Histopathological Changes in Rat Hypothalamus After Propylthiouracil Induced Hypothyroidism and The Protective Role of Folic Acid

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Summary

Background: Hypothyroidism means that the thyroid gland can't make enough thyroid hormone to keep the body running normally. We studied the histopathological and immunohistochemical changes in the hypothalamus of hypothyroid rat at the post-pubertal stage, in addition to the ameliorating role of folic acid.

Material and Method: Twenty male albino rats were equally divided into four groups; the first and second groups were the control and folic acid groups respectively while the third group was the hypothyroid group in which rats received propylthiouracil (PTU) in drinking water for 6 weeks to induce hypothyroidism. The fourth group was hypothyroid rats treated with folic acid for four weeks and dissected after 6 weeks. In order to ensure the hypothyroid state, the level of serum T3 and TSH through the dose period were regularly determined.

Results: The levels of TSH in hypothyroid rats were significantly higher while the levels of T3 were significantly lower in hypothyroid rats when compared to control rats group. A variety of changes were observed in the hypothalamus of hypothyroid compared with control rats. Microscopically, the hypothalamus of hypothyroid rats showed diffuse vacuolar degeneration and damages neurons especially in both SCN and SON.

Conclusion: Treatment of hypothyroidism with folic acid depressed the histopathological alteration.

Key words: Hypothyroidism; PTU; Hypothalamus; Rats; SCN; SON

Propiltiourasil ile Oluşturulan Hipotiroidizmdede Suçan Hipotalamusunda Histopatolojik Değişiklikler ve Folik Asidin Koruyucu Rolü

Özet


Matiereyal ve Metod: Yirmi albino çifçi 4 eşit gruba ayrıldı; ilk ve ikincisi grupler kontrol ve folik asit grupları oldu. Üçüncü grup hipotiroidi gelişirmek üzere 6 hafta boyunca içme sulardında propiltiourasil (PTU) alan hipotiroidi grupu oldu. Dördüncü grup 4 hafta süreyle folik asit tedavisi uygulanan ve 6 hafta sonra disseke edilen hipotiroidi grup oldu. Hipotiroididen emin olmak üzere düzenleni olarak serum T3 ve TSH düzeyleri monitorize edildi.

Sonuçlar: Kontrol gruplarına oranla hipotiroidili suçanın TSH düzeyleri önemli ölçüde yüksek ve T3 değerleri ise düşük idi. Kontrol grupu suçanalara gore hipotiroid hipotalamusunda
INTRODUCTION

Thyroid hormones are recognized as key metabolic hormones that play a critical role in brain development, mediating important effects within the central nervous system throughout life, regulate the metabolism and function of various neurotransmitters, their receptors, second-messengers, and gene expression. Normal brain development requires the presence of thyroid hormones that are essential for cell migration, dendrite and axon outgrowth, synapse formation, myelination and gliogenesis. It is now well established that the mammalian brain is a direct target organ of thyroid hormone, both during development and in adult individuals. Alterations in their normal levels cause some biochemical and clinical abnormalities such as hypothyroidism and hyperthyroidism. Hypothyroidism is an underactive thyroid gland that can not make enough thyroid hormone to keep the body running normally. It is a very common disorder that occurs in mild or severe forms in 3 to 5% of the population. PTU is an anti-thyroid drug which inhibits both the synthesis of thyroid hormones in the thyroid gland, and the conversion of thyroxine (T4) to its active form, triiodothyronine (T3), in peripheral tissues. A reduction, or absence, of thyroid hormone during brain maturation yields molecular, morphological and functional alterations in the cerebral cortex. Hypothyroidism causes major defects in synaptic organization. On other words, the early hyperthyroidism in rats alters thyroid states and caused some malformations as decrease in body, brain and cerebellar weight.

Hypothalamus is a collection of small but critical nuclei in the diencephalon that lies just inferior to the thalamus. It governs reproductive, homeostatic and circadian functions. The hypothalamus integrates autonomic and endocrine functions with behavior. The hypothalamus coordinates the peripheral expression of emotional states. The function of these vital systems can be altered by various causes ranging from food mishandling, dependency & substance withdrawal, stress or psychological responses to simple functional deficits, hyperactivity, hypoactivity or learning disabilities. Hypothalamic nuclei refer to well-defined cell groups and can usually be localized by coronal sections of Nissl stained tissue. Hypothalamic areas are a more diffusely defined heterogeneous collection of cells and fibers that are not separated from each other by distinct boundaries. In the anterior division there are an important three hypothalamic nuclei (Suprachiasmatic nucleus, paraventricular nucleus and supraoptic nucleus), while in the arcuate and posterior regions there are another two hypothalamic nuclei (Arcuate nucleus and dorsomedial hypothalamic nucleus) were located. The majority of hypothalamic nuclei are located medially. An important exception is the supraoptic nucleus, which is both lateral and anterior.

Vitamin B9 (folic acid) is essential to numerous body functions ranging from nucleotide biosynthesis to the remethylation of homocysteine. Children and adults both require folic acid in order to produce healthy red blood cells and prevent anemia. Folic acid can be used to help treat Alzheimer's disease, depression, anemia, and certain types of cancer. Folic acid, with its antioxidant activity, is a little studied compound in the sense of human intervention trials. This water-
soluble vitamin has gathered abundant attention because of its role in the pathogenesis of cardiovascular disease, neural tube defects and cancer prevention.\(^{(19)}\) There is little information about the relation between hypothyroidism and the changes in hypothalamus structure so the present study represents a contribution to declare the effect of low thyroid hormone status on the histopathological changes in the hypothalamus of hypothyroid rat at the post-pubertal stage, in addition to the ameliorating role of folic acid.

**MATERIAL AND METHODS**

The experiments were performed on 20 male albino rats (Rattus norvigicus) weighing 120±10g and of 6-7 week's age. They were obtained from our laboratory farms, Zoology Department, Faculty of Science, Tanta University, Egypt. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch; Egyptian Company of Oils and Soap Kafr El-Zayat; Egypt) and water available ad libitum. The temperature in the animal room was maintained at 23±2°C with a relative humidity of 55±5%. Light was on a 12:12 hr light-dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were randomly and equally divided into four groups (5 animals each).

**Group 1:** (G\(_1\)) Control group in which animals never received any treatment.

**Group 2:** (G\(_2\)) Folic acid group in which, rats received folic acid (El Nasr Pharmaceutical Chemicals Co.; 0.011 \(\mu\text{mol/g of body weight}\)) for four weeks.\(^{(17)}\)

**Group 3:** (G\(_3\)) Hypothyroid rats group in which, rats received 0.05% 6-n-propyl-2-thiouracil (PTU) daily in drinking water for 6 weeks.\(^{(22,29)}\)

**Group 4:** (G\(_4\)) Co-treatment group in which, animals received 0.05% PTU in drinking water and folic acid simultaneously according to Matte et al.\(^{(17)}\) The dose period of PTU was six weeks as in hypothyroid rats group. However, folic acid was administered orally for 4 weeks form the second to sixth week after evidence of hypothyroidism had been established at the end of the second week.

At the end of the experimental period, rats were euthanized with inter peritoneal injection with sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from the inferior vena cava of each rat in heparinized glass tubes to estimate thyroid hormone levels in different groups under study. Serum was separated by centrifugation at 3000 rpm for 15 minutes. Blood serum was analyzed to determine the thyroid hormones (T3 and TSH). Serum T3 was assayed by using commercial test supplied by the Diagnostic systems Laboratories (DSL), Taxes, USA. Serum TSH was assayed by using commercial Kit supplied by Coat-A-Count TSH IRMA, Los Angeles, USA.

**Histopathological investigation (H&E staining):**

The skulls were opened with fine scissors and the brains were excised and fixed in 4% paraformaldehyde in phosphate buffered saline (0.1M, Ph 7.4 PBS) for 24 hours at 4°C. Fixed brains were dehydrated through a graded series of ethanol and embedded in paraffin according to standard procedures. Paraffin sections (5\(\mu\text{m}\) thick) were mounted on gelatin chromalum–coated glass slides and used for Haematoxylin and eosin stains as a routine method after Bancroft and Stevens.\(^{(5)}\)

**Transmission Electron Microscopy Studies:**

Small pieces (1 mm) of control and treated hypothalamic tissues were cut and fixed in 3% glutaraldehyde (PH7.4) in phosphate buffer and post fixed in 2% osmium...
tetroxide in phosphate buffer. Following fixation, tissues were dehydrated at increasing concentrations of ethanol. They were then embedded in araldite resin. Ultrathin sections were cut using an ultratome. Ultrathin sections were stained by uranyl acetate saturated in 70% ethanol, and lead citrate. Tissue sections were evaluated using a JEOL transmission electron microscope JEM-1200. Ex, Japan.

RESULTS

In order to ensure the hypothyroid state we regularly determined the serum T3 and TSH through the dose period. Levels of T3 and TSH are shown in Table 1. While T3 level significantly (p < 0.05) decreased while serum TSH significantly (p < 0.05) increased in hypothyroid rats group as compared to control group. The histopathological examination of rat hypothalamus sections in control and folic acid groups showed normal architecture as shown in Figures (1A, 1B, 1E, 1F). A variety of changes were observed in the hypothalamus of hypothyroid compared with control rats. Microscopically, the brains of hypothyroid rats showed diffuse vacuolar degeneration and damages neurons especially in both SCN and SON (Figs. 1C, 1G). A large number of degenerating (i.e., shrunken, argyrophilic) neurons were observed in the SCN and SON with clear oedema and apoptosis were more significant and moderate neurofibrillary degeneration is quite illustrated. In addition, mild spongy changes in hypothalamic nuclei, such as in SCN and SON were observed in the hypothalamus of hypothyroid group (Figs. 1C, 1G). Decrease in the number of parvocellular cells in SON of hypothyroid rats as compared with control groups (Figs. 1G). Neural cells looked globular and spindle shaped, and a strong degenerative change was observed. Also, the number of glial cells remarkably increased in all of the brain lesions that were examined. A few numbers of degenerating neurons in SCN and mild vacuolation in addition to decrease number of parvocellular cells were observed in hypothalamic sections in co-treated hypothyroid rat with folic acid (Figs. 1D, 1H). No damage or changes in neurons or nerve fibers were observed in the PVN, DMH and ARN of the hypothalamus of the different experimental groups under study (data not shown).

Hypothalamic coronal sections from different groups under study were examined by transmission electron microscopy. No significant difference in the occurrence or severity of axonal degeneration was seen between the controls and folic acid-exposed groups (Figs. 2A-2D). However, damaged in most of neuronal axons and some of axons exhibiting thin myelin, demyelination and intramyelinic edema were only observed in hypothyroid rats group (Figs. 3A, 3B). In co-treated hypothyroid rats group (Figs. 3C, 3D) shown little irregular damage in nuclear membrane and the number of mitochondria were increased.
Table 1: Serum T₃ and TSH levels in different groups under study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T₃  (ng/dl)</th>
<th>TSH (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>103-176</td>
<td>0.05-0.10</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>156±13.6</td>
<td>0.07±0.009</td>
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<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>105-186</td>
<td>0.04-0.10</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>157±14.9</td>
<td>0.05±0.012</td>
</tr>
<tr>
<td>p&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hypothyroid</td>
<td></td>
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<tr>
<td>Range</td>
<td>44-66</td>
<td>2.90-4.60</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>56±4.9</td>
<td>3.78±0.347</td>
</tr>
<tr>
<td>p&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>p&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<tr>
<td><strong>Group IV</strong></td>
<td></td>
<td></td>
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<tr>
<td>Co-treatment</td>
<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>38-43</td>
<td>3.80-5.10</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>40±6.8</td>
<td>4.18±0.235</td>
</tr>
<tr>
<td>p&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>p&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>p&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>NS</td>
<td>NS</td>
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</table>

p<sup>(a)</sup> = value vs. control group (I). p<sup>(b)</sup> = value vs. folic acid group (II).
p<sup>(c)</sup> = value vs. hypothyroid group (III). p<sup>(d)</sup> = value vs. co-treatment group (IV).

Figure 1: High power micrographs of hypothalamic coronal sections of rat brain stained with H&E showed the SCN and SON. A: Normal structure of SCN in control rats. B: Normal structure of SCN in folic acid group. C: SCN of hypothyroid rats showed moderate inflammation, oedema and diffuse vacuolar degeneration. D: A few numbers of degenerating neurons in SCN of co-treated hypothyroid rats with folic acid. E-H: High power micrographs of rat SON coronal sections stained with HE showing two types of cells, the small cells called parvocellular cells (arrows) and large cells called magnocellular cells (arrow head). E&F: Normal structure of SON in control and folic acid groups. G: SON of hypothyroid rats showed a large number of degenerating neurons, oedema and moderate neurofibrillary. H: SON in co-treated hypothyroid rats with folic acid showing vacuolation and decrease number of parvocellular cells.
Figure 2: Photo electron micrographs (TEM) of rat hypothalamus A&B: Control rat hypothalamus shown normal structure of neuron and axons. C&D: Normal structure of neuron and axons in hypothalamus in control and folic acid groups.

Figure 3: Photo electron micrographs (TEM) of rat hypothalamus A&B: Damaged neurons and degenerated axons in rat hypothalamus in hypothyroid group. N, nucleus; Ax, axon; M, mitochondria C&D: Rat hypothalamus in co-treated hypothyroid rat group showed little irregular damage nuclear membrane and an increased in the number of mitochondria.
DISCUSSION

The present study revealed significant decrease in serum T₃ level and significant increase in serum TSH level in the hypothyroid group when compared to the control and folic acid groups. This might be considered as a sound argument in the induction of hypothyroidism indicating that PTU is a good choice as an antithyroid drug for induction of hypothyroid state. This finding is compatible with other studies that used PTU as an antithyroid drug for induction of hypothyroidism at different ages. In treated hypothyroid rats with folic acid group, T3 and TSH levels returned to its normal range after withdrawing PTU from the drinking water confirming the nature of PTU as a reversible goitrogen. Several reports are listed on the harmful effect of thyroid hormone deficiency during the development. Many studies have characterized the neuroanatomical consequences of developmental hypothyroidism. Early work by Eayrs demonstrated that perinatal hypothyroidism could alter the density and size of neuronal perikarya within specific brain regions, as well as fiber density and orientation within adult cortical layers. Additionally, Berbel et al. have published a series of studies characterizing the effect of developmental hypothyroidism on a variety of anatomical features, including spine density of pyramidal neurons in the cerebral cortex, the organization of callosal connections, and other features. Also, thyroid hormone deficiency during a brief perinatal period produces severe neurological defects in humans and experimental animals. During critical periods of development, hypothyroidism causes abnormalities of the CNS such as incomplete maturation of neuronal and glial cells, reduction in synaptic densities and myelin deficits.

In our histopathological results, a variety of changes were observed in the hypothalamus of hypothyroid compared with control rats. Microscopically, the brains of hypothyroid rats showed diffuse vacuolar degeneration and damages neurons especially in both SCN and SON. A large number of degenerating neurons were observed in the SCN and SON with clear oedema and apoptosis were more significant and moderate neurofibrillary degeneration is quite illustrated. Decrease in the number of parvocellular cells in SON of hypothyroid rats as compared with control groups. No cellular damage or nerve fibers were observed in the PVN, DMH and ARN of the hypothalamus of the hypothyroid rats. In contrast, there was no significant difference between the hypothyroid and co-treated hypothyroid with folic acid groups in all the examined areas of the hypothalamus. Our results agree with Alvarez-Dolado et al. who reported that thyroid hormone deficiency in experimental animals causes an array of abnormalities in the CNS of which alterations of cell migrations are of special relevance. Also, Schwartz; Schwartz et al. and Ahmed et al. reported that, thyroid hormone deficiency results in multiple morphological alterations in neonatal rat brain. Thyroid hormone deficiency also causes specific defects in cell migration and differentiation because of the regulatory effects of thyroid hormones on the processes of terminal brain differentiation such as dendritic and axonal growth, synaptogenesis, neuronal migration and myelination. The current study indicated and recommended that, folic acid as a treatment was better if it is administered as an adjuvant after returning to the euthyroid state. If confirmed in human beings, these results could propose that folic acid can be used as an adjuvant therapy with thyroxin replacement therapy in hypothyroidism disorders.
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