairment of E-maze learning, which is considered to be involved in hippocampal or cerebral cortical functions, persists at weaning and after maturation.[42] Therefore, the alterations in the hippocampal formation that occur during developmental hypothyroidism are critical for the neurological and behavioral defects in the postnatal period.

It has also been previously reported that hypothyroidism induced by methimazole during lactation leads to spatial learning and memory impairments in 8-week-old male offspring rats when they are examined using Morris water maze.[43]

Models of adult-onset hypothyroidism

PTU treatment or thyroidectomy has been used to understand the neurological changes and their molecular mechanisms related to hypothyroidism in adult rats. Adult hippocampal neurogenesis has been observed to play an important role in depressive-like behaviors and hippocampal-dependent learning and memory.[44-46] Therefore, the altered hippocampal neurogenesis observed in the models of adult-onset hypothyroidism could contribute to the cognitive and behavioral deficits associated with this condition.[18,46] Moreover, electrophysiological and biochemical studies in hypothyroid adult rats suggest that memory dysfunction may be related to the impairment of long-term potentiation (LTP) in the CA1 region of the hippocampus.[19,47] Recent studies have shown that T4 replacement therapy restores impaired LTP in CA1 of hypothyroid rats and normalizes most of the molecular changes induced by hypothyroidism, supporting the idea that T4 replacement can restore some of the hypothyroidism-induced cognitive impairments.[17]

Nowadays, it is well known that hypothyroidism during the fetal and/or neonatal period leading to several histological changes in the neonatal rat brain, such as decreased synaptic connectivity, delayed myelination, interrupted neuronal migration, and modifications in neurotransmission, cause harmful effects on the development of the CNS.[7] The hippocampus integrates the encoding, storage, and recall of memories while binding the spatio-temporal and sensory information that make up experience and maintain episodes in their correct context. The rapid and accurate processing of data relies on interconnected networks corresponding to the anatomical subfields of DG, CA3, and CA1.[48] A reduction in the volume of the hippocampus and even the whole brain of the hypothyroid rats has also been reported.[49] Through morphological experiments, the diminished density of neurons in all pyramidal cell layers of the hippocampal areas including CA1, CA2, CA3, CA4, and DG in both hemispheres of 15-day-old hypothyroid rat has been reported which is accompanied by the altered density of astroglial cells in each hemisphere of the brain.[50] The diminished density of neurons in developmental hypothyroidism is consistent with the findings by Gong *et al*., who found a reduced number of surviving cells in the hippocampus of young hypothyroid rats.[51] Astrocytes are the main glial cells connected with the development of the nervous system. These cells are involved in neuronal migration and maturation, myelination, ionic regulation, metabolism of neurotransmitters, and synaptic integration.[52,53] It has been reported that the glial fibrillary acidic protein (GFAP) positive cells are elevated in CA1 of hypothyroid hippocampus.[50]

Mechanism Effect

The exact mechanisms responsible for learning and memory damage due to THs deficiency have not been surely understood. The cholinergic system as a vital neurotransmitter system involved in learning and memory has been shown to have an important mediatory role in the effects of THs on cognition. Maturation of the cholinergic system has been suggested to be affected by THs.[2] Other studies have suggested that cognitive impairment in hypothyroidism is likely to be related to abnormal brain development, decreased interneuronal connectivity and in particular, impairment of synaptic plasticity in the hippocampus.[54] On the other hand, some studies have suggested that impairment related to hypothyroidism might be related to the changes in the expression of c-jun and c-fos proteins and extracellular signal-regulated kinases (ERKs) levels in the hippocampus during the critical periods of brain development.[40,55] It also seems that other proteins including synapsin I, synaptotagmin I, and syntaxin which have a role in neurotransmitter release may have a role in the hypothyroidism-associated memory impairments.[56] Changes in glutamate release have also been considered another possible mechanism.[57-59] Researchers have also demonstrated that cyclic-AMP response element binding protein (CREB) and mitogen-activated protein kinases (MAPKs) may be a contributory factor in the impairment of hippocampal-dependent learning and memory in hypothyroidism conditions.[60] Some of the possible mechanisms are summarized in Figure 1.

Oxidative stress

THs has been known to be related to the oxidative and anti-oxidative status of organisms; thus, it seems to play an important role in free radical production.[61] Oxidative stress, described by enhancing in the concentration of reactive oxygen species (ROS), has been suggested to occur in many biologic and pathological conditions.[62] Some studies have reported an imbalance in antioxidant and pro-oxidant criteria in overt hypothyroidism.[63] It has been well known that ROS are produced in a huge amount in metabolically active organs including the brain. ROS are considered neurotoxic molecules which have some harmful effects on essential macromolecules such as enzymes and cytoskeletal proteins via the oxidation of targets. An impaired performance in cognitive functions has been known to be observed following an excessive ROS production.[64,65] All of these events have been considered responsible for the decreased level of neuronal density in the hippocampal regions including CA3 area of hypothyroid rats,[66] the later of which could take part in learning, memory, and cognitive impairments associated with hypothyroidism.[67] It has also been previously confirmed that hypothyroidism-associated

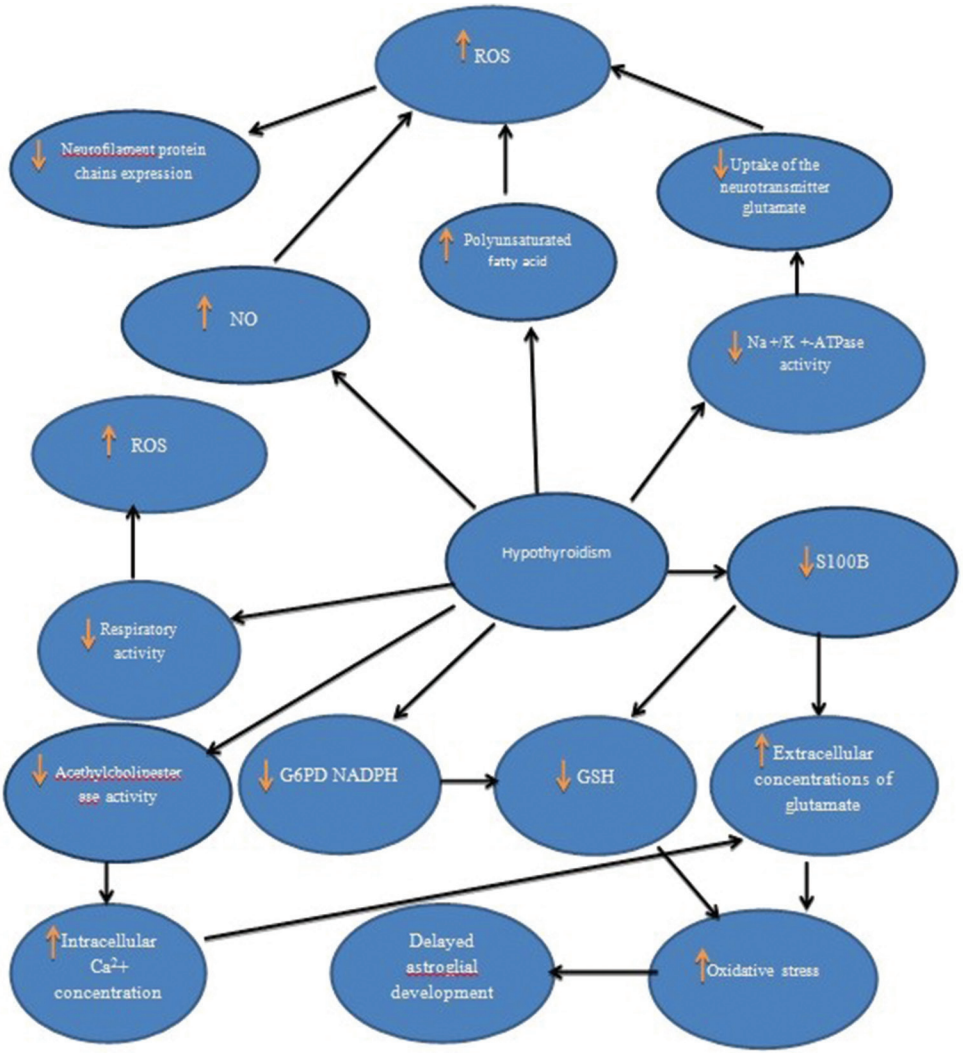

Figure 1: Possible mechanism(s) of impairing effects of hypothyroidism on memory and cognition. Hypothyroidism elevates reactive oxygen species generation via different pathways. Continuous lines represent the different pathways which finally lead to reactive oxygen species production

learning and memory impairment during neonatal and juvenile growth in rats is accompanied by hippocampal and cortical tissues oxidative damage.[68]

In fact, many studies have reported that hypothyroidism affects antioxidant defense system in various regions of rats' brain. A summary of representative studies relating the hypothyroidism and parameters of oxidative stress in the brain is presented in Table 1.

It is worth mentioning that the selective oxidative stress which occurs in hypothyroidism is not due to the direct pharmacological effect of methimazole since T4 replacement reverses the effects of methimazole to the values similar to those of the euthyroid group.[75]

A decreased level of body temperature due to hypothyroidism has been argued as a determining factor for the effects of hypothyroidism on the brain functions. It has been reported that hypothermia has neuroprotective properties. On the other hand, hypothermia has been suggested to decrease cerebral metabolism and energy consumption, which may decrease the extent of degenerative processes such as excitotoxic cascade, apoptotic and necrotic cell death, microgliall activation, oxidative stress, and inflammation.[78] In the experimental models which are used to study the effects of hypothermia, temperature is usually lowered to about 4°C–12°C from the basal temperature, either in all the whole animal[79] or in tissue culture.[80] However, the hypothyroid rats have been reported to have body temperature of only 0.5°C lower than that of control rats.[75] Thus, it is unlikely that such a small difference in the body temperature could affect the results of hypothyroidism on the brain functions.

Several genes related to myelination and synaptogenesis are known to be regulated by THs.[81] THs promote the biogenesis and assembly of the cytoskeletal proteins including tubulin, actin, and vimentin.[82,83] More recently, it has been reported that progressive hypothyroidism leads to 50%–80% decline in the expression of all three

Table 1: Effects of hypothyroidism on the activity/abundance of antioxidant enzymes and the oxidative status in different parts of the brain

Tissue	SOD	GPx	CAT	GSH	Lpx	Chl	Crb	Species
Homogenate				↑	↑			Rat[69]
	↑		↑	↓	↑		↑	Rat neonate[70]
Mitochondria	↓↓	↓	↓		↑			Rat neonate[71]
	↑	↑			↑		↑	Rat[72]
Cortex	↓	↑	↓		↓			Rat neonate[73]
	↑	↑	—		↑			Rat old[65]
	↓				↑			Rat[74]
					↑			Rat[75]
Hippocampus				↓	↑			Rat neonate[50]
	↑				↑			Rat[76]
Cerebellum	↓	–	↑		↓			Rat juvenile[77]
	↑	–	–		↑			Rat neonate[77]
Medulla	↓↓	↓	↓		↑			Rat neonate[71]

Antioxidant enzymes and substrates – SOD: Superoxide dismutase (no distinction is made between Cu/Zn-SOD and Mn-SOD), GPx: Glutathione peroxidase. Oxidative status – Lpx: Lipid peroxidation (measured as TBARS or malondialdehyde production). Chl: Chemiluminescence, Crb: Carbonylated proteins, CAT: Catalase, GSH: Reduced glutathione, TBARS: Thiobarbituric acid-reactive substances: of thiobarbituric acid reactive substances, ↓: Decrease; ↑: Increase; —: No change. Double arrows represent a highly significant effect (<0.001)

neurofilament (NF) protein chains (NF-L, NF-M, and NF-H) in the developing rat brain, both at messenger RNA and protein level.[84] Previous studies have described that, in primary cultures of neurons, hypothyroidism causes the altered intracellular distribution of NFs with characteristic accumulation in the hillock and in the proximal axon region.[85,86] A similar downregulation of NF gene expression and abnormal intracellular accumulation of NF has been reported in several neurodegenerative diseases.[87] On the other hand, oxidative stress is considered to play a major role in the abnormal accumulation of NF leading to the blockade of the axonal transport system as well as cell degeneration and death.[88-90] Therefore, it might be suggested that NF-accumulation due to brain tissues oxidative damage has a role in the learning and memory damage induced by hypothyroidism.

Recently, it has been reported that congenital hypothyroidism is connected with c-Jun-N-terminal kinase (JNK) activation, cytoskeleton dis-regulation, as well as downregulation of astrocyte glutamate transporters leading to decreased glutamate uptake with the subsequent influx of Ca^{2+} through N-methyl-D-aspartate (NMDA) receptors in rat hippocampus.[50] The Na^{+}-coupled ^{14}C-MeAIB (α-methylaminoisobutyric acid) accumulation in hippocampal cells also seems to elevate the intracellular Ca^{2+} concentration via opening voltage-dependent calcium channels (VDCC). The excessive influx of Ca^{2+} through NMDA receptors and VDCCs is followed by the overload of Ca^{2+} within the cells, which sets off glutamate excitotoxicity and oxidative stress.

It is worth mentioning that hypothyroidism can also affect the phosphorylating system associated with the cytoskeleton of both neuron and astrocyte intermediate filament (IF), but supplementation with T3 does not restore either neuronal and astrocyte IF hyper-phosphorylation. On the one hand, the phosphorylation of GFAP and lysine-serine-proline (KSP) repeats on NF-H is partially reversed by T4. Therefore, it could be reasonably argued that this hormone might restore, at least in part, the homeostasis of the phosphorylating system associated with the cytoskeleton.[50] On the other hand, these results are in line with the previous studies reporting that hypothyroid-induced hyperphosphorylation of IF in the cerebral cortex of young rats is not reversed by short-term exposure to T3.[91] NF-M and NF-H KSP repeats are among the preferential targets for the MAPKs, including JNK, and misregulated phosphorylation of KSP sites, in particular those of NF-H, regulates NF axonal transport.[92] In this connection, hyperphosphorylation of these sites plays a role in several neurological disorders.[93] Significantly, the activation of JNK is considered a molecular switch in stress signal transduction. Several stimuli, including ROS, have been suggested to be able to activate JNK pathway, playing critical roles in regulating cell fate, including gene expression, cell proliferation, and programmed cell death.[94] Also, Sherrin *et al*. demonstrated a role for hippocampal JNK in memory and synaptic plasticity.[95] Thus, it could be reasonably argued that ROS generation in the hypothyroid hippocampus elicits the JNK signaling pathway, upsets the homeostasis of the cytoskeleton, and leads to delayed hippocampal development.

In CNS, astrocytes are the main protectors of neurons from excitotoxicity and this protection is conferred mainly by the clearance of extracellular glutamate.[96,97] According to these findings, diminished glutamate uptake, elevated Ca^{2} + uptake, diminished levels of glutamate transporters (gLutamate aSpartate transporter; GLAST and glutamate transporter; GLT-1), and diminished

glutamine synthetase (GS) activity in hippocampal slices from hypothyroid rats support possible glutamate-induced excitotoxicity in this brain structure. In this connection, in astrocytes, the GS enzyme rapidly converts glutamate into glutamine,[96,98] which serves as an important precursor for the synthesis of the primary excitatory neurotransmitter by neurons through the neuronal enzyme glutaminase. The hypothyroid condition clearly shows an interruption of glutamate-glutamine cycle, by misregulating the excitatory neurotransmitter metabolism (by inhibiting GS) and transport (by decreasing GLAST and GLT-1 levels). These events may lead to neuronal excitotoxicity due to the overload of extracellular glutamate concentration.[99] Moreover, regarding the fact that GS expression and activity are upregulated by T3, a diminished GS activity in hypothyroid condition has been evidenced by some studies.[50] On the other hand, purines, pyrimidines, and certain amino acids which are critical for the synthesis of myelin components are, in part, supplied by the GS pathway. It is suggested that the effects of T3 on myelination could be partly mediated through the GS gene regulation.[100] Therefore, a delay in myelination and decreased level of myelin amount in hypothyroid rat brain[101,102] could be connected with the decreased GS activity/expression.

It has also been demonstrated that hypothyroidism modulates the composition of fatty acids of the cellular membrane in the liver. Hypothyroidism increases the levels of polyunsaturated $n-3$ and $n-6$ series (e.g., 22: 6n − 3 and 18: 2n − 6) and decreases the levels of monosaturated $n-7$ and $n-9$ fatty acids.[31] In addition, the change in plasma membrane composition could, in turn, modify the activity of the Na^+/K^+-ATPase as well as other transmembrane ion exchangers.[103] In fact, it has been described as the reduction of Na^+/K^+-ATPase activity in the hippocampus of hypothyroid rats.[104,105] The decrease in enzymatic activity might alter the sodium/potassium transmembranal gradient and diminish the uptake of the neurotransmitter glutamate[106] or stimulate the reversed uptake of glutamate.[107] This increase could, in turn, produce mitochondrial calcium overload, decline ATP production, and activate calcium-dependent phospholipases, proteases, and endonucleases.[108] Those biochemical events may increase ROS production and as a result, the lipid peroxidation.[109]

In the CNS, S100 calcium binding protein B (S100B) is generally found in the cytoplasm and nucleus of astrocytes, where it controls cytoskeleton and cell proliferation, along with other members of the S100 family. A part of this protein content (<1%) is secreted in a regulated manner.[110] As a result, S100B can be found in the cerebrospinal fluid and in serum. S100B protein levels (intra-and extracellular) have been used as a parameter of astrocyte activation and/or death in several situations of brain injury.[111-114] It has also been reported that hypothyroidism leads to the increased level of S100B in the serum while diminishing the content of this protein in the hippocampus of developing rats.[50] These results are in line with the previous reports demonstrating the increased S100 serum levels in perinatal acidosis[115] and higher S100B in the serum of newborns with hypoxic ischemic encephalopathy.[113] Several extracerebral sources for instance adipocytes, chondrocytes, lymphocytes, bone marrow cells and melanoma cells have been suggested that influence on the serum S100B content.[116] On the one hand, regarding the fact that hypothyroidism in developing rats leads to abnormal and delayed brain development,[7] it might be suggested that serum high and the brain low levels of S100B are the result of delayed glial maturation and/or decreased glial cell number in rats. A diminished level of brain tissue S100B content, glutamate transporters, and GS in addition to GFAP substance is all connected with astrocyte maturity, and reinforces the possibility of delayed astroglial development. It is also demonstrated that an elevated level of extracellular glutamate reduces S100B release in hippocampal astrocytes.[117]

Beside the reduced level of glutamate transporters in cerebral cortex and hippocampus of hypothyroid animals,[91] it seems that diminished intracellular S100B levels are connected with glutamate excitotoxicity. In addition, decreased glutamate uptake results in the decreased level of intracellular glutamate, which finally diminishes glutathione (GSH) precursor.[98] Glutamate and GSH metabolisms intricately interact as a result of complex mechanisms and these interactions might be altered in hypothyroidism.

GSH is a critical component of astrocyte neuroprotective functions. In this connection, the decreased level of GSH content which is accompanied by the increased level of thiobarbituric acid reactive substances (TBARS) in the hippocampus has been suggested to induce neurotoxic damage in hypothyroid rats. Moreover, a high level of O_2 consumption gives rise to the particular vulnerability of the brain to oxidative stress.[118,119] GSH-dependent redox systems rely on the continuous supply of nicotinamide adenine dinucleotide phosphate (NADPH) as the major electron donor. The cellular supply of NADPH is mainly believed to be provided by glucose-6-phosphate dehydrogenase (G6PD) activity.[120] In this connection, the inhibition of reported G6PD might result in the reduced supply of NADPH and following oxidative stress generation in the hippocampus of hypothyroid animals and might also be related to the decreased levels of GSH.[50] GSH synthesis is regulated by the enzymes of the gamma-glutamyl cycle and a defect in these enzymes connected with GSH metabolism could be related to neurological disorders. Gamma-glutamyl transpeptidase (GGT) is an astroglial ecto-enzyme which transfers the gamma-glutamyl moiety of GSH to an acceptor amino acid; therefore, its activity can generate the precursors of neuronal GSH.[118,121] GSH levels as well as GGT and G6PD activities may illustrate

the deficiencies in the antioxidant defense system in hypothyroid rat brain. It seems that the inhibition of GGT activity might diminish the reservoir of glutamate necessary to GSH synthesis, resulting in the reduced level of this important antioxidant in the brain.[50] However, this phenomenon requires further investigation.

Cholinesterase inhibition reported in hippocampal slices from hypothyroid animals could also be related to the modifications in brain cognition.[50] According to Wu *et al*., the elevated acetylcholine levels could be involved in the activation of nicotinic acetylcholine receptors, allowing for the influx of both extracellular Na^+ and Ca^{2+}.[122] Thus, taking into account these findings, the elevated Ca^{2+} uptake as a result of hypothyroid condition could be probably connected with reduced acethylcholinesterase activity reported herein.[50] Furthermore, the Na^+-coupled ^{14}C-MeAIB accumulation into hippocampal cells due to hypothyroidism might lead to a rapid increase in the intracellular Ca^{2+} concentration. To support this idea, Young *et al*. described the stimulation of Ca^{2+} signaling in response to MeAIB in STC-1 cells.[123]

Nitric oxide system

Another important molecule involved in the oxidative pathway leading to cell damage is nitric oxide (NO). Under pathological conditions, NO possibly promotes oxidative damage through the formation of the highly reactive metabolite peroxynitrite.[124,125] On the other hand, NO is known to play a critical role in biologic systems.[126] It acts like a diffusible intercellular signaling molecule in the brain and spinal cord.[127] NO synthase (NOS) is the enzyme that produces NO from l-arginine. The gaseous neurotransmitter NO has been associated with different forms of learning and memory and in several forms of synaptic plasticity; it is thought to be connected with memory formation. This connection has been well documented via pharmacological studies, in which a variety of substances and methods have been utilized to inhibit NOS. Results from knockout studies in mice have represented that mice deficient in endothelial NOS and neuronal NOS (nNOS) expression exhibit impaired LTP.[60,128,129]

It has also been observed that nitrergic neurons, the neurons that produce NO, elevate in number after spatial learning in rats, which can be elucidated as the up-regulation caused by behavioral training.[130] This evidence shows that NO participates in the memory process. In addition, many studies have suggested a relationship between glutamate NMDA receptors and NO system in terms of learning and memory.[128,131] Several studies have also revealed that NOS inhibitors impair the consolidation of memory[132,133] and block the induction of LTP.[132,134]

A relationship between THs and the NO system has been confirmed.[135-137] Many studies have shown that THs regulates NOS gene expression in the brain.[75,138,139] The results of several studies have demonstrated that the effects of TH on the nNOS are controversial. Sinha *et al*. demonstrated that THs are able to inhibit nNOS in rat embryonic neocortex.[138] It is also suggested that hypothyroidism elevates nNOS activity and NO in amygdala and hippocampus.[75] Hosseini *et al*. also demonstrated that hypothyroidism increases NO level in the hippocampus which is accompanied by the impairment of learning and memory.[139] In another study, TH has increased nNOS activity in the cortex and cerebellum.[140]

It is proposed that the elevation of NO level in hypothyroid rats can cause neuronal damage and stress oxidative in the brain. From one viewpoint, under neurotoxic conditions, NO can cause oxidative damage through the formation of the highly reactive metabolite peroxynitrite. Therefore, by reacting with superoxide, NO produces peroxynitrite, a powerful oxidant, which can damage many biological molecules.[124,125,141] Moreover, NO induces a mitochondrial dysfunction due to the damaging of the complexes of the respiratory chain (complex I–III, II–III, and cytochrome c oxidase), which finally leads to more formation of superoxide radical.[142,143] In addition, an elevation in ROS level could be trigger the chain reactions of free radicals and lead to oxidative damage to membrane lipids, proteins, nucleic acid, and carbohydrates.[143] An increased level of NO-induced ROS could be a factor that elucidates the histopathological damage observed in the brain of hypothyroid rats. Interestingly, it has been proposed that NO, which is mainly produced by nNOS in the mammalian brain, acts as a negative regulator of cell proliferation in the adult brain.[144]

Conclusion

Although the exact mechanisms involved in the learning, memory, and cognition impairments induced by hypothyroidism is still unknown. It seems that hypothyroidism changes the oxidative stress status in different regions of the brain which triggers consequent pathways. It is also suggested that there are relationships between oxidative stress and other hypothyroidism-affected biochemical events including Na^+/K^+-ATPase activity, polyunsaturated fatty acid, nNOS, uptake of the neurotransmitter glutamate, acethylcholinesterase activity, and intracellular Ca^{2+} concentration which creates a multivariate condition with the outcome of brain tissues oxidative damage. It is also suggested that deficiency of antioxidant system plays a role in the regulation of signaling pathways related to cell proliferation and cell death during hypothyroidism. The direct regulation of transcription and translation by changes in active oxygen metabolism is well documented, which is in turn in the tight control of the THs. However, to get an insight into how these signaling molecules are influenced during hypothyroidism, a detailed examination is warranted.

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Conflicts of interest

There are no conflicts of interest.

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