Article published online: 2022-06-17

ARTICLE

Effect of Triiodothyronine (T3) Supplementation on the Cerebral Neurotransmitters in Offspring of Hypothyroid Rats

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Published: 07 November 2012
Ibnosina J Med BS 2012,4(6):231-241
Received: 28 January 2012
Accepted: 11 June 2012
This article is available from: http://www.ijmbs.org

Abstract

Introduction: Nervous system growth and differentiation are intimately interrelated with the presence of thyroid hormones (THs) in early development stages. Hypothyroidism during the fetal and postnatal life results in an irreversible mental retardation syndrome. **Aim of the study:** Assessment of the effect of 3,5,3'-triiodo-L-thyronine (T_3) on changes in the cerebral neurotransmitters level in hypothyroid rats male offspring and the possible role of Na^+, K^+-ATPase activity. **Materials and methods:** Hypothyroidism during pregnancy and lactation in one group (hypothyroid group) was induced by the antithyroid drug, methimazole (MMI) that was added to drinking water at a concentration of 0.02%. In the second group (T_3-treated hypothyroid group), MMI was stopped and animal's offsprings were given T_3 (20 µg/100 g body weight in 0.01 N NaOH, i.p.) for one week. The third group is the control group; neither the mother nor the offspring received any drug. The hypothyroid state in mothers during pregnancy was confirmed by measuring total thyroxine (TT_4) and total triiodothyronine (TT_3) at gestational day 10. At the end of experiment, the offsptings were sacrificed and free thyroxine (FT_4) and free triiodothyronine (FT_3) in sera and neurotransmitters (dopamine, norepinephrine and serotonin) and Na^+, K^+-ATPase activity were measured in the cerebral homogenate. **Results:** Maternal hypothyroidism induced a significant decrease in the cerebral level of dopamine, norepinephrine, serotonin and Na^+, K^+-ATPase activity when compared with euthyroid group. Treatment with T_3 significantly increased the cerebral level of neurotransmitters and Na^+, K^+-ATPase activity when compared with the hypothyroid group. **Conclusion:** T_3 supplementation during the postnatal period through its effect on the cerebral Na^+, K^+-ATPase effectively reversed the effect of maternal methimazole-induced hypothyroidism on the cerebral level of dopamine, norepinephrine and serotonin.

Keywords: Thyroid hormone; Neurotransmitter; Antithy-

roid drug; Dopamine; Norepinephrine and Serotonin.

Introduction

Thyroid hormones (THs) play a critical role in the development and physiological functions of diverse body organs especially the brain (1,2). THs enter the brain throughout two routes (3). The main route is through the blood–brain barrier and the second less important route is via the choroid plexus–cerebrospinal fluid (4). THs receptors are expressed in the main cell types of the brain namely neurones, oligodendrocytes, and astrocytes (5,6). THs exert special effects on the brain development by regulating gene expression (7,8). Also they exert non-genomic, thyroid receptor-independent pathways in the cytoplasm, plasma membranes and/or organelles, including the regulation of ion channels and the activation of various signaling cascades (9).

During brain development THs are necessary for cell migration, dendrite and axon outgrowth, synapse formation, myelination and gliogenesis (7,9,10). Hypothyroidism in adults resulted in functional defects include inability to create long-term potentiation in rats hippocampus, in addition to impaired learning and memory in rats and humans (11,12). THs also affect the characteristics of various neurotransmitter systems and neurotransmission in the brain (4,11,13,14). Catecholaminergic and serotonergic systems play a functional role in activity, mood, and learning, hence in behavior (10,15). Thyroid hormone-dependent brain development is best studied in neonatal rats (16). This is due to the interspecies differences in developmental schedules (7). At birth, the rat's brain is equivalent to the human brain at 5–6 months of gestation, and at 10 days of postnatal age the rat's brain is alike to the human brain at birth (4). Therefore, at birth the brain and hypothalamico-pituitary system of the rats are not fully developed compared to those of the human newborn (17).

The present study aims to test the hypothesis that T_3 supplementation alters the cerebral neurotransmitters in rat's male offspring whose mothers were rendered hypothyroid during pregnancy and lactation via modulation of Na^+, K^+-ATPase activity.

Materials and Methods

Animals

Eighteen mature virgin White Albino female rats weighting about 170–190 g and 9 mature males were used. They were obtained from Animal house, Faculty of Medicine, Assiut University. The animals were housed in clean properly ventilated cages and were maintained on standard laboratory diet and these animals were maintained on normal daily light/dark periods of 12 hour each. Free access to food and water was allowed throughout the study period. All experimental protocols followed the guidelines of the Animal Committee of the Faculty of Medicine of Assiut University. Daily vaginal smear examination of each female was carried out to determine the estrus cycle. Estrous females exhibited the presence of cornified cells in vaginal smear. Mating was induced by housing pro-esterous females with males in separate cages at ratio of two females and one male overnight for 1 or 2 consecutive days. In the next morning, the presence of vaginal plug determined the first day of gestation. Then, the pregnant females were transferred into separate cages from males to start the experiment. The adult female rats from the 1st day of pregnancy [gestation day (GD) 1] to the first 3 weeks of lactation period [lactation day (LD) 21] were divided into three groups as follows 1) Group I (Euthyroid group): Six control rats were received tap water. 2) Group II (Hypothyroid group): six rats who rendered hypothyroid by administration of an antithyroid agent, methimazole [(2-mercapto-1-methylimidazole: MMI; CAS No. 60-56-0), obtained from Sigma Chemical Co. (St. Louis, MO)], at a concentration of 0.02% (w/v) to induce maternal hypothyroidism. The used dose of MMI was based on the dose range that induced changes in neuronal or oligodendroglial parameters in previous studies (18,19). MMI was dissolved in distilled water and administrated in the drinking water to the dams from first day of gestation to postnatal day 21 (GD 1 - PND 21). After completion of this time period, administration of MMI was stopped and litter rats were given once daily injection of the vehicle for T_3 (0.01 N NaOH, i.p.) for one week. MMI blocks the synthesis of thyroxine (T_4) and triiodothyronine (T_3) and can cross the placenta readily, reaching fetal to maternal serum ratios of approximately 1:1 (2) and 3) Group III (T_3-treated hypothyroid group): rats were treated with 0.02% (w/v) MMI in drinking water from first day of gestation to postnatal day 21 (GD 1 - PND 21). After completion of this time period, administration of MMI was stopped and litter rats were given once daily injection of T_3 (20 μg/100 g body weight in 0.01 N NaOH, i.p.) for one week.

The mothers' blood was taken from optic vein at gestational day 10 to estimate the total triiodothyronine (TT_3) and total thyroxine (TT_4) in euthyroid, hypothyroid and T_3-treated hypothyroid status. At the end of experiment, six male litters were taken from each group this is because of high mortality rate and in order to examine the same litters throughout the experimental period. Then, litters were

weighed and sacrificed by decapitation and blood samples were taken from jugular vein. Then, blood samples were centrifuged at 3000 round per minute (rpm) for 30 minute. The clear, non-hemolysed supernatant serum were quickly removed and kept at -20^0 C until use. The litter's cerebral cortex was separated from the skull base after cutting all the cranial nerves, weighed and washed using chilled saline solution. Then, the cerebrum was dried on a filter paper and homogenized for 5 minutes by using a Teflon homogenizer (Glas-Col, Terre Haute, USA). The cerebrum was divided into two longitudinal equal halves. One half was homogenized in 1M perchloric acid for the determination of neurotransmitters (dopamine, norepinephrine and serotonin). The other half was homogenized in isotonic solution (0.9% NaCl) to be used for the assay of Na^+, K^+-ATPase activity. The homogenate was centrifuged by cold centrifuge Minifuge-2. (Heraeus Christ GmbH, Osterode, Germany) at 5,000 g for 20 min and the resultant supernatant was kept in deep freezer at -20^0 C until use.

Measurement of T_4, T_3 and growth hormone in sera

Enzyme-linked immunosorbent assays (ELISA) were performed for measuring concentrations of TT_4 and TT_3 in sera of the mothers and free thyroxine (FT_4), free triiodothyronine (FT_3) and growth hormone (GH) in sera of their offspring using commercial kits (BioSource, Europe, Belgium) with monoclonal antibodies against each substance and following the instruction attached with each kit. The apparatus used was Statfax-2100.

Measurement of cerebral neurotransmitters levels

Estimation of dopamine, norepinephrine and serotonin level in the cerebral homogenate was done by HPLC (Agilent 1200 series). 100 µl of the cerebral homogenate was injected directly to the Zorbax Eclipse XDB-C18 column (150 mm×4.6 mm, 5µ and C18) under the following conditions: mobile phase mixture of acetic acid and ammonium acetate buffer pH 3.1 (1:99 v/v), flow rate 0.1 ml/min, UV 285 nm. The resulting chromatogram identifying each neurotransmitter position and area under curve for each sample was compared to that of the standard curve made by Eurochrom HPLC Software, version 1.6. The concentration of neurotransmitter was determined from the formula: concentration of neurotransmitter in

Table 1. Effect of T_3 supplementation on body and brain weight and maternal serum levels of total tri-iodothyronine (TT_3, ng/ml) and total thyroxine (TT_4, ug/dl) of rat offspring whose mothers rendered hypothyroid during pregnancy and lactation periods.

Parameters	Euthyroid group	Hypothyroid group	T_3-treated hypothyroid group
Body weight (g)	50.2 ± 4.2	31.40 ± 4.72***	39.60 ± 3.21** #
Brain weight (g)	1.10 ± 1.13	0.86 ± 0.89***	0.89 ± 0.93***
Maternal TT_3 (ng/dl)	7.13 ± 2.78	0.59 ± 0.30*	6.75 ± 3.02#
Maternal TT_4 (ug/dl)	11.89 ± 6.04	3.17 ± 1.56***	9.39 ± 2.53#
Offspring FT_3 (ng/dl)	2.32 ± 0.31	0.42 ± 0.18***	1.32 ± 0.16** #
Offspring TT_4 (ug/dl)	4.96 ± 0.15	0.98 ± 0.16***	2.23 ± 0.85*** ###
Offspring growth hormone (ng/dl)	2.41 ± 0.69	0.77 ± 0.22***	1.59 ± 0.21* #

All values are mean ± SD, *: $p < 0.05$ **: $p < 0.01$ ***: $p < 0.001$ versus euthyroid group, #: $p < 0.05$ versus the hypothyroid group.

Table 2. Effect of T_3 supplementation on cerebral neurotransmitter levels (dopamine, norepinephrine and serotonin) and Na^+, K^+-ATPase activity of rat offspring whose mothers rendered hypothyroid during pregnancy and lactation periods.

Parameters	Euthyroid group	Hypothyroid group	T_3-treated hypothyroid group
Dopamine (mg/g)	1.00 ± 0.11	0.10 ± 0.01***	0.53 ± 0.10#
Norepinephrine (mg/g)	0.53 ± 0.49	0.01 ± 0.001***	0.43 ± 0.19#
Serotonin (mg/g)	1.94 ± 0.08	1.32 ± 0.12***	1.77 ± 0.11##
Na^+, K^+-ATPase activity (umol pi/ mg protein)	4.90 ± 0.64	3.11 ± 0.27***	3.87 ±0.48**#

All values are mean ± SD, **: p < 0.01, ***: p < 0.001 versus Euthyroid group, #: p < 0.05, ##: p < 0.01 versus the hypothyroid group.

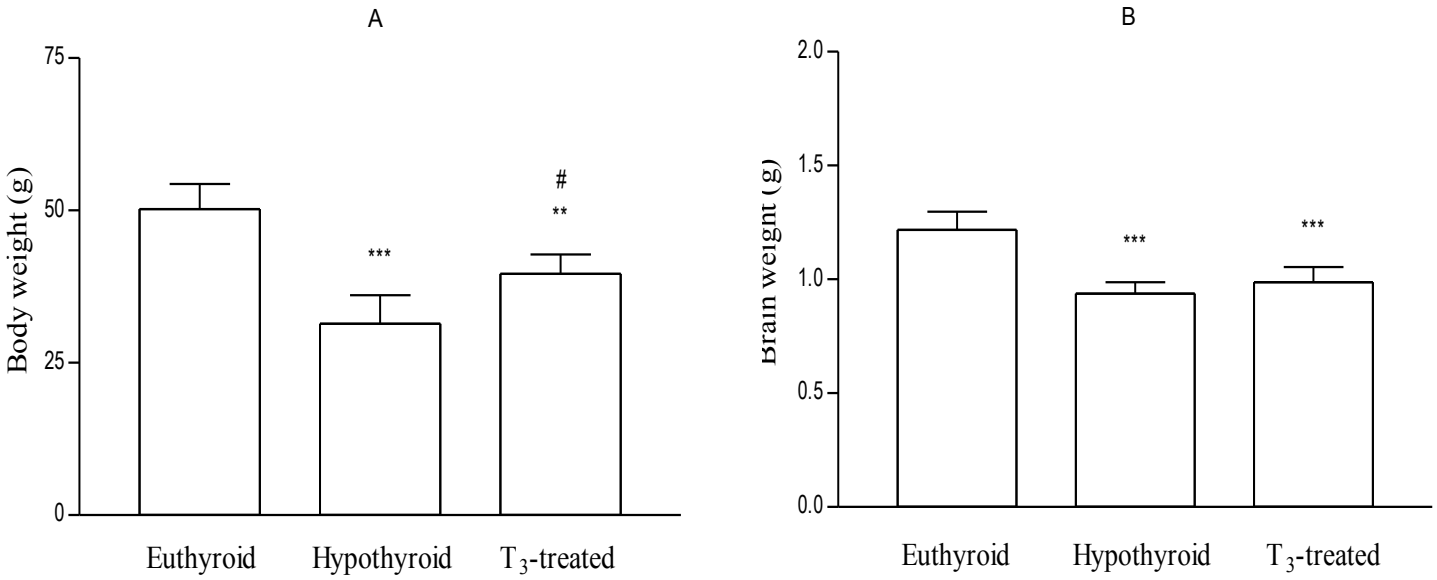

Figure 1. Effect of T3 supplementation on offspring body weight (A) and brain weight (B). Maternal MMI administration resulted in a significant reduction in the body and brain weight of their offspring. The body weight is reverted by T3 supplementation. Bars represent mean ± SD, ** p < 0.01 and *** p < 0.001 versus the euthyroid group, # p < 0.05 versus the hypothyroid group.

sample (mg/g) = concentration of standard (µg/ml) x volume of homogenization/weight of tissue (g) x area of sample under curve/area of standard under curve (13).

Measurement of Na^+, K^+-ATPase activity

Measurement of Na^+, K^+-ATPase activity was followed the procedure of Reading and Isbir (20) and depended on the measurement of inorganic phosphate that is resulted from addition of 3 mM of disodium adenosine triphosphate (Na_2ATP) to the medium during the incubation period. The medium was incubated in a 37°C water bath for 5 min with a mixture of 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$,

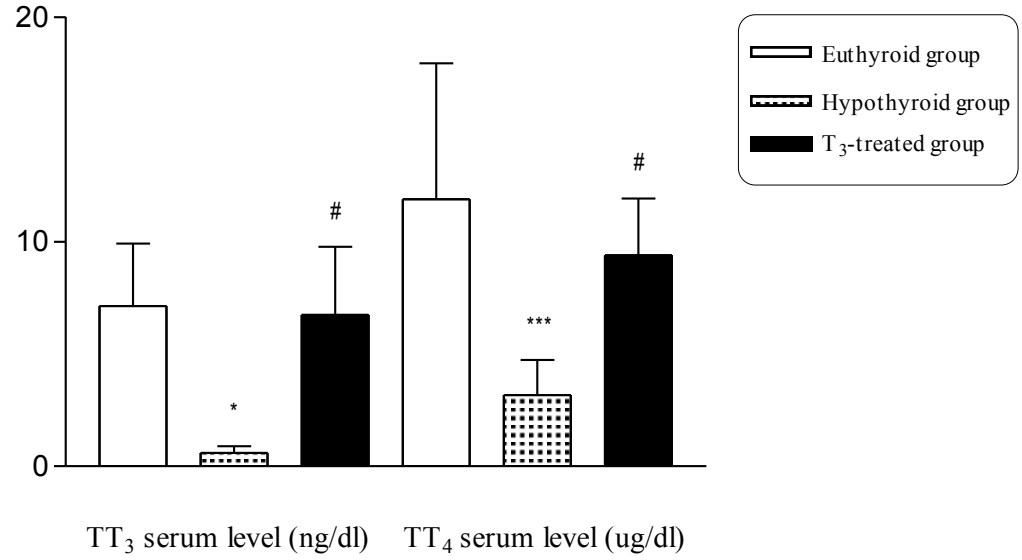

Figure 2. Effect of methimazole (MMI) administration on maternal serum level of TT3 and TT4. MMI administration resulted in a significant reduction of maternal serum level of TT3 and TT4. T3 supplementation resulted in a significant increase in maternal serum level of TT3 and TT4 as compared with hypothyroid group. Bars represent mean ± SD, * $p < 0.05$ and *** $p < 0.001$ versus the euthyroid group, # $p < 0.05$ versus the hypothyroid group.

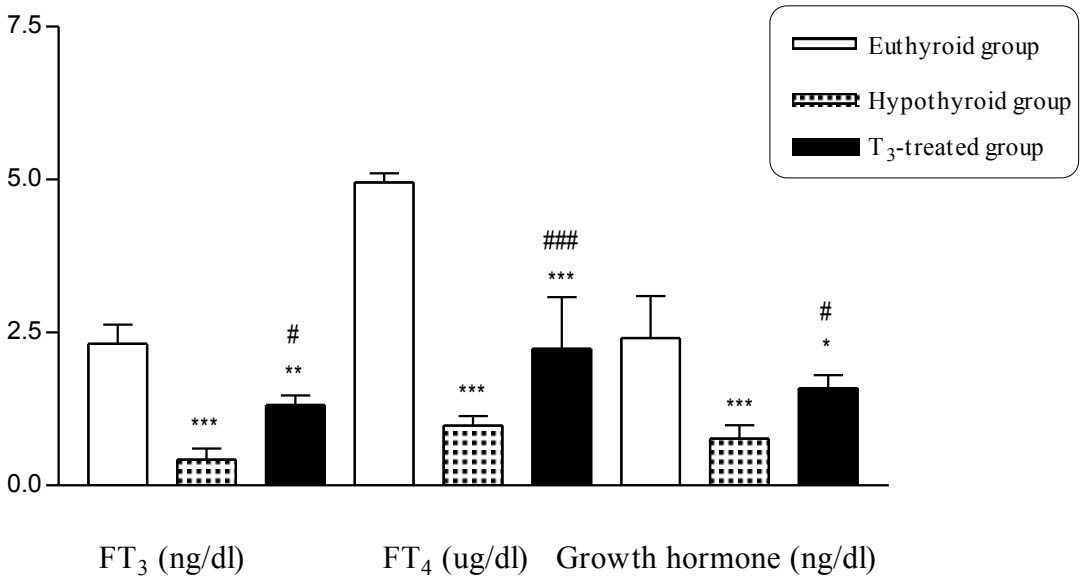

Figure 3. Effect of T3 supplementation on FT3, FT4 and growth hormone serum level of rat offspring. MMI administration resulted in a significant reduction of offspring serum level of FT3, FT4 and growth hormone. T3 supplementation resulted in a significant increase in offspring serum level of FT3, FT4 and growth hormone as compared with hypothyroid group. Bars represent mean ± SD, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus the euthyroid group, # $p < 0.05$ and ### $p < 0.001$ versus the hypothyroid group.

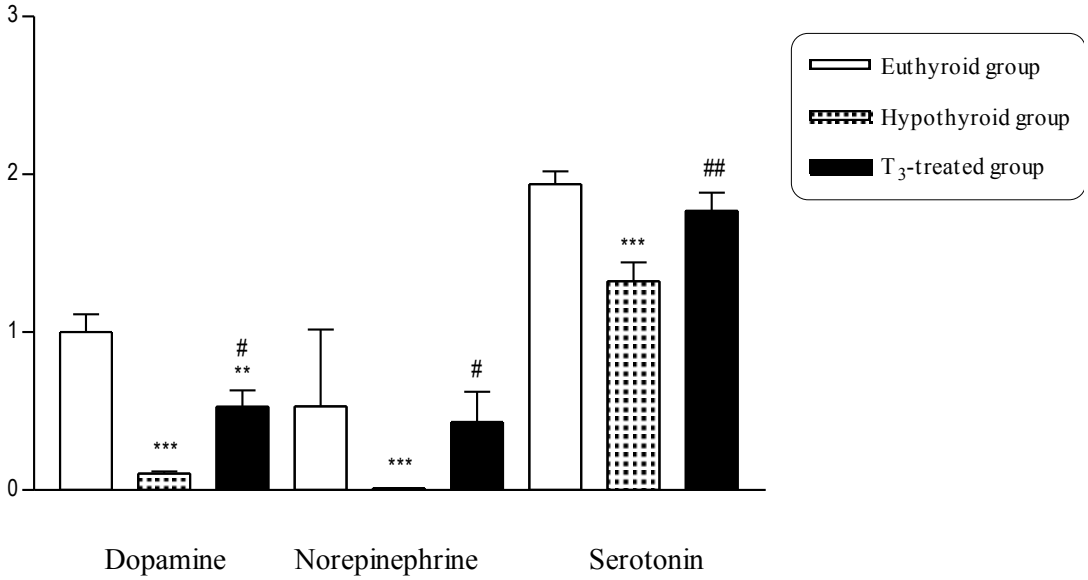

Figure 4. Effect of T3 supplementation on the cerebral neurotransmitter level (dopamine, norepinephrine and serotonin) of rat offspring. MMI administration resulted in a significant reduction in the cerebral neurotransmitter level. T3 supplementation resulted in a significant increase in the cerebral neurotransmitter level as compared with hypothyroid group. Bars represent mean ± SD, *** $p < 0.001$ versus the euthyroid group, # $p < 0.05$ and ## $p < 0.01$ versus the hypothyroid group.

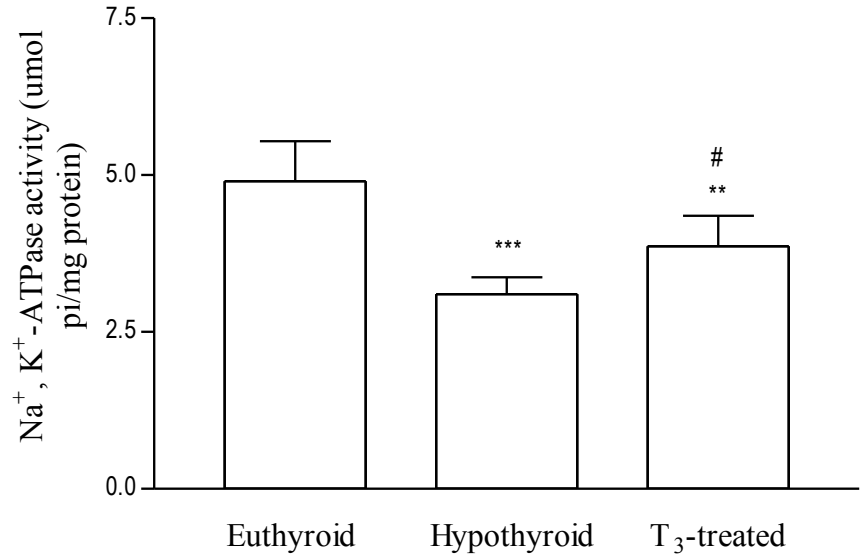

Figure 5. Effect of T3 supplementation on the cerebral Na+, K+-ATPase activity of rat offspring. MMI administration resulted in a significant reduction in the cerebral Na+, K+-ATPase activity. T3 supplementation resulted in a significant increase in the cerebral Na+, K+-ATPase activity as compared with hypothyroid group. Bars represent mean ± SD, ** $p < 0.01$ and *** $p < 0.001$ versus the euthyroid group, # $p < 0.05$ versus the hypothyroid group.

0.1 mM EDTA and 30 mM Tris HCl (pH 7.4). Following the pre-incubation period, Na_2ATP at a final concentration of 3 mM, was added to each tube and the tubes were incubated at 37°C for 30 min. After the incubation, the tubes were placed in an ice bath, and the reaction was terminated. Subsequently, the level of inorganic phosphate was determined using a spectrophotometer (UV/visible spectrophotometer) at an excitation wavelength of 690 nm. The specific activity of the enzyme was expressed as umol pi/mg protein. The protein concentration of the supernatant was measured by the Lowry method (21) using bovine serum albumin as standard protein.

Statistical analysis

Data were analyzed using Minitab Version 14 (Minitab Inc., State College, Philidelphia, PA. USA) and Graph-Pad prism version 5. Shapiro-Wilkes test was used to determine whether data were normally or abnormally distributed. Our data were non-categorical data normally distributed and are expressed as mean ± standard deviation (SD). The data were analyzed using one-way ANOVA test, followed by Bonferroni post hoc test to determine significance. A probability value of $P < 0.05$, $P < 0.01$ and $P < 0.001$ are considered statistically significant, highly significant and very highly significant, respectively.

Results

Offspring body weight and brain weight

The effect of T_3 supplementation on the body weight and brain weight of rat's offspring whose mothers were rendered hypothyroid was during pregnancy and lactation is shown in Figure 1. The body weight of hypothyroid group was significantly reduced compared to the euthyroid group and this effect was partially reversed in T_3-hypothyroid treated group when compared with the hypothyroid group (Table 1). The brain weight of the hypothyroid group was significantly lower than that of the euthyroid group and this effect was not reverted to normal level after T_3 supplementation (Table 1).

Maternal TT_3 and TT_4 serum levels

The effect of MMI administration on TT_3 and TT_4 serum level of pregnant rats is shown in Figure 2. Administration of MMI resulted in a significant decrease in maternal serum level of TT_3 and TT_4 compared with the euthyroid group. Treatment with T_3 significantly increased the level of TT_3 and TT_4 compared with the hypothyroid group, (Table 1).

Offspring FT_3, FT_4 and growth hormone serum level

The effect of T_3 supplementation on FT_3, FT_4 and growth hormone serum level of rats' offspring whose mothers were rendered hypothyroid during pregnancy and lactation is shown in Figure 3. Administration of MMI resulted in a significant decrease in offspring serum level of FT_3 and FT_4 compared with euthyroid group. Treatment with T_3 significantly increased the level of FT_3 and FT_4 compared with the hypothyroid group (Table 1). Maternal hypothyroidism resulted in a significant decrease in offspring serum level growth hormone compared with the euthyroid group. Treatment with T_3 significantly increased the level of growth hormone compared with the hypothyroid group (Table 1).

Offspring cerebral neurotransmitters level

The effect of T_3 supplementation on the cerebral neurotransmitter level (dopamine, norepinephrine and serotonin) of rats' offspring whose mothers were rendered hypothyroid during pregnancy and lactation is shown in Figure 4. Administration of MMI resulted in a significant decrease in the cerebral level of dopamine, norepinephrine and serotonin compared with the euthyroid group. Treatment with T_3 significantly increased the level of cerebral neurotransmitters compared with the hypothyroid group, (Table 2).

Offspring cerebral Na^+, K^+-ATPase activity

The effect of T_3 supplementation on the cerebral Na^+, K^+-ATPase activity of rats' offspring whose mothers were rendered hypothyroid during pregnancy and lactation is shown in Figure 5. Maternal hypothyroidism resulted in a significant decrease in the cerebral Na^+, K^+-ATPase activity when compared with the euthyroid group. Treatment with T_3 significantly increased the Na^+, K^+-ATPase activity compared with the hypothyroid group (Table 2).

Discussion

Thyroid hormones have been implicated in mammalian brain maturation and function. The present study evaluated the influence of T_3 supplementation on cerebral neurotransmitters (dopamine, norepinephrine and serotonin) and Na^+, K^+-ATPase activity in rats' male offspring whose mothers were rendered hypothyroid during pregnancy and lactation. MMI administration to female rats during pregnancy and lactation resulted in hypothyroidism in mothers as indicated by decrease in serum level of TT_3 and TT_4 in mothers. Furthermore, thyroid gland function of rats' offspring maternal hypothyroidism resulted in decreasing serum level of FT_3 and FT_4 in their offspring.

T_3 supplementation resulted in an increase in offspring FT_3 and FT_4 serum level. These results are concordant with several previous reports, which found that rats' mothers, which received antithyroid drugs such as MMI rendered their offspring hypothyroid (22-25). MMI exerts hypothyroidism by preventing incorporation of iodine into tyrosyl residues of thyroglobulin and inhibiting the coupling of iodotyrosyl residues to form iodothyronine, thus inhibiting the formation of THs (4,13). In the present study, maternal MMI-induced hypothyroidism resulted in a significant reduction in the body weight of their offspring and administration of T_3 ameliorated to some extend these effects. Also, this study demonstrated that maternal hypothyroidism resulted in decreased serum level of growth hormone in their offspring.

Such results are in agreement with the findings of others who demonstrated that exposure to anti-thyroid agents during gestation to the end of lactation resulted in decrease in body weight of the offspring (2,9,18,26-28). Our results are concordant with those of Morreale et al. (29) who reported that administration of MMI to pregnant rats resulted in the reduction of both plasma and pituitary growth hormone and with those of Wasniewska et al. (30) who reported that THs stimulate secretion of growth hormone and insulin like growth factor-1. Moreover, Ahmed et al. (13) proposed that the THs regulate the growth and development, in part, via their influence on GH secretion and action. Our results showed that the brain weight of rats' offspring was smaller in MMI-induced hypothyroidism compared to euthyroid group and this effect is not reverted after T_3 supplementation. Our findings are consistent with those of Zhang et al. (31) who found that neonatal brain weight was smaller in propylthiouracil-induced hypothyroidism than the control group and compatible with those Hasegawa et al. (2) who documented that perinatal hypothyroidism resulted in irreversible reduction in the brain volume. Results of the present study is at variance with others who demonstrated a significant recovery of the hippocamal volume of rats with transient postnatal hypothyroidism treated with thyroid hormones (32,33). Deregulation of dopaminergic neurons lead to major consequences like Parkinson's disease, depression or schizophrenia (34). Loss of serotonergic neurons in the brain is associated with sudden infant death syndrome (35). The current study showed that MMI-induced maternal hypothyroidism decreased the cerebral neurotransmitter level, dopamine, norepinephrine and serotonin and T_3 supplementation ameliorated this effect.

The results of our study are in concurrence with others (36,37) who demonstrated that neonatal hypothyroidism decreased the concentrations of norepinephrine and dopamine. Vaccari et al (38) suggested that the development of dopaminergic system is delayed in hypothyroid rats and Ahmed et al. (13) found that maternal hypothyroidism significantly decreases the concentrations of neurotransmitters (norepinephrine, dopamine and serotonin) in the brain homogenates of offspring rats.

Our results do not support those of Mano et al. (39) who showed a significant increase in the cerebral cortex norepinephrine and dopamine levels in hypothyroid rats. Whereas our results are more compatible with those of Sidneva and Adamskaia (40) who have shown that thyroidectomy produced no significant changes in the dopamine, noradrenaline or adrenaline concentration. Ito et al. (41) speculated that synthesis, turnover rate and steady levels of serotonin are depressed in the brain of offspring and adult hypothyroid rats.

In humans, serotonin plasmatic level positively correlated to T_3 concentrations, being increased in hyperthyroidism, and decreased in hypothyroidism (42).

Both reports from Cleare et al. (43) and Heal et al. (44) demonstrated that administration of thyroid hormones to animals with induced hypothyroidism and to euthyroid animals caused an increase in the cortical serotonin. Conversely, Henley et al. (45) found an inverse relationship between the circulating thyroid hormone level and serotonin in the brain.

This study revealed that maternal hypothyroidism during pregnancy and lactation resulted in a significant reduction in the cerebral Na^+, K^+-ATPase activity of their offspring and this effect is partially reversed by T_3 supplementation. Na^+, K^+-ATPase activity is essential for uptake, storage, and metabolism of catecholamines (19,46) and of serotonin (47). Thus, decreased cerebral Na^+, K^+-ATPase activity in neonatal hypothyroidism and its reversal by T_3 supplementation explained the changes in the cerebral neurotransmitter levels as a consequence of thyroid hormone deprivation during serious stage of brain development. These results Concur with those of Billimoria et al. (48) and Katyare et al. (49) who reported that neonatal hypothyroidism resulted in a significant reduction in the Na^+, K^+-ATPase activity. Furthermore, Ahmed et al. (13) demonstrated that maternal hypothyroidism decreased the Na^+, K^+-ATPase activity.

In conclusion, T_3 supplementation during the postnatal period through its effect on the cerebral Na^+, K^+-ATPase effectively reversed the effect of maternal methimazole-induced hypothyroidism on the cerebral level of dopamine, norepinephrine and serotonin.

Further studies concerned with combination of adjustable

dose and duration of T_3 are required to verify the best strategy for treatment.

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