Int. J. Devl Neuroscience 28 (2010) 437–454

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones–neurotransmitters and adenosinergic system interactions

O.M. Ahmed [a,*], S.M. Abd El-Tawab [a], R.G. Ahmed [b]

[a] Zoology Department, Division of Physiology, Faculty of Science, Beni Suef University, Beni Suef, Egypt
[b] Zoology Department, Division of Comparative Anatomy and Embryology, Faculty of Science, Beni Suef University, Egypt

ARTICLE INFO

Article history:
Received 24 January 2010
Received in revised form 16 June 2010
Accepted 18 June 2010

Keywords:
Development
Hypothyroidism
Hyperthyroidism
Neurotransmitters
Adenosinergic system

ABSTRACT

The adequate functioning of the maternal thyroid gland plays an important role to ensure that the offspring develop normally. Thus, maternal hypo- and hyperthyroidism are used from the gestation day 1 to lactation day 21, in general, to recognize the alleged association of offspring abnormalities associated with the different thyroid status. In maternal rats during pregnancy and lactation, hypothyroidism in one group was performed by antithyroid drug, methimazole (MMI) that was added in drinking water at concentration 0.02% and hyperthyroidism in the other group was induced by exogenous thyroxine (T4) (from 50 μg to 200 μg/kg body weight) intragastric administration beside adding 0.002% T4 to the drinking water. The hypothyroid and hyperthyroid states in mothers during pregnancy and lactation periods were confirmed by measuring total thyroxine (TT4) and triiodothyronine (TT3) at gestational day 10 and 10 days post-partum, respectively; the effect was more pronounced at the later period than the first. In offspring of control maternal rats, the free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) concentrations were pronouncedly increased as the age progressed from 1 to 3 weeks. In hypothyroid group, a marked decrease in serum FT3, FT4 and GH levels was observed while there was a significant increase in TSH level with age progress as compared with the corresponding control. The reverse pattern to latter state was recorded in hyperthyroid group. The thyroid gland of offspring of hypothyroid group, exhibited some histopathological changes as luminal obliteration of follicles, hyperplasia, fibroblastic proliferation and some degenerative changes throughout the experimental period. The offspring of hyperthyroid rats showed larger and less thyroid follicles with flattened cell lining epithelium, decreased thyroid gland size and some degenerative changes along the experimental period. On the other hand, the biochemical data revealed that in control offspring, the levels of iodothyronine 5′-monodeiodinase (5′-DI), monoamines, γ-aminobutyric acid (GABA), acetylcholinesterase (AchE), ATPase-enzymes (Na^+,K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase) follow a synchronized course of development in all investigated brain regions (cerebrum, cerebellum and medulla oblongata). In addition, the depression in 5′-DI activity, monoamines levels with age progress in all investigated regions, was more pronounced in hypothyroid offspring, while they were increased significantly in hyperthyroid ones in comparison with their respective controls. Conversely, the reverse pattern was recorded in level of the inhibitory transmitter, GABA while there was a disturbance in AchE and ATPases activities in both treated groups along the experimental period in all studied regions. In conclusion, the hypothyroid status during pregnancy and lactation produced inhibitory effects on monoamines, AchE and ATPases and excitatory actions on GABA in different brain regions of the offspring while the hyperthyroid state induced a reverse effect. Thus, the maternal hypothyroidism and hyperthyroidism may cause a number of biochemical disturbances in different brain regions of their offspring and may lead to a pathophysiological state. These alterations were age dependent.

1. Introduction

Several reports have been published on the essential role of the thyroid hormones (THs) for mammalian and non-mammalian brain development (Ahmed et al., 2008; Koibuchi, 2008; Leonard, 2008; Di Paola et al., 2010; Sigrun and Heike, 2010). More importantly, the

* Corresponding author.
E-mail addresses: osamamoha@yahoo.com, r_g_a_ahmed@yahoo.com (O.M. Ahmed).

0736-5748/$36.00 © 2010 ISDN. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijdevneu.2010.06.007

best-defined animal model of thyroid hormone-dependent brain development is the neonatal rat (Legrand, 1986; Schwartz et al., 1997). Probably, the main reason is the interspecies differences in developmental schedules. The rat born with a relatively undeveloped brain and with the thyroid–pituitary-hypothalamic axis not yet fully matured (Oppenheimer and Schwartz, 1997). The rat brain at birth is at the same stage as human brain at 5–6 months of gestation and rat brain at 10 days of postnatal age is equivalent to the human brain at birth (Porterfield and Hendrich, 1993; Ahmed et al., 2008). Thus, the study of the effects of various diseases states on the development of brain and the thyroid–pituitary-hypothalamic axis in the early days after birth in the rat offspring may offer good preclinical data which may be useful and valuable in studying the brain development of human beings.

Besides the crucial role of THs in brain development, recent investigations have highlighted the involvement of these hormones in affecting the characteristics of various neurotransmitter systems and neurotransmission in brain of mammals (Carageorgiou et al., 2005, 2007; Ahmed et al., 2008). The effects of THs imbalances on brain cholinergic neurons are regionally selective (Ahmed et al., 2008). Acetylcholine (Ach) is a very important neurotransmitter for the central nervous system. In cholinergic synapses, acetylcholinesterase (AChE) plays a critical role in the control of acetylcholine hydrolysis during synaptic transmission (Valenzuela et al., 2005). As well, type II deiodinase (D2) activity increases the production of 3,5,3′-triiodothyronine (T3) in the brain and hypophysis through deiodination of thyroxine (T4), and consequently also the local production of serotonin (Kirkegaard and Faber, 1998). Also, Ito et al. (1977) emphasized that the accumulation rates of serotonin and dopamine in hyperthyroidism increased in pons-medulla and mesodiencephalon, respectively while in hypothyroidism, these monoamines decreased in cerebral hemispheres and mesodiencephalon of rats. The effects of hyperthyroidism on the inhibitory neurotransmitter, γ-aminobutyric acid and excitatory amino acid, glutamate levels in brain are more difficult to interpret. In one study, hyperthyroidism induced by intraperitoneal T4 injection resulted in lower GABA and higher glutamate levels in hypothalamus and thalamus (Upadhyaya and Agrawal, 1993) but in another study, there was no effect on whole brain GABA or glutamate levels (Chatterjee et al., 1989). THs are involved in the modulation of the adenosinergic system in the central nervous system (CNS) (Ahmed et al., 2008). The neonatal rat hypothyroidism significantly altered the kinetic properties of Na^+,K^+-ATPase originating from the synaptic membranes (Billimoria et al., 2006). Na^+,K^+-ATPase is essential for Na^+–K^+-pump that helps to maintain the resting membrane potential of neurons and nerve fibres (Kaplan, 2002). In addition, the synaptic plasma membrane Na^+,K^+-ATPase is implicated in metabolic energy production as well as in the uptake, storage, and the metabolism of catecholamines and serotonin (Carageorgiou et al., 2007). Brain Ca^{++}- and Mg^{++}-ATPases which play an important role in regulation of the intracellular and extracellular Ca^{++} and Mg^{++} concentrations, was reported to be affected by THs (Ahmed et al., 2008). The Ca^{++}-ATPase gets impaired in brain due to the decrease in ATP synthesis in the hypothyroid state (Katyare and Rajan, 2005). The low intracellular Ca^{++} concentration inside nerve cells is maintained, in face with high extracellular concentration by various mechanisms including Ca^{++}-ATPase (Mata and Fink, 1989). On the other hand, Mg^{++}-ATPase is another important enzyme implicated in the maintenance of high intracellular Mg^{++} whose changes can control the rates of protein synthesis and cell growth (Sanui and Rubin, 1982; Carageorgiou et al., 2007).

As most of the previous publications deals with the effect of thyroid hormones on the structural development of the brain and neurons, the present study aims to assess and compare the effects of hypothyroidism and hyperthyroidism in pregnant and lactating albino rats on different subsets of different neurotransmitters levels and ATPases activity as well as thyroxine deiodination in various brain regions of their offspring during the course of postnatal development.

2. Materials and methods

2.1. Experimental animals

The present study was carried out on white albino rat (*Rattus norvegicus*); 46 mature virgin females weighting about 170–190 g and 11 mature males. They were obtained from the National Institute of Ophthalmology, Giza, Egypt. The adult rats were kept under observation in the department animal house for 2 weeks to exclude any intercurrent infection and to acclimatize the new conditions. The culled animals were marked, housed in stainless steel separate bottom good aerated cages at normal atmospheric temperature (23 ± 2 °C) and fed on standard rodent pellet diet manufactured by the Egyptian Company for oil and soap as well as some vegetables as a source of vitamins. Tap water was used for drinking *ad libitum* and these animals were maintained normal daily light/dark periods of 12 h each. All animal procedures are in accordance with the general guidelines of animal care and the recommendations of the Canadian Committee for Care and use of animals (Canadian Council on Animal Care, 1993). All efforts were made to minimize the number of animals used and their suffering.

Daily examination of vaginal smear of each virgin 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 proesterous females with male in separate cage at ratio of two females and one male overnight for 1 or 2 consecutive days. In the next morning, the presence of sperm in vaginal smear determined the first day of gestation. Then, the pregnant females were transferred into separate cages from males to start the experiment.

2.2. Experimental schedule

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 allocated into three groups as follows:

- *Hypothyroid group*: Fifteen rats were rendered hypothyroid by administration of antithyroid agent, methimazole (MMI) (Sigma Chemical Company, USA), an inhibitor of triiodothyronine (T3) and thyroxine (T4) synthesis (Ornellas et al., 2003; Hasebe et al., 2008), in drinking water at concentration of 0.02% (weight per volume; w/v) (Venditti et al., 1997) directly after mating (GD 1—LD 21).
- *Hyperthyroid group*: Further fifteen rats were rendered hyperthyroid by exogenous thyroxine (T4) (Eltroxine tablets; GlaxoWellcome, Germany) intragastric administration in increasing doses beginning from 50 μg to reach 200 μg/kg body weight (b. wt.) beside adding 0.002% (w/v) T4 to the drinking water (Guerrero et al., 1999; Abdel-Moneim, 2005; Ahmed, 2006) directly after mating (GD 1—LD 21).
- *Control group*: Sixteen control rats received tap water.

The mother sera (6 per group) were taken during the pregnancy at day 10 and after pregnancy at day 10 to estimate the total triiodothyronine (TT3) and total thyroxine (TT4) in control, hypothyroid and hyperthyroid status. The blood samples were taken from optic vein and centrifuged at 3000 round per minute (rpm) for 30 min (min). The clear, non-hemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at −30 °C until use.

After the pregnancy, the sacrifice of control, hypothyroid and hyperthyroid offspring was done at the end of the 1st, 2nd and 3rd postnatal weeks under mild diethyl ether anaesthesia. The blood samples were taken from jugular vein, centrifuged and kept at −30 °C until use. The sera from offspring were pooled within each litter. On the other hand, the thyroid gland of these offspring was removed immediately after a rapid anaesthesia, dropped into the fixative of choice for general histological structure (haematoxylin and eosin stain; Bancroft and Stevens, 1982). Also, the cerebrum, cerebellum and medulla oblongata were quickly removed, separated and homogenized by using a Teflon homogenizer (Glas-Col, Terre Haute in USA) and kept in deep freezer at −30 °C until use. Then, these regions were divided into two longitudinal equal halves: one half was homogenized in 75% methyl alcohol (99.9% methanol HPLC-grade) and used for monoamines and γ-aminobutyric acid (GABA) determinations. The other half was homogenized at concentration 10% (w/v) in isotonic solution (0.9% NaCl) to be used for the assay of iodothyronine 5′-monodeiodinase (5′-DI) and acetylcholinesterase (AchE).

2.3. The hormonal and biochemical examinations

2.3.1. Estimation of serum T4, T3, TSH and GH levels

Estimation of total thyroxine (TT4), total triiodothyronine (TT3) in sera of mothers and free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) in sera of their offspring were determined in Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive Medicine, New Children Hospital, Faculty of Medicine, Cairo University according to the methods

of Thakur et al. (1997), Maes et al. (1997), Larsen (1982), Smals et al. (1981), Mandel et al. (1993) and Reutens (1995), respectively. The kits for these hormones were obtained from Calbiotech INC (CBI), USA.

2.3.2. Estimation of iodothyronine 5′-monodeiodinase (5′-DI) activity

5′-DI activity, in cerebrum (CR), cerebellum (CB) and medulla oblongata (MO) of rat offspring, was estimated according to the method of Kahl et al. (1987) and Gupta and Kar (1998). The method depends on the incubation of tissue supernatant with exogenous T4 for 1 h in the presence of dithiothreitol (DTT). Also, the T3 liberated as a result of enzyme action was estimated by method of Maes et al. (1997). The kit obtained from Calbiotech INC (CBI), USA.

2.3.3. High performance liquid chromatography (HPLC) analysis

2.3.3.1. Estimation of monoamine (MA) concentrations. The MA concentration was determined according to the method of Pagel et al. (2000). The brain regions (CR, CB and MO) of rat offspring were weighed and homogenized in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenates were spun at 3000 rpm for 10 min and the supernatant was immediately extracted from the trace elements and lipids by the use of solid phase extraction CHROMABOND column NH2 phase Cat. No.730031. The sample was then injected directly to the AQUA column (150 mm × 4.6 mm, 5 μ and C18) which obtained from phenomenex USA under the following conditions: mobile phase 97/3 20 mM potassium phosphate, pH 3.0/methanol, flow rate 1.5 ml/min, UV 270 nm. Additionally, norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5-HT) were separated after 12 min. The resulting chromatogram identifying each monoamines 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 MA was determined from the formula: concentration of MA in sample (μg/g) = concentration of standard (μg/ml) × volume of homogenization/weight of tissue (g) × area of sample under curve/area of standard under curve.

2.3.3.2. Estimation of γ-aminobutyric acid (GABA) concentration (Chakrabarti and Poddar, 1989). GABA concentration was estimated according to the method of Chakrabarti and Poddar (1989). A 10% (w/v) homogenates were prepared in 0.25 M cold sucrose. Protein-free filtrates of brain homogenates were prepared by mixing the homogenates with equal volumes of 10% trichloroacetic acid (TCA). The mixture was centrifuged in cold at 3000 rpm for 15 min. GABA content was measured using sodium tartrate and copper tartrate, respectively, after developing fluorophores by ninhydrin with the protein-free filtrate. The GABA content was calculated from the formula: concentration of GABA in sample (μg/g) = concentration of standard (μg/ml) × volume of homogenization/weight of tissue (g) × area of sample under curve/area of standard under curve.

2.3.4. Estimation of acetylcholinesterase (AchE) activity

The method used in our study was the modified of Ellman's method (Ellman, 1978) using acetyl-thiocholiniodide as substrate. Add 0.1 ml homogenate in the assay system [0.15 ml phosphate buffer (20 mM, pH 7.6) and 0.05 ml substrate (0.1 M acetyl-thiocholiniodide)]. Then, the reaction was stopped by 1.8 ml DTNB (5,5′-dithiobis-2-nitrobenzoic acid) phosphate ethanol reagent [12.4 mg DTNB was dissolved in 120 ml of 96% ethanol, 80 ml distilled water and 50 ml of 0.1 M phosphate buffer (pH 7.6)] after 10 min at 38 °C. The developed color was measured immediately at 412 nm.

2.3.5. Estimation of ATPase (adenosine 5′-triphosphatase) activities

The assay of the enzyme activities (Na^+,K^+-, Ca^{2+}- and Mg^{2+}-ATPase) followed the procedure of Hesketh et al. (1978) and Elekwa et al. (2005) and monitored the inorganic phosphate (Pi) released from adenosine triphosphate (ATP). Enzyme activities were expressed as mg Pi/g.

2.4. Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD analysis to discern the main effects and compare various groups with each other. *F*-probability for each variable expresses the general effect between groups. A two-way analysis of variance was also applied to evaluate the effect of age, treatment and their interaction during the experimental period. The data are presented as mean ± standard error (SE) and values of $P > 0.05$ are considered statistically non-significant while those of $P < 0.05$, $P < 0.01$ and $P < 0.001$ are considered statistically significant, highly significant and very highly significant, respectively.

3. Results

3.1. Histoarchitecture of thyroid glands (Figs. 1–3)

At the end of the 1st postnatal week, the thyroid gland of normal offspring showed normal distribution, morphology and architecture of follicles (Fig. 1A). These follicles with colloid in their luminae vary from irregular rounded to tubular shape and lined with single layer of cuboidal cell lining epithelium. Parafollicular cells were observed between follicles. The offspring of hypothyroid mothers exhibited hypertrophy of the thyroid gland and hyperplasia of its follicles (Fig. 1B_1). Many follicles showed luminal obliteration and some others were devoid of colloid (Fig. 1B_1 and B_3). There were also marked fibroblast proliferations between follicles (Fig. 1B_2), hyperemic blood vessels (Fig. 1B_1 and B_3) and oedema (Fig. 1B_3) at this age. The offspring of hyperthyroid mothers showed a marked atrophy of the thyroid gland and a reduction in the number of its follicles which were observed with more or less cuboidal or flattened cell lining epithelium (Fig. 1C_1 and C_2). Moreover, there were oedema and rich colloid in the follicular luminae (Fig. 1C_1 and C_2). Some follicles contain colloid vacuole (Fig. 1C_1) and others were atrophied (Fig. 1C_2) at this period.

With the age progress to the 2nd week of birth, there was a marked increase in the size of the thyroid gland of normal offspring due to the increase in the size and number of the thyroid follicles associated with a gradual increase in the amount of the colloid till it fills the major part of the follicular luminae (Fig. 2A). In offspring of hypothyroid mothers at the end of 2nd postnatal week, the same previous lesions observed at the end of the 1st postnatal week were found but the follicles appeared with smaller size (Fig. 2B_1–B_3). Furthermore, atrophy in some follicles (Fig. 2B_1–B_3) and haemorrhage (Fig. 2B_3) were also found. In addition to the previous histological perturbations observed in the thyroid of hyperthyroid offspring at the end of the 1st postnatal week, there were marked destructive changes and haemorrhage (Fig. 2C_2), and severe hyperemic blood vessel (Fig. 2C_1 and C_2) at the end of the 2nd postnatal week.

At the end of the 3rd postnatal week, the whole thyroid follicular luminae in the thyroid gland of normal offspring were filled with a homogenous colloid and the glands showing cuboidal lining epithelium of the follicles and their follicles became well developed (Fig. 3A). In the offspring of the hypothyroid mothers at the end of the 3rd week, the previous alterations observed at the end of the 1st and 2nd weeks were more pronounced (Fig. 3B_1–B_3) in addition to the presence of destructive changes (Fig. 3B_2) and irregular follicles (Fig. 3B_3). Notably, many follicles were atrophied at the end of the 3rd postnatal week (Fig. 3B_2). The offspring of hyperthyroid mothers at the end of the 3rd postnatal week exhibited an atrophy of both thyroid gland and its follicles which become more severe as compared to the previous earlier postnatal periods (Fig. 3C_1 and C_2). There were also severe oedema (Fig. 3C_1 and C_2) and degenerative changes (Fig. 3C_2) at end of the 3rd week.

Generally, degenerative sings, in both treated groups, as pyknosis, oedema and destructions in some follicles were observed at the end of the 3rd postnatal week (Fig. 3B_2 and C_2), where the follicles became very irregular and abnormal at this age (Fig. 3B_3 and C_2).

3.2. Biochemical examinations

3.2.1. Serum-hormonal levels (thyroid function) (Tables 1–3)

The present work comprised the disturbance induced in serum-hormonal system of pregnant rats and their offspring in response to administrations of methimazole (MMI) and thyroxine (T4) to mothers from gestation day (GD) 1 to lactation day (LD) 21.

3.2.1.1. Maternal total thyroxine (TT4) and total triiodothyronine (TT3) concentrations (Tables 1 and 3). Table 1 shows higher serum TT4 and TT3 of adult female rats at day 10 post-partum than those at gestational day 10 in control group. Generally, administration of MMI to female rats from GD 1 to LD 21 resulted in a marked decrease (LSD; $P < 0.01$) of serum TT4 and TT3 levels (characteristic of hypothyroidism); at day 10 during the pregnancy, TT4 and TT3 levels were significantly lower (LSD; $P < 0.01$) in hypothyroid rats than in controls and remained lower at day 10 after the birth

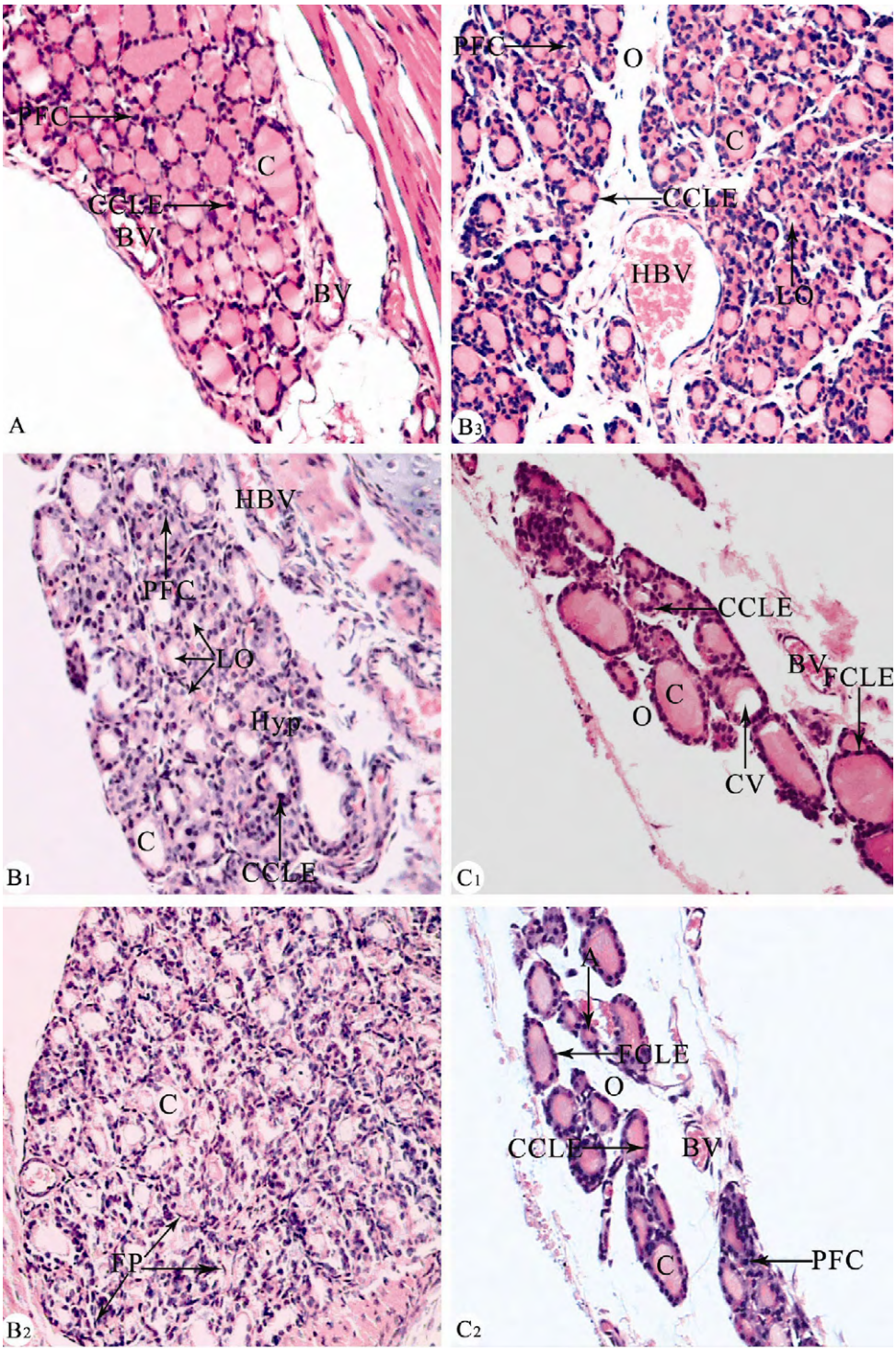

Fig. 1. Sagittal sections in the thyroid gland of rat newborns at the end of the 1st postnatal week in normal (A), hypothyroid (B_1–B_3) and hyperthyroid offspring (C_1 and C_2) (H. & E. stain, 400×). A: Atrophy; BV: blood vessel; C: colloid; CCLE: cuboidal cell lining epithelium; CV: colloid vacuoles; FCLE: flattened cell lining epithelium; FP: fibroblast proliferation; HBV: hyperemic blood vessel; Hyp: hyperplasia; LO: luminal obliteration; O: oedema and PFC: parafollicular cell.

(Table 1). Conversely, the administration of exogenous T4 during the same previous periods exhibited the reverse pattern of changes; serum TT4 and TT3 levels increased significantly (LSD; $P < 0.01$) at day 10 during pregnancy and after birth.

Considering one-way ANOVA analysis of TT4 and TT3, the general effect between groups was very highly significant ($P < 0.001$) throughout the experiment. In addition, two-way analysis of variance of TT4 verified that the effect of age, hyperthyroidism and their interaction was very highly significant and a similar pattern was also observed in hypothyroid group except for the effect of age which was non-significant ($P > 0.05$) (Table 3). On the other hand, the effect of hyperthyroidism on TT3 was very highly significant while the effect of age alone was significant ($P < 0.05$) and their interaction was highly significant ($P < 0.01$). As the effect of hypothyroidism on TT3 was very highly significant, the effect of age and their interaction was highly significant (Table 3).

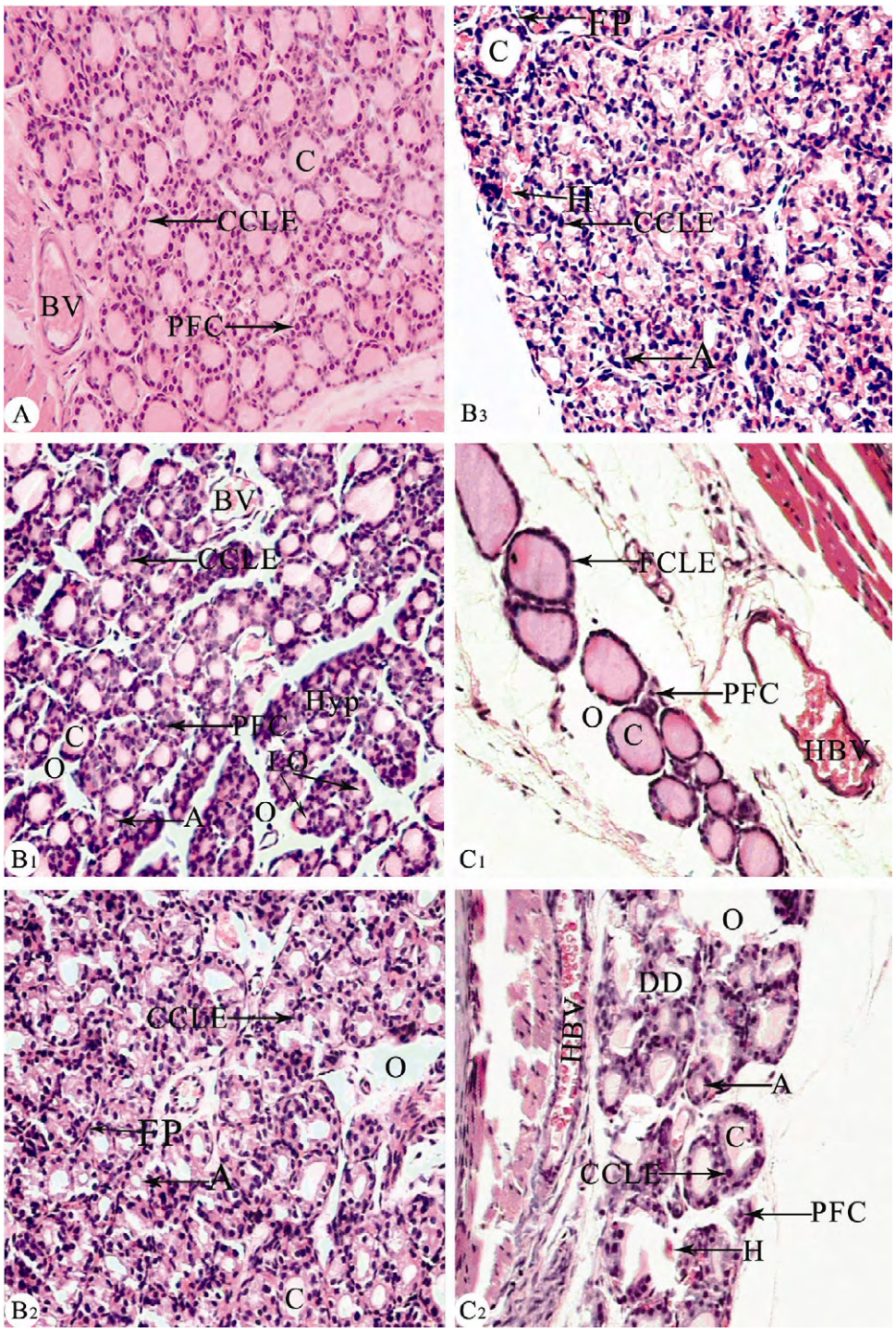

Fig. 2. Sagittal sections in the thyroid gland of rat newborns at the end of the 2nd postnatal week in normal (A), hypothyroid (B_1–B_3) and hyperthyroid offspring (C_1 and C_2) (H. & E. stain, 400×). A: Atrophy; BV: blood vessel; C: colloid; CCLE: cuboidal cell lining epithelium; DD: destructive degeneration; FCLE: flattened cell lining epithelium; FP: fibroblast proliferation; H: haemorrhage; HBV: hyperemic blood vessel; Hyp: hyperplasia; LO: luminal obliteration; O: oedema and PFC: parafollicular cell.

3.2.1.2. Free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) concentrations in offspring (Tables 2 and 3). The effects of thyroid dysfunction, hypo- and hyperthyroidism, on serum FT4, FT3, TSH and GH levels at the end of the 1st, 2nd and 3rd weeks after birth of rat offspring, are allotted in Table 2. In control rat offspring, the concentrations of these parameters were increased with the age progress in all investigated periods. At all testing periods, the baseline levels of serum FT4, FT3 and GH were decreased significantly (LSD; $P < 0.01$) below control values in offspring of hypothyroid mothers whose serum TSH levels were significantly elevated (LSD; $P < 0.01$). However, FT4, FT3 and GH levels in offspring of hyperthyroid mothers were increased significantly (LSD; $P < 0.01$); their serum TSH levels were significantly lower (LSD; $P < 0.01$) as the age progressed from the 1st to 3rd postnatal weeks as compared with the corresponding controls (Table 2). Moreover, at the end of the 3rd week, TSH levels in hyperthyroid group were very low as compared to the levels in the age-matched controls (0.150 ± 0.022 vs. 1.750 ± 0.021).

With regard to one-way ANOVA of FT4, FT3, TSH and GH, the general effect between groups was found to be very highly signifi-

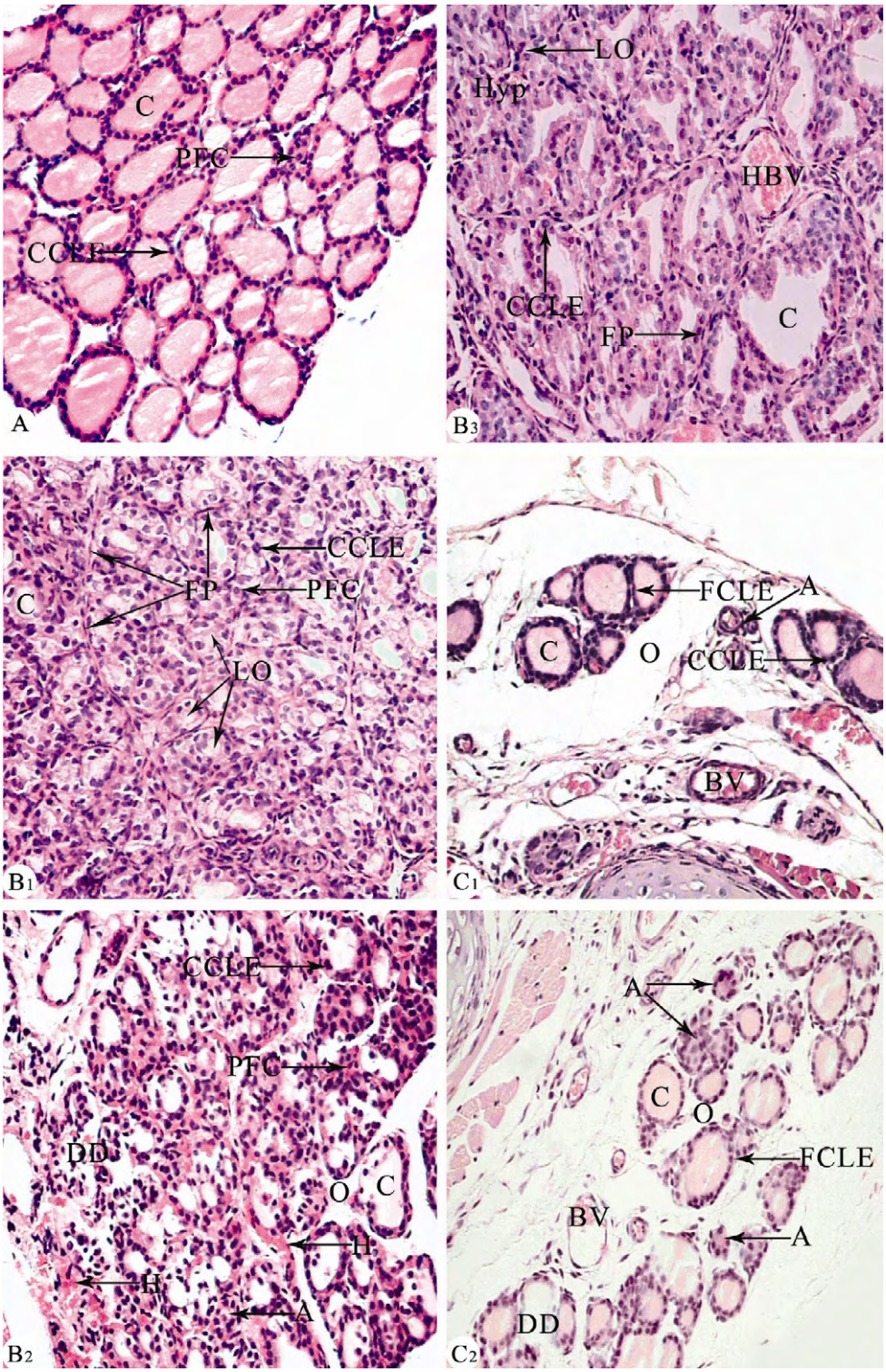

Fig. 3. Sagittal sections in the thyroid gland of rat newborns at the end of the 3rd postnatal week in normal (A), hypothyroid (B_1–B_3) and hyperthyroid newborns (C_1 and C_2) (H. & E. stain, 400×). A: Atrophy; BV: blood vessel; C: colloid; CCLE: cuboidal cell lining epithelium; DD: destructive degeneration; FCLE: flattened cell lining epithelium; FP: fibroblast proliferation; H: haemorrhage; HBV: hyperemic blood vessel; Hyp: hyperplasia; LO: luminal obliteration; O: oedema and PFC: parafollicular cell.

cant ($P < 0.001$) throughout the experiment. Furthermore, two-way analysis of variance recorded that the effect of hypothyroidism and its interaction with age on all previous parameters was very highly significant. The effect of age of hypothyroid offspring alone was significant ($P < 0.05$) on FT3 level while it was very highly significant on FT4 and TSH (Table 3). The effect of age, hypothyroidism and their interaction was very highly significant on GH level. On the other hand, the effect of hyperthyroidism and age was very highly significant on FT4 and FT3 levels but the effect of their interaction was non-significant ($P > 0.05$). Also, the effect of hyperthyroidism and its interaction with age on TSH level was very highly significant and on GH level was non-significant ($P > 0.05$) while the effect of age alone was significant on TSH and very highly significant on GH (Table 3).

Table 1
Effect of thyroid status on total thyroxine (TT4, μg/100 ml) and total triiodothyronine (TT3, ng/100 ml) concentrations in serum of pregnant rats.

Status	Hormones	
	TT4	TT3
At day 10 during pregnancy		
Control	4.495 ± 0.137^{c}	77.501 ± 0.670^{d}
Hypothyroid	2.745 ± 0.039^{d}	31.001 ± 0.894^{e}
Hyperthyroid	5.250 ± 0.068^{b}	90.000 ± 0.894^{b}
At day 10 after post-partum		
Control	5.535 ± 0.060^{b}	87.050 ± 4.896^{b}
Hypothyroid	1.600 ± 0.084^{e}	13.501 ± 0.670^{f}
Hyperthyroid	10.450 ± 0.335^{a}	129.003 ± 5.021^{a}
LSD at 5% level	0.289	1.900
LSD at 1% level	0.389	2.560
F-probability	$P < 0.001$	$P < 0.001$

Data are expressed as mean ± SE. Number of animals in each group is six.
For each parameter, values which share the same superscript symbols are not significantly different.
F-probability expresses the effect between groups, where $P < 0.001$ is very highly significant.

3.2.2. Effects on brain iodothyronine 5′-monodeiodinase (5′-DI) activity (Tables 4 and 5)

The 5′-DI activity of studied brain regions in all experimental groups, control, hypo- and hyperthyroidism, was shown in Table 4. The data showed that the activity of 5′-DI, in all investigated regions of the control offspring, was gradually increased with the age progress during the experimental period. Hypothyroid offspring, at the end of the 1st and 2nd weeks, were associated with a highly significant decrease (LSD; $P < 0.01$) in the 5′-DI yield in all examined brain regions. As the age progressed to the end of the 3rd week, the 5′-DI activity of hypothyroid group was severely decreased (LSD; $P < 0.01$) and this depletion was in the following order: cerebellum > cerebrum > medulla oblongata. The 5′-DI yield, in hyperthyroid offspring, was profoundly increased (LSD; $P < 0.01$) in all examined periods and regions in respect to control group (Table 4).

With regard to one-way ANOVA, the general effect between groups was very highly significant ($P < 0.001$) in all investigated regions (Table 5). Concerning two-way analysis of variance, it was found that the effect of age, hypothyroidism and their interaction was very highly significant in all studied regions and the same trend was also observed in cerebrum and medulla oblongata of hyperthyroid group (Table 5). In cerebellum, while the effect of age and hyperthyroidism was very highly significant, the effect of their interaction was highly significant ($P < 0.01$).

3.2.3. Effects on brain monoamine (MA) concentrations (Tables 4 and 5)

Table 4 reveals that the control values of norepinephrine (NE), epinephrine (E) dopamine (DA) and serotonin (5-HT) were markedly increased in an age-dependent manner in all tested regions to reach maximum values at the end of the 3rd postnatal week. Compared with control offspring, hypothyroid ones showed an enormous decrease in the concentration of NE, E, DA and 5-HT which was more pronounced (LSD; $P < 0.01$) as the period extended

Table 2
Effect of thyroid status on free thyroxine (FT4, ng/100 ml), free triiodothyronine (FT3, pg/100 ml), thyrotropin (TSH, ng/100 ml) and growth hormone (GH, ng/100 ml) concentrations in serum of rat offspring.

Periods	Status	Hormones			
		FT4	FT3	TSH	GH
1 Week	Control	2.801 ± 0.044^{f}	42.501 ± 1.119^{f}	0.951 ± 0.022^{f}	1.750 ± 0.067^{f}
	Hypothyroid	1.950 ± 0.021^{g}	36.504 ± 0.670^{g}	2.451 ± 0.067^{c}	0.950 ± 0.022^{g}
	Hyperthyroid	3.351 ± 0.067^{d}	50.503 ± 0.670^{e}	0.701 ± 0.044^{g}	1.950 ± 0.021^{e}
2 Week	Control	3.159 ± 0.067^{e}	54.500 ± 1.119^{d}	1.350 ± 0.068^{e}	2.551 ± 0.022^{d}
	Hypothyroid	1.450 ± 0.066^{h}	27.008 ± 0.894^{h}	3.751 ± 0.068^{b}	0.750 ± 0.021^{h}
	Hyperthyroid	3.801 ± 0.044^{b}	59.507 ± 0.223^{c}	0.401 ± 0.044^{h}	2.751 ± 0.023^{c}
3 Week	Control	3.550 ± 0.022^{c}	66.001 ± 1.341^{b}	1.750 ± 0.021^{d}	3.301 ± 0.044^{b}
	Hypothyroid	0.551 ± 0.022^{i}	19.000 ± 0.670^{e}	4.051 ± 0.021^{a}	0.501 ± 0.045^{i}
	Hyperthyroid	4.150 ± 0.067^{a}	69.501 ± 0.670^{a}	0.150 ± 0.022^{i}	3.652 ± 0.021^{a}
F-probability		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
LSD at 5% level		0.148	2.492	0.134	0.103
LSD at 1% level		0.198	3.355	0.181	0.139

Data are expressed as mean ± SE. Number of animals in each group is six.
For each variable, values which share the same superscript symbols are not significantly different.
F-probability expresses the effect between groups, where $P < 0.001$ is very highly significant.

Table 3
Two-way analysis of variance (ANOVA) for TT4 and TT3 concentrations in serum of pregnant rats and for FT4, FT3, TSH and GH concentrations in serum of their offspring.

Source of variation	*F*-probability					
	TT4	TT3	FT4	FT3	TSH	GH
Control-hypothyroid						
General effect	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Hypothyroid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Time	$P > 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$
Hypothyroid–time interaction	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Control-hyperthyroid						
General effect	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Hyperthyroid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P > 0.05$
Time	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.001$
Hyperthyroid–time interaction	$P < 0.001$	$P < 0.01$	$P > 0.05$	$P > 0.05$	$P < 0.001$	$P > 0.05$

Where $P > 0.05$ is non-significant, $P < 0.05$ is significant, $P < 0.01$ is highly significant and $P < 0.001$ is very highly significant.

Table 4
Effect of thyroid status on iodothyronine 5′-monodeiodinase (5′-DI, ng/100 mg) activity and norepinephrine (NE, μg/g), epinephrine (E, μg/g), dopamine (DA, μg/g) and serotonin (5-HT, μg/g) concentrations in different brain regions at various postnatal ages of rat offspring.

Postnatal weeks	Status	5′-DI			NE			E		
		CR	CB	MO	CR	CB	MO	CR	CB	MO
1 W	Control	15.009 ± 0.894^{f}	18.500 ± 0.670^{e}	23.502 ± 0.223^{f}	0.256 ± 0.011^{e}	0.197 ± 0.007^{e}	0.341 ± 0.021^{e}	0.172 ± 0.002^{f}	0.274 ± 0.010^{d}	0.165 ± 0.006^{f}
	Hypothyroid	9.010 ± 0.448^{g}	9.002 ± 0.447^{f}	14.001 ± 0.890^{g}	0.151 ± 0.004^{f}	0.129 ± 0.001^{f}	0.204 ± 0.008^{g}	0.116 ± 0.002^{g}	0.110 ± 0.004^{f}	0.116 ± 0.002^{g}
	Hyperthyroid	23.500 ± 0.670^{d}	35.002 ± 0.895^{c}	35.000 ± 0.448^{d}	0.371 ± 0.012^{d}	0.261 ± 0.004^{d}	0.411 ± 0.003^{d}	0.290 ± 0.007^{d}	0.289 ± 0.008^{d}	0.210 ± 0.004^{d}
2 W	Control	21.508 ± 0.670^{e}	27.008 ± 0.448^{d}	30.506 ± 0.224^{e}	0.460 ± 0.009^{c}	0.485 ± 0.008^{c}	0.499 ± 0.006^{c}	0.221 ± 0.012^{e}	0.345 ± 0.010^{c}	0.203 ± 0.002^{d}
	Hypothyroid	6.502 ± 0.223^{h}	5.000 ± 0.449^{g}	9.001 ± 0.448^{h}	0.245 ± 0.020^{e}	0.170 ± 0.009^{e}	0.260 ± 0.009^{f}	0.163 ± 0.013^{f}	0.160 ± 0.002^{e}	0.145 ± 0.003^{f}
	Hyperthyroid	39.504 ± 0.223^{b}	48.006 ± 0.448^{b}	50.50 ± 0.670^{b}	0.586 ± 0.016^{b}	0.612 ± 0.016^{b}	0.661 ± 0.011^{b}	0.471 ± 0.010^{b}	0.505 ± 0.012^{b}	0.309 ± 0.002^{c}
3 W	Control	29.001 ± 0.448^{c}	37.004 ± 0.894^{c}	40.50 ± 0.670^{c}	0.572 ± 0.017^{b}	0.637 ± 0.006^{b}	0.626 ± 0.026^{b}	0.426 ± 0.020^{c}	0.521 ± 0.012^{b}	0.349 ± 0.009^{b}
	Hypothyroid	3.500 ± 0.671^{i}	1.501 ± 0.223^{h}	4.000 ± 0.445^{i}	0.371 ± 0.012^{d}	0.242 ± 0.013^{d}	0.404 ± 0.008^{d}	0.240 ± 0.003^{e}	0.270 ± 0.018^{d}	0.181 ± 0.007^{e}
	Hyperthyroid	48.001 ± 0.894^{a}	60.502 ± 1.566^{a}	63.000 ± 0.448^{a}	0.779 ± 0.006^{a}	0.842 ± 0.019^{a}	0.790 ± 0.010^{a}	0.618 ± 0.001^{a}	0.771 ± 0.008^{a}	0.491 ± 0.003^{a}
LSD at 5% level		1.788	2.227	1.552	0.038	0.030	0.037	0.029	0.029	0.014
LSD at 1% level		2.408	2.999	2.090	0.051	0.041	0.050	0.039	0.040	0.019

Postnatal weeks	Status	DA			5-HT		
		CR	CB	MO	CR	CB	MO
1 W	Control	0.163 ± 0.008^{e}	0.275 ± 0.006^{f}	0.171 ± 0.007^{f}	0.314 ± 0.006^{g}	0.238 ± 0.001^{f}	0.213 ± 0.002^{f}
	Hypothyroid	0.106 ± 0.001^{f}	0.134 ± 0.008^{h}	0.105 ± 0.071^{g}	0.175 ± 0.008^{i}	0.125 ± 0.003^{g}	0.107 ± 0.008^{h}
	Hyperthyroid	0.370 ± 0.007^{c}	0.318 ± 0.004^{e}	0.206 ± 0.002^{e}	0.419 ± 0.013^{e}	0.320 ± 0.011^{e}	0.387 ± 0.006^{c}
2 W	Control	0.281 ± 0.009^{d}	0.408 ± 0.005^{d}	0.248 ± 0.006^{d}	0.465 ± 0.012^{d}	0.385 ± 0.010^{d}	0.325 ± 0.007^{d}
	Hypothyroid	0.160 ± 0.011^{e}	0.201 ± 0.004^{g}	0.164 ± 0.003^{f}	0.264 ± 0.007^{h}	0.209 ± 0.006^{f}	0.170 ± 0.009^{g}
	Hyperthyroid	0.434 ± 0.011^{b}	0.506 ± 0.008^{c}	0.348 ± 0.013^{c}	0.521 ± 0.009^{c}	0.412 ± 0.013^{c}	0.491 ± 0.012^{b}
3 W	Control	0.463 ± 0.009^{b}	0.576 ± 0.009^{b}	0.399 ± 0.010^{b}	0.588 ± 0.013^{b}	0.460 ± 0.012^{b}	0.469 ± 0.009^{b}
	Hypothyroid	0.291 ± 0.011^{d}	0.268 ± 0.004^{f}	0.260 ± 0.012^{d}	0.376 ± 0.011^{f}	0.311 ± 0.014^{e}	0.276 ± 0.006^{e}
	Hyperthyroid	0.595 ± 0.014^{a}	0.663 ± 0.020^{a}	0.507 ± 0.011^{a}	0.667 ± 0.011^{a}	0.571 ± 0.011^{a}	0.592 ± 0.005^{a}
LSD at 5% level		0.028	0.027	0.029	0.030	0.031	0.021
LSD at 1% level		0.036	0.036	0.036	0.041	0.041	0.029

Where CR is cerebrum, CB is cerebellum and MO is medulla oblongata. For each variable, values which share the same superscript symbols are not significantly different.

Table 5
Two-way analysis of variance (ANOVA) for 5′-DI, NE, E, DA, 5-HT, GABA and AchE in different brain regions at various postnatal ages of rat offspring.

Source of variation	*F*-probability																					
	5′-DI			NE			E			DA			5-HT			GABA			AchE		
	CR	CB	MO	CR	CB	MO	CR	CB	MO	CR	CB	MO	CR	CB	MO	CR	CB	MO	CR	CB	MO
Control-hypothyroid																						
General effect	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hypothyroid	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Age	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hypothyroid–age interaction	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.01$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Control-hyperthyroid																						
General effect	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hyperthyroid	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.05$	$P<0.001$
Age	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hyperthyroid–age interaction	$P<0.001$	$P<0.01$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.01$	$P>0.05$	$P<0.01$	$P<0.05$	$P<0.01$	$P<0.05$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P>0.05$	$P<0.05$	$P<0.01$

Where, CR is cerebrum, CB is cerebellum and MO is medulla oblongata. $P>0.05$ is non-significant, $P<0.05$ is significant, $P<0.01$ is highly significant and $P<0.001$ is very highly significant.

to the end of the 3rd postnatal week in all studied regions and the opposite pattern occurred in hyperthyroid group. Notably, the elevation in concentration of E only was non-significant (LSD; $P>0.05$) at the end of the 1st week in cerebellum only of hyperthyroid group (Table 4). Also, the increase of DA in hyperthyroid group was significant (LSD; $P<0.05$) at the end of the 1st week in medulla oblongata only while this increase was highly significant (LSD; $P<0.01$) in other regions. Also, at the end of the 2nd and 3rd weeks, the DA level of hyperthyroid group was significantly increased (LSD; $P<0.01$) in all tested regions. Also, in hyperthyroid group, the elevation in concentration of 5-HT was highly significant (LSD; $P<0.01$) in all examined periods and regions except in cerebellum where this elevation was non-significant (LSD; $P>0.05$) at the end of the 2nd week only in respect to control group. In hyperthyroid group, the DA and 5-HT levels showed their highest profile (LSD; $P<0.01$) at the end of the 3rd postnatal week in all investigated regions (Table 4).

With regard to one-way ANOVA of NE, E, DA and 5-HT, the general effect between groups was very highly significant ($P<0.001$) in all investigated regions (Table 5).

Two-way analysis of variance verified that the effect of age, hypothyroidism (or hyperthyroidism) and their interaction was very highly significant ($P<0.001$) in cerebrum and cerebellum for NE and in all examined brain regions for E. Also, in medulla oblongata, the effect of hypothyroidism (or hyperthyroidism) and age in NE was very highly significant but their interaction was highly significant ($P<0.01$) (Table 5).

In addition, two-way analysis of variance of DA verified that in all tested regions, the effect of age, hypothyroidism and their interaction was very highly significant ($P<0.001$). The effect of age and hyperthyroidism was very highly significant in all investigated regions (Table 5). Also, the effect of age and hyperthyroid interaction was highly significant ($P<0.01$) in cerebrum and medulla oblongata, however the interaction was non-significant ($P>0.05$) in cerebellum.

Concerning two-way analysis of variance of 5-HT, it was established that in all investigated regions, the effect of age and hypothyroidism (or hyperthyroidism) was very highly significant ($P<0.001$). The effect of age and hypothyroidism interaction was very highly significant in medulla oblongata while it was significant ($P<0.05$) in cerebrum and cerebellum. On the other hand, the effect of age and hyperthyroidism interaction was non-significant ($P>0.05$) in cerebrum and medulla oblongata but the effect of their interaction was highly significant ($P<0.01$) in cerebellum (Table 5).

3.2.4. γ-Aminobutyric acid (GABA) concentration (Fig. 4 and Table 5)

Table 4 shows that the concentration of GABA of control rat offspring exhibited a stepwisely increase with the age progress in all tested regions. On the other hand, an increase (LSD; $P<0.01$) in the level of GABA was detected in all investigated regions and ages of hypothyroid group. Contrary, a marked drop (LSD; $P<0.01$) in the concentration of GABA in hyperthyroid group was noticed in all studied regions and periods. This drop continued quite regularly to reach its lowest mean level (LSD; $P<0.01$) at the end of the 3rd postnatal week in all tested regions (Fig. 5).

With regard to one-way ANOVA, the general effect between groups was very highly significant ($P<0.001$) in all investigated regions (Table 5). Two-way analysis of variance recorded that in all studied regions, the effect of age, hypothyroidism and their interaction was very highly significant and the same pattern of probabilities was recorded in control-hyperthyroid effect (Table 5).

3.2.5. Effects on acetylcholinesterase (AchE) activity (Fig. 5 and Table 5)

Data regarding the effects of maternal hypo- and hyperthyroidism on AchE activity of their offspring with the age progress

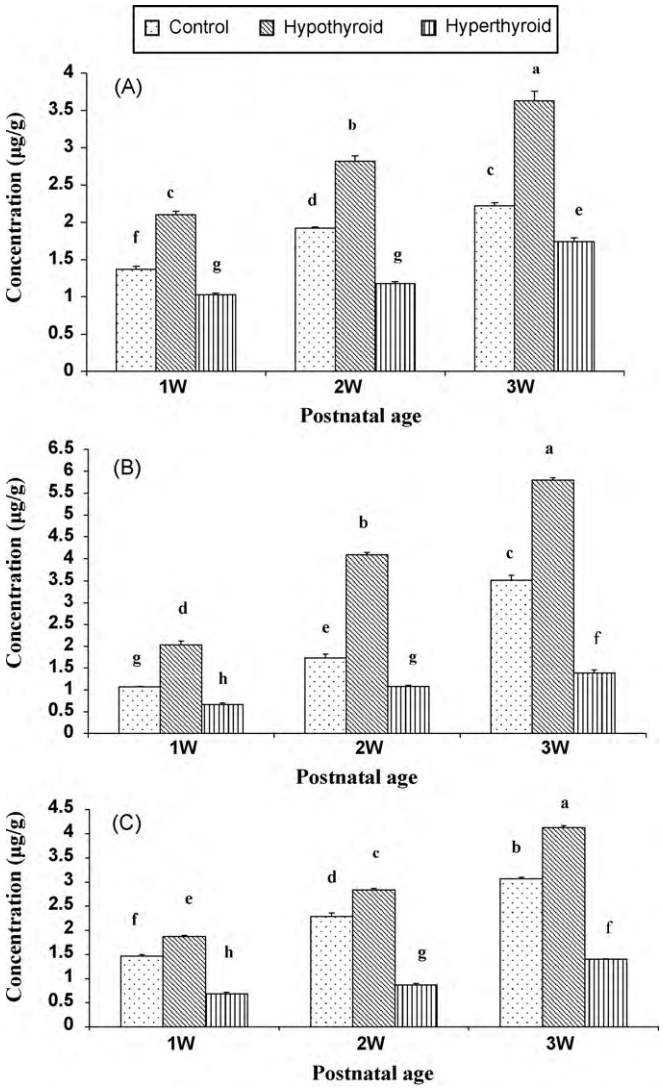

Fig. 4. Effect of thyroid status on GABA concentration in cerebrum (A), cerebellum (B) and medulla oblongata (C) of rat offspring (W: week). A (LSD at 5%: 0.168; LSD at 1%: 0.224); B (LSD at 5%: 0.199; LSD at 1%: 0.268); C (LSD at 5%: 0.102; LSD at 1%: 0.138). Bars, which share the same symbol(s), are not significantly different.

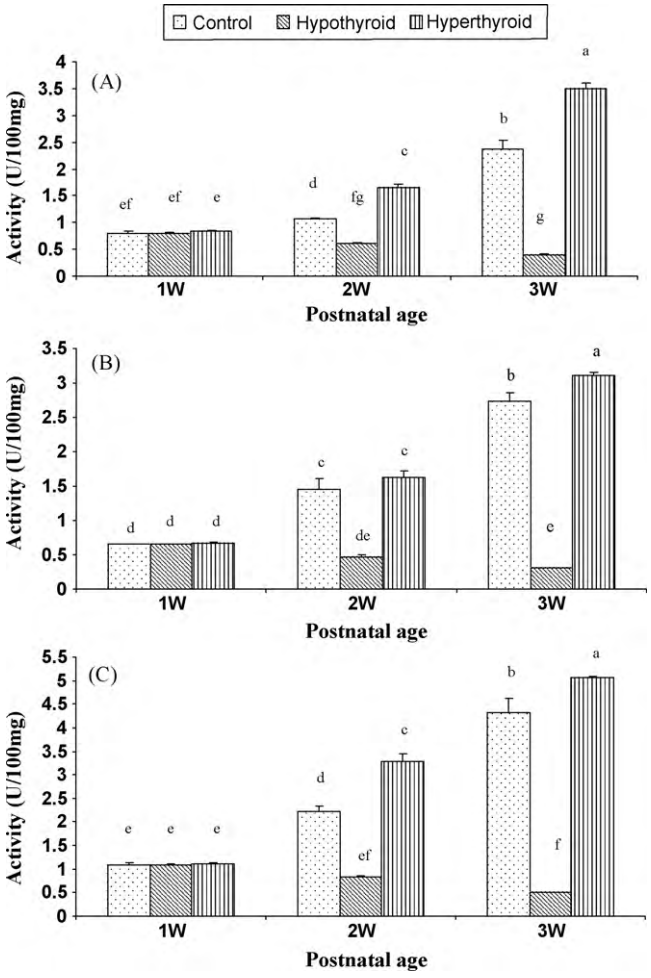

Fig. 5. Effect of thyroid status on AchE activity in cerebrum (A), cerebellum (B) and medulla oblongata (C) of rat offspring. A (LSD at 5%: 0.213; LSD at 1%: 0.287); B (LSD at 5%: 0.223; LSD at 1%: 0.301); C (LSD at 5%: 0.351; LSD at 1%: 0.472).

during the tested periods are represented in Fig. 5. The control values of this enzyme were markedly increased in an age-dependent manner in all investigated brain regions to reach maximum values at the end of the 3rd week after birth. This behavioral pattern was disrupted as a result of hypo- or hyperthyroidism. At the end of the 1st postnatal week, the AchE level, in hypo- and hyperthyroid offspring, tended to be nearby those of control values in all tested regions. Contrary to controls, during the 2nd and 3rd weeks, AchE level was severely depressed (LSD; $P < 0.01$) in hypothyroid offspring in all studied regions. In cerebrum and medulla oblongata, while AchE level of hyperthyroid offspring, at the end of the 2nd week, was increased significantly (LSD; $P < 0.01$) in comparison with their respective controls, the increase in its level in cerebellum was non-significant (LSD; $P > 0.05$). At the end of the 3rd week, in hyperthyroid group, the AchE level was enormously increased (LSD; $P < 0.01$) in all studied regions in comparison with the corresponding controls (Fig. 5).

Considering one-way ANOVA, it was demonstrated that the general effect between groups was very highly significant ($P < 0.001$) in all investigated regions (Table 5). Moreover, two-way analysis of variance revealed that in all tested regions, the effect of age, hypothyroidism and their interaction was very highly significant and the same pattern of probabilities occurred in cerebrum concerning control-hyperthyroid effect (Table 5). Also, in cerebellum, the effect of age, hyperthyroidism and their interaction was very highly significant ($P < 0.001$), significant ($P < 0.05$) and non-significant ($P > 0.05$), respectively. In medulla oblongata, the effect of either age or hyperthyroidism was very highly significant though the effect of their interaction was highly significant ($P < 0.01$) (Table 5).

3.2.6. *ATPase (adenosine 5′-triphosphatase) activities (Figs. 6, 7 and 8 and Table 6)*

3.2.6.1. Sodium–potassium adenosine 5′-triphosphatase (Na^+,K^+-ATPase) activity. The control values of Na^+,K^+-ATPase were stepwisely increased in all investigated regions with the age progress (Fig. 6). In hypothyroid group, while a decrease in the Na^+,K^+-ATPase activity was highly significant (LSD; $P < 0.01$) in medulla oblongata only at the end of the 1st week and in all studied regions at the end of the 2nd and 3rd weeks, this decrease was non-significant (LSD; $P > 0.05$) in cerebrum and cerebellum at the end of the 1st week as compared to control one. On the other hand, in hyperthyroid group, Na^+,K^+-ATPase activity was significantly increased (LSD; $P < 0.05$) in cerebrum only at the end of the 1st and 3rd weeks while this activity was highly significantly increased (LSD; $P < 0.01$) at the end of the 2nd week of the latter region and at all tested ages in other tested regions in comparison with con-

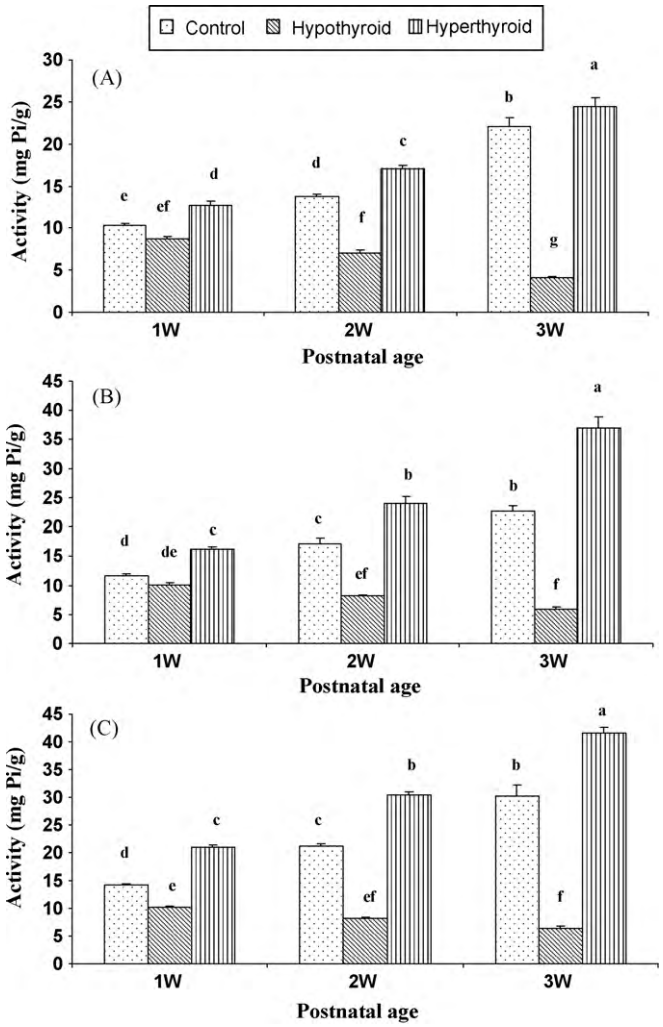

Fig. 6. Effect of thyroid status on Na^+–K^+-ATPase activity in cerebrum (A), cerebellum (B) and medulla oblongata (C) of rat offspring. A (LSD at 5%: 1.887; LSD at 1%: 2.542); B (LSD at 5%: 2.488; LSD at 1%: 3.351); C (LSD at 5%: 2.393; LSD at 1%: 3.223).

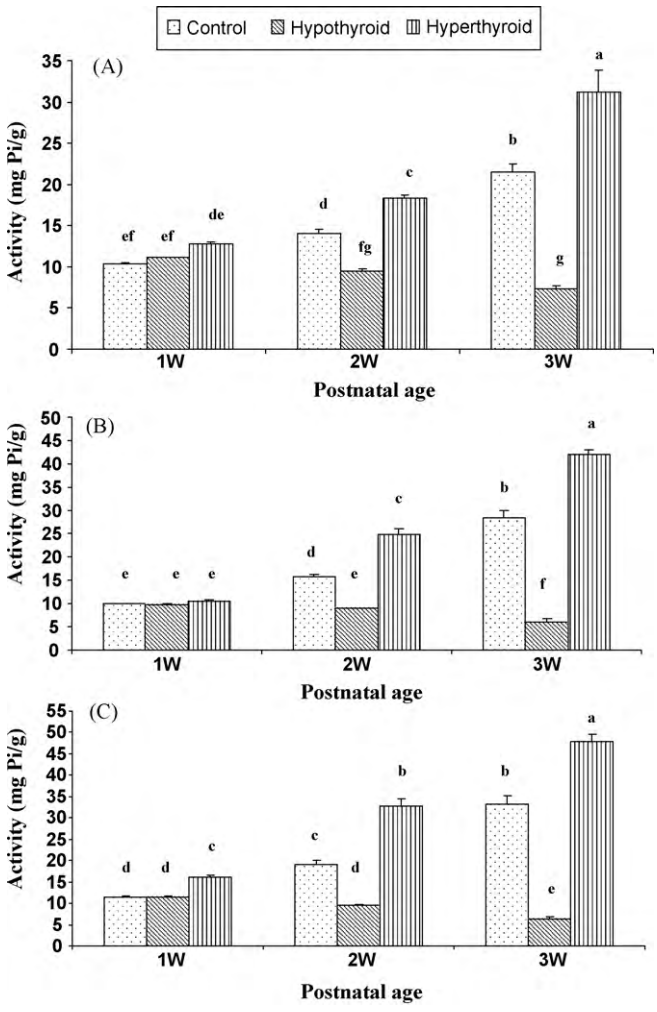

Fig. 7. Effect of thyroid status on Ca^{2+}-ATPase activity in cerebrum (A), cerebellum (B) and medulla oblongata (C) of rat offspring. A (LSD at 5%: 1.788; LSD at 1%: 2.408); B (LSD at 5%: 2.885; LSD at 1%: 3.885); C (LSD at 5%: 2.419; LSD at 1%: 3.259).

trol values. Notably, at the end of the 3rd week of hypothyroid offspring, Na^+,K^+-ATPase activity exhibited about 5-folds decrease in cerebrum and medulla oblongata and about 4-folds decrease in cerebellum when compared to control values (Fig. 6).

One-way ANOVA analysis recorded that the general effect between groups was very highly significant ($P < 0.001$) (Table 6) in all investigated regions. In addition, two-way analysis of variance depicted that in all studied regions, the effect of age, hypothyroidism and their interaction was very highly significant and a similar pattern of probabilities was attained in cerebellum concerning control-hyperthyroid effect (Table 6). In cerebrum and medulla oblongata, the effect of hyperthyroidism and age was very highly significant although their interaction was non-significant ($P > 0.05$).

3.2.6.2. Calcium adenosine 5′-triphosphatase (Ca^{2+}-ATPase) activity. The data presented in Fig. 7 demonstrated a gradual increase of Ca^{2+}-ATPase activity in the control offspring in all tested regions to reach its maximum values at the end of the 3rd week after birth. Being compared to control offspring, no significant differences (LSD; $P > 0.05$) in the activity of Ca^{2+}-ATPase were found at the end of the 1st week of hypothyroid group in all investigated regions and in cerebellum only of hyperthyroid ones at this age. Interestingly, in all tested regions, this activity was significantly lower (LSD; $P < 0.01$) in hypothyroid offspring than in controls at the end of the 2nd week and remained very lower at the end of the 3rd week (Fig. 7). On the other hand, the hyperthyroid offspring had a higher level (LSD; $P < 0.01$) of Ca^{2+}-ATPase than control in cerebrum and medulla oblongata only at the end of the 1st week and in all studied regions at the end of the 2nd and 3rd weeks (Fig. 7).

Regarding one-way ANOVA, the general effect between groups was very highly significant ($P < 0.001$) in all tested regions (Table 6). Moreover, two-way analysis of variance revealed that in all investigated regions, the effect of age, hypothyroidism and their interaction was very highly significant and the same pattern of probabilities was achieved in cerebellum and medulla oblongata concerning control-hyperthyroid effect (Table 6). On the other hand, in cerebrum, the effect of hyperthyroidism and age was very highly significant although their interaction was only significant ($P < 0.05$).

3.2.6.3. Magnesium adenosine 5′-triphosphatase (Mg^{2+}-ATPase) activity. The data showing the effects of maternal hypo- and hyperthyroidism on Mg^{2+}-ATPase activity of their offspring with the age progress are demonstrated in Fig. 8. The results of the control rat offspring indicated a gradual increase in Mg^{2+}-ATPase activity in all investigated regions with development (during the first 3 postnatal weeks).

At the end of the 1st week, as the Mg^{2+}-ATPase activity of hypothyroid group was significantly increased (LSD; $P < 0.05$) in

Table 6
Two-way analysis of variance (ANOVA) for Na^+–K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in cerebrum, cerebellum and medulla oblongata their offspring.

Source of variation	*F*-probability								
	Na^+–K^+-ATPase			Ca^{2+}-ATPase			Mg^{2+}-ATPase		
	CR	CB	MO	CR	CB	MO	CR	CB	MO
Control-hypothyroid effect									
General effect	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hypothyroid	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Age	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hypothyroid–age interaction	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Control-hyperthyroid effect									
General effect	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hyperthyroid	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Age	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Hyperthyroid–age interaction	$P>0.05$	$P<0.001$	$P>0.05$	$P<0.05$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$

Where $P>0.05$ is non-significant, $P<0.05$ is significant, $P<0.01$ is highly significant and $P<0.001$ is very highly significant.

the cerebrum only, a decrease occurred in other tested ages and this decrease (LSD; $P<0.01$) was more announced as the age progressed to the end of the 3rd week. Furthermore, it is apparent from Table 4 that no significant differences (LSD; $P>0.05$) in cerebellum and medulla oblongata were noticed between control and hypothyroid groups at the end of the 1st and 2nd weeks. However, as development proceeded to the 3rd week, hypothyroid offspring had lower values (LSD; $P<0.01$) in cerebellum and medulla oblongata as compared to the corresponding control ones. In contrast, the Mg^{2+}-ATPase activity of hyperthyroid group increased significantly (LSD; $P<0.01$) in all investigated regions and ages (Fig. 8).

Regarding one-way ANOVA, the general effect between groups was very highly significant ($P<0.001$) in all tested regions (Table 6). Two-way analysis of variance verified that the effect of age, hypothyroidism (or hyperthyroidism) and their interaction, in all tested regions, was very highly significant (Table 6).

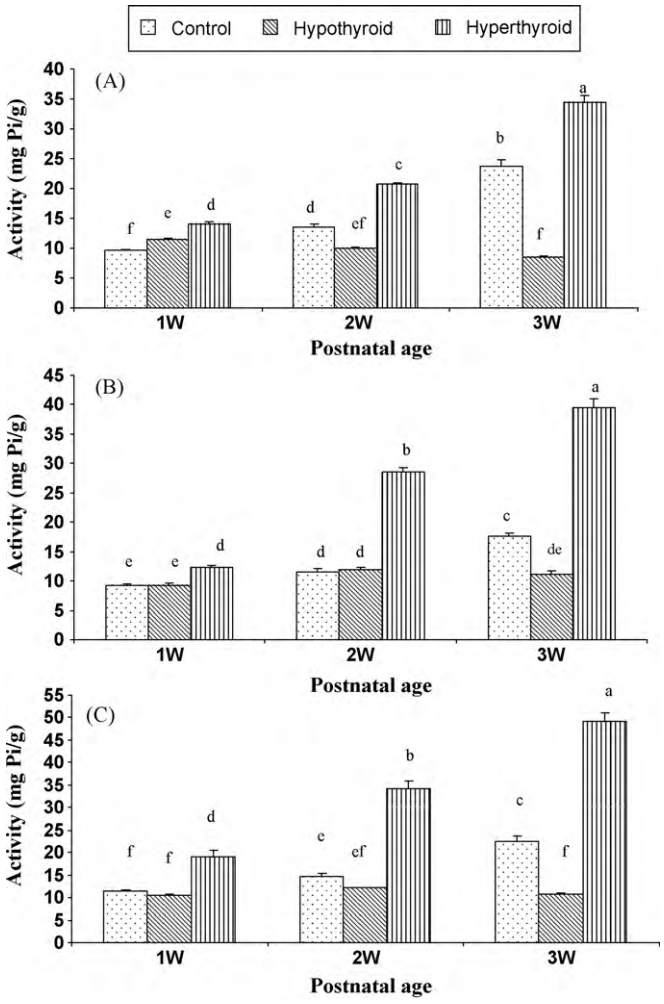

Fig. 8. Effect of thyroid status on Mg^{2+}-ATPase activity in cerebrum (A), cerebellum (B) and medulla oblongata (C) of rat offspring. A (LSD at 5%: 1.758; LSD at 1%: 2.368); B (LSD at 5%: 2.088; LSD at 1%: 2.812); C (LSD at 5%: 3.038; LSD at 1%: 4.091).

4. Discussion

Because of thyroid hormones (THs) potentially influences brain development postnatally in the rat (Ahmed et al., 2008), the current study highlights the alterations in brain development during the early postnatal period in rat offspring of control, hypothyroid and hyperthyroid mothers. This study assesses the effects of maternal hypo- and hyperthyroidism on the histoarchitecture and function of thyroid gland and various biochemical parameters in different brain regions (cerebrum, cerebellum and medulla oblongata) of rat offspring at the end of the 1st, 2nd and 3rd postnatal weeks.

The present study revealed that administration of methimazole (MMI) in drinking water (0.02%, w/v) to adult female rats during pregnancy and weaning periods induced hypothyroidism in mothers and their offspring as indicated by decrease in serum total thyroxine (TT4) and total triiodothyronine (TT3) levels in mothers and free thyroxine (FT4) and free triiodothyronine (FT3) levels in offspring. This result coincides with several studies. Neonatal rats receiving the antithyroid drug as MMI that crosses the placenta and in their mother's milk, are rendered hypothyroid (MacNabb et al., 2000; Cristovao et al., 2002; Ramos et al., 2002; Mookadam et al., 2004; Hasebe et al., 2008). The mechanism by which MMI exerts hypothyroidism was explained by Awad (2002) and Ahmed et al. (2008) who reported that MMI interferes with incorporation of iodine into tyrosyl residues of thyroglobulin (TG) and inhibits the coupling of iodotyrosyl residues to form iodothyronine, thus inhibiting the synthesis of THs.

In our study, the administration of T4 to adult female rats in drinking water at 0.002% (w/v) beside gastric intubation of 50–200 μg/kg body weight during pregnancy and weaning periods induced a marked hyperthyroidism in mothers and their offspring. This hyperthyroidism was assured by elevated levels of serum TT3 and TT4 in mothers and FT3 and FT4 in their offspring. Similarly to the MMI, THs from mothers may cross to the fetus through placenta or may pass in mother's milk to the neonates (Higuchi et al., 2005). Taking the previous information about the two experimental models together, it can be concluded that the mother's thyroid state during pregnancy and lactation periods affects the thyroid status of their offspring. This suggestion was supported by

other publications (Varas et al., 2002; Awad, 2002; Ahmed et al., 2008).

The thyroid gland of control rat offspring exhibited gradual increases in the size and number of the follicles. The whole size of thyroid lobe was gradually increased with the age progress. The new thyroid follicles were formed by a process of extracellular budding from the parent ones and mitotic cell division (Ahmed et al., 2008). The increase in the size of the thyroid lobe with proceeding offspring age was attributed to the increase in the thyroid interreticular tissue, stroma and its vascularity in addition to the increase in the number and size of thyroid follicles as revealed in rat (Saleh et al., 1986) and mice (Hisao et al., 1980). In accordance with our results, it was reported that rats are born with a less developed thyroid system (Jahnke et al., 2004) and the full maturation of thyroid system function is complete by 4 weeks after birth (Fisher and Klein, 1981). Nearly, the same thought was supposed in opossum (*Didelphis virginana*) by Krause and Cutts (1983) who demonstrated that as development progresses, the follicles increase in size and number where thyroid gland shows its adult features at 35 days after birth.

In view of thyroid function of control rat offspring, the current study revealed gradual increases of serum FT4, FT3 and thyrotropin (TSH) levels at the end of the 1st, 2nd and 3rd postnatal weeks. Also, growth hormone (GH) was markedly elevated in an age-dependent manner. These results are concomitant with those of Obregón et al. (1984) and Morreale de Escobar et al. (1985) who stated that after the fetal thyroid gland starts secreting THs, the fetal T4 and T3 pool as well as the circulating T4 level increased steadily until the thyroid gland is completely developed. With regard to the control maternal THs, in the present study, their serum levels were lower during the pregnancy at gestation day 10 than those at day 10 post-partum. This state may reflect the higher transfer of THs from pregnant females to their fetuses during pregnancy and/or more efficiency of thyroid gland to secret THs after birth. The steady increase in serum FT4 and FT3 levels in offspring with the age progress, in the present study, may reflect the histological changes in the thyroid gland which showed a marked and gradual increase in the size and number of thyroid follicles. The gradual increase of TSH is necessary for the development and growth of thyroid gland during this sensitive period because it increases the size of the follicles and it increases the rate of synthesis, secretion and iodination of glycoprotein into colloid, the rate of breakdown of thyroglobulin (TG) and the liberation of THs into circulation (Ahmed et al., 2008). Furthermore, it was reported that the maturation of the pituitary–thyroid axis is intrinsically controlled by gestational age rather than by serum thyroid hormone levels (Hashimoto et al., 1991a). Alternatively, THs, through their nuclear receptor, play a crucial role in regulating differentiation, growth, and metabolism in higher organisms (DeVito et al., 1999; Ahmed et al., 2008). These hormones are also a necessary component for the physiological growth of a young organism, stimulating the secretion of GH and insulin-like growth factor (Wasniewska et al., 2003). In addition, GH is a key factor controlling postnatal growth and development (Zhou et al., 2005; Wong et al., 2006). In turn, these observations imply that the THs may regulate the growth and development, in part, via their influence on GH.

The thyroid gland of rat offspring of hypothyroid dams showed luminal obliteration, hypertrophy, hyperplasia, interfollicular fibroblast proliferation, decrease or absence of colloid in follicular luminae, hyperemic blood vessel, oedema, destruction in some follicles and haemorrhage. These changes were more pronounced with the age progress from postnatal day 1 to 21. These histopathological alterations were associated with a tremendous decrease in serum FT4 and FT3 and elevation of TSH levels as well as a potential decrease in serum GH level in an age-dependent manner. MMI is a potent reversible antithyroid drug which acts by inhibiting the incorporation of iodine into the thyroid hormone precursor protein TG (Cooper, 1984). The inhibition of THs synthesis results in the depletion of stores of iodinated TG leading to decrease or absence of colloid in the thyroid follicles later on (Awad, 2002). Also, in accordance with our study, Potter et al. (1982) found that in sheep, the fetal hypothyroidism due to iodine deficiency showed thyroid hyperplasia at gestational day 70. Shibutani et al. (2009) reported that hypothyroidism by 3 or 12 ppm of 6-propyl-2-thiouracil or 200 ppm of MMI caused thyroid follicular cell hypertrophy. York et al. (2004) revealed that male rat pups administrated, in drinking water, ammonium perchlorate, which blocks iodine uptake into thyroid gland, induced hypertrophy and hyperplasia of the thyroid follicles as well as decrease in the thyroid follicle size. The sustained or continuous elevation of circulating TSH, in the present study, due to absence negative feedback by the THs levels leads to increase in the follicular cell size and their dispersion leading to obliteration of follicular luminae. Concomitant with the present results, Morreale de Escobar et al. (1993) emphasized that both plasma and pituitary GH decreased in hypothyroid fetuses from MMI-treated pregnant rats while their plasma TSH was elevated. Thus, it is worth mentioning that maternal THs deficiency may disturb the secretion of other pituitary hormones in their offspring (Tamasy et al., 1984).

On the other hand, the thyroid gland of neonates born from exogenous thyroxine-induced hyperthyroid mothers exhibited profound thyroatrophy, decrease in the size and number of follicles with flattened cell lining epithelium, colloid vacuoles, oedema, hyperemic blood vessel, haemorrhage, dilated blood vessel and deformation in some follicles. These deleterious changes were more pronounced with the age progress from postnatal day 1 to 21. These histopathological perturbations were associated with increases in serum FT4 and FT3 levels, gradual decrease in TSH level and gradual increase in GH level in an age-dependent manner. In accordance with our study, Hashimoto et al. (1995) and Higuchi et al. (2001) hypothesized that maternal hyperthyroidism during pregnancy leads to a hyperthyroid state in fetus and neonates. These authors attributed the state of hyperthyroidism in fetuses or early neonates to passive transfer of maternal T4 from a mother with hyperthyroidism or thyrotoxicosis through placenta and in mother's milk. The decrease in serum TSH level is attributed to negative feedback effect of the excess circulating THs levels on the anterior lobe of pituitary gland. The lowering in serum TSH level, in the current study, may play a crucial role in the atrophy of the thyroid gland due to loss of stimulatory effect of this hormone on the gland. These suggestions are supported by Fisher et al. (2000) and Higuchi et al. (2005) who reported that the exposure of the fetal hypothalamic-pituitary–thyroid system to a higher-than-control thyroid hormone (T4) concentration might impair its physiologic maturation, because there is a continuous significant decrease in the TSH/fetal T4 ratio during the development. The lowered TSH level, in hyperthyroid offspring, results in decreased colloid formation from the follicular cells (Pyer et al., 1981). This explains the lack of colloid in the luminae of thyroid gland in this experimental model of hyperthyroidism. Furthermore, neonatal rat hyperthyroidism (Varma and Crawford, 1979) results in permanent imprinting regarding growth and thyroidal development. Also, Segni and Gorman (2001) speculated that untreated childhood thyrotoxicosis causes accelerated growth. Generally, it is observed from the above mentioned results that a transient and moderate deficiency or increase of maternal THs can have deleterious consequences on thyroid function of both mothers and their offspring.

It was found that the activity of iodothyronine 5′-monodeiodinase (5′-DI) of control rat offspring was increased tremendously in all investigated brain regions (cerebrum, cerebellum and medulla oblongata) from the 1st to 3rd week after birth. As

suggested by Silva and Matthews (1984), the local 5′-deiodination of serum T4 is the main source of T3 for the brain of rat. The latter authors added that the production of T3 by developing brain is a very active process in agreement with the need of THs during this period. Also, Takeuchia et al. (2006) found that type I deiodinase (D1), in rodents, plays a major role in maintaining circulating T3 levels.

The depression in 5′-DI activity, with the age progress in all studied regions, was more pronounced in hypothyroid offspring while this activity in hyperthyroid ones was increased significantly in comparison with their respective controls. Pregnant rats were rendered hypothyroid by the treatment with n-propylthiouracil which blocks thyroid hormone synthesis by inhibiting the iodination of thyroglobulin and by decreasing the activity of deiodinase D1 (Sigrun and Heike, 2010). In 1981, Kaplan et al. confirmed that the deiodination rates of T3 tyrosyl ring were significantly lower in homogenates of hypothyroid tissue than in homogenates of control tissue for all CNS regions of rat except the spinal cord. In addition, Kaplan (1986) and Sharifi and St Germain (1992) found that D1 decreased in hypothyroidism and increased in hyperthyroidism in animal tissues. In addition, a small reduction in circulating levels of T4 in the dam alters cortical neuronal migration in animals (Ausó et al., 2004; Kester et al., 2004). On the other hand, several investigators attributed that D1's homeostatic function may be due to siphon T4 away from the type II deiodinase (D2) pathway, therefore providing some degree of protection against the development of hyperthyroidism when serum T4 concentrations increase (Bianco et al., 2002; Bianco and Larsen, 2005; Köhrle et al., 2005; Kuiper et al., 2005; Bianco and Kim, 2006). Adding to the complexity of these studies, it is worth mentioning that the changes in serum T3 level in both treated groups may arise from alterations in the monodeiodination pathway of T4. Thus, we conclude that a change in maternal T4 concentration in both treated groups, together with the transport of T4 to the feto-placental compartment, may cause indirectly some alterations in the availability of T3 in the brain of their offspring.

Biochemically, the control concentrations of monoamines [norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5-HT)] were increased tremendously in all studied brain regions (cerebrum, cerebellum and medulla oblongata) from the 1st to the 3rd postnatal weeks. Ahmed (2004) and Ahmed et al. (2007) recorded that the normal monoamines (NE, E, DA and 5-HT) contents were significantly and gradually increased with the age progress between postnatal days 7 and 21 in cerebrum, cerebellum and medulla oblongata of rat offspring. THs also regulate the development of monoaminergic neurotransmission system (Aszalós, 2007; Ahmed et al., 2008). Generally, the gradual increase in control monoamines in all investigated regions in our study may be due to the increase in THs with the age progress.

Our results showed marked decreases of monoamines (NE, E, DA and 5-HT) concentrations in all examined regions of hypothyroid group along the duration of the experiment. These results are in concurrence with many other publications. Singhal et al. (1975) and Puymirat (1985) revealed that the neonatal hypothyroidism induced either by ^{131}I or by an antithyroid drug as MMI decreases the concentrations of NE and DA. Furthermore, Ito et al. (1977) speculated that the accumulation rates of 5-HT and DA decreased in cerebral hemispheres and mesodiencephalon of hypothyroid rat. Thus, the present study supports the suggestion that the development of dopaminergic system is delayed in hypothyroid rat (Vaccari et al., 1990). It was also reported that the synthesis, turnover rate and steady levels of 5-HT are reportedly depressed in the brain of offspring and adult hypothyroid rats (Ito et al., 1977). Indeed, if there were a decrease in brain 5-HT levels, it would produce an increase in brain thyroid releasing hormone (TRH), which can consequently stimulate the secretion of TSH (Morley, 1981). More recently, Ahmed et al. (2008) inferred that the thyroid maldevelopment may cause a disturbance in the synthesis and release of catecholamine (CA).

Hyperthyroid group exhibited a marked increase in the monoamine levels (NE, E, DA and 5-HT) during the experimental period in all examined regions. These findings are in harmony with several publications. Jacoby et al. (1975) found that experimental hyperthyroidism in rats (15 μg T4/100 g/b. wt. for 25 days) accelerated the accumulation of CA and 5-HT. Neonatal hyperthyroidism induced by daily administration of L-triiodothyronine results in an increased turnover of NE (Singhal et al., 1975). The increment in monoamine levels and neurotransmission in various brain regions, in hyperthyroidism, may be attributed by several authors to enhanced synthesis (Ito et al., 1977; Atterwill, 1981) and stimulation of CA receptors (Gur et al., 1999; Bauer and Whybrow, 2001) or alteration in the metabolism of biogenic amines due to thyrotoxicosis (Upadhyaya et al., 1992). In the light of these observations, we can conclude that the deleterious effect of the THs during the development may lead to CNS pathophysiology. These findings agree with Ahmed et al. (2008).

According to the study herein, the γ-aminobutyric acid (GABA) contents of control group were gradually increased in an age-dependent manner in all studied brain regions to reach maximum values at the end of the 3rd week after birth. Such results are in agreement with the findings of several authors. Cutler and Dudzinski (1974) speculated that a linear increase in GABA content was found in the cerebral cortex and hypothalamus of rat until 4 weeks of age and only slight developmental changes in GABA were found in the medulla and spinal cord. Cheluja et al. (2007) indicated that the uptake of GABA is regulated differentially during postnatal development of rat. Represa and Ben-Ari (2005) reported that GABA has critical roles in early neuronal development, even before synapses are formed. Obviously, THs play an important role in GABA production, metabolism, release and reuptake as well as the function of GABA receptors (Wiens and Trudeau, 2006; Aszalós, 2007; Ahmed et al., 2008).

The current data showed that in all studied regions and periods, the contents of the inhibitory neurotransmitter, GABA, significantly increased in hypothyroid group while the opposite occurs in hyperthyroid group in comparison with their corresponding controls. Homogenates of corpus striatum from young rats made hypothyroid by PTU treatment from birth to 6 weeks of age had slightly increased GABA uptake (Kalaria and Prince, 1985). Mason et al. (1987) revealed that the homogenates from rats rendered hypothyroid by surgical thyroidectomy demonstrated greater GABA uptake than controls. The latter investigators added that this effect of long-term hypothyroidism was assumed to reflect a genomic action of TH that was reduced due to lower TH levels. Perhaps, THs inhibit GABA transporter synthesis. Thus, lower TH levels resulted in an increased number of transporters in neuron membranes and therefore greater GABA uptake by brain homogenates (Mason et al., 1987). This suggests that neural impairment that results from disruptions in control TH function during development could be due, at least partially, to TH effects on GABA function (Represa and Ben-Ari, 2005).

On the other hand, the effects of hyperthyroidism on GABA level in brain are controversial (Ahmed et al., 2008). Thus, depending on brain region studied, GABA in hyperthyroidism has been recorded to decrease (Sandrini et al., 1991; Upadhyaya and Agrawal, 1993), or slightly decrease (Messer et al., 1989) or no change (Chatterjee et al., 1989; Messer et al., 1989), or increase (Hashimoto et al., 1991b). This reflects the secondary effects of THs and the complex structure of the brain in different experimental animals. From previous studies, it may be suggested that the induced changes in GABA may be intimately related to the alterations in monoamine levels in both treated groups due to both maternal hypo- and hyperthyroidism.

In view of the current results, the acetylcholinesterase (AchE) activity, in control rat offspring, increased enormously with the age progress in all tested regions. These findings are concomitant with the results of several studies. The AchE activities have been found to increase with development in brain of rat (Muller et al., 1985). Notably, Skopec et al. (1981) found that the hemispheres of rat had the greatest AchE activity while the cerebellum had the lowest one. The latter observation goes parallel with our results. Cholinesterase (chE), which increased with the age progress in different brain regions of rat offspring (Ahmed, 2004; Ahmed et al., 2007), has a role in neural development (Brimijoin and Koenigsberger, 1999). TH has been shown to regulate several neurotransmitter systems, including the development of cholinergic terminals and enzymes for cholinergic transmission in various brain regions of rat (Evans et al., 1999). Generally, the present work revealed that monoamines, GABA and AchE levels tend to attain their maturity levels at the end of the 3rd week in all studied brain regions of control offspring; this observation is concomitant with THs and 5′-DI increment at this age. The same thought was recorded by Porterfield and Hendrich (1993).

In addition, the results reported in our study assured that the AchE level, in hypo- and hyperthyroid offspring, were more or less similar to those of control values at the end of the 1st postnatal week. However, AchE level was severely depressed in hypothyroid offspring in all examined regions during the 2nd and 3rd weeks. As AchE level in hyperthyroid offspring, at the end of the 2nd week, was increased significantly in cerebrum and medulla oblongata, its level was non-significant in cerebellum as compared to the levels in the age-matched control controls. Moreover, as the age progressed to the end of the 3rd week, in hyperthyroid group, the AchE level was markedly increased in all investigated regions in relation to controls.

Hypothyroidism in developing rats impairs synaptic transmission and has devastating effects on neurological functions that may be permanent (Gilbert and Paczkowski, 2003). Also, hypothyroidism decreases choline acetyltransferase (ChAT) quantities in rat brain regions (Evans et al., 1999) because THs serve as positive regulatory factors for the ChAT gene (Quirin-Stricker et al., 1994). In hyperthyroid rats, Virgili et al. (1991) demonstrated that the ChAT activity was increased in the prefrontal cortex and striatum. Recently, Carageorgiou et al. (2007) found that in hyperthyroidism, AChE activity was significantly increased only in the rat hippocampus whereas in hypothyroidism, AChE activity was significantly decreased in the frontal cortex and increased in the hippocampus. These changes may cause the impairment in the cholinergic functions, development of neurons and the tissues of the CNS of rats (Rao et al., 1990). Thyroid dysfunction has been shown to influence AChE activity in both developing and adult rats (Salvati et al., 1994). Based on the previous data and reports, it can be suggested that both hypo- and hyperthyroidism may influence the ontogenesis of AchE activity in postnatal brain regions of rat offspring, where the enzyme activity was markedly affected during the 2nd and 3rd week.

ATPase-enzyme (Na^+,K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase) activities of control rat offspring were stepwisely increased in all investigated regions with the age progress. During development, there is an increase in Na^+,K^+-ATPase activity in the rat brain (Bertoni and Siegel, 1978) accompanied by a marked change in the brain's ionic composition (Valcana and Timiras, 1969) and increase in the number of functional sodium pump sites (Bertoni and Siegel, 1978). Notably, Valcana and Timiras (1969) have shown that a component of the increase in Na^+,K^+-ATPase activity in developing rat brain is due to the presence of THs. Bruno et al. (2003) elucidated that THs are associated with an increase in the adenosine transport in brain and that adenosine plays an important modulatory role in physiological and pathological situations. T3 and T4 have membrane-initiated actions modulating Ca^{2+} channels supporting a role for THs as modulators of signal transduction pathways in the CNS of rats (Zamoner et al., 2006). Interestingly, it was reported that THs modulate the cellular sodium current, inward rectifying potassium current, sodium pump (Na^+,K^+-ATPase) and calcium pump (Ca^{2+}-ATPase) activities (Davis et al., 2010).

Furthermore, in all studied regions, the hypothyroid group exhibited a decrease in the activity of Na^+,K^+-ATPase and Ca^{2+}-ATPase at the end of the 2nd and 3rd weeks while Mg^{2+}-ATPase was decreased only at the end of the 3rd week. Also, a reduction in the Na^+,K^+-ATPase activity was observed in medulla oblongata at the end of the 1st week and in cerebrum at the end of the 2nd week for Mg^{2+}-ATPase. However, at the end of the 1st week, the Mg^{2+}-ATPase activity of hypothyroid group was significantly increased in the cerebrum. Concomitant with these results, Pacheco-Rosado et al. (2005) and Carageorgiou et al. (2007) recorded that the deficiency of THs produce a deficiency of Na^+,K^+-ATPase in different brain regions in rats. Also, the neonatal hypothyroidism results in an impairment of Na^+,K^+-ATPase activity and alterations in its kinetic properties (Billimoria et al., 2006; Katyare et al., 2006). Atterwill et al. (1985) postulated that neonatally induced hypothyroidism leads to a selectively greater impairment of the ontogenesis of the activity of cerebellar α form of Na^+,K^+-ATPase. Katyare and Rajan (2005) revealed that the Ca^{2+}-ATPase function can get further impairment due to decrease adenosine triphosphate synthesis in the hypothyroid rat brain. Hypothyroidism also induces a decrease in the activity of adenosine-metabolizing enzymes in different brain fractions in rats (Mazurkiewicz and Saggerson, 1989). Bruno et al. (2005) attributed that in rats, the decreased thyroid function as well as a potential increase in the adenosine levels and a lower availability of ATP as an excitatory neurotransmitter could be contributing to the severity of hypothyroidism during aging. Thyroid dysfunction is frequently associated with disturbances of Ca^{2+} and inorganic phosphate homeostasis in rats (Kumar and Prasad, 2003). Matsuzaki (1976) reported that Mg^{2+}-activated adenosine triphosphatase increased markedly in rats after methylthiouracil treatment.

On the other hand, in the study herein, Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of hyperthyroid group were considerably increased at all studied ages and regions as compared to control group. Furthermore, the activity of Ca^{2+}-ATPase elevated in cerebrum and medulla oblongata only at the end of the 1st week and in all investigated regions at the end of the 2nd and 3rd weeks in relation to control values. dos Reis-Lunardelli et al. (2007) found that Na^+,K^+-ATPase activity is increased in parietal cortex in L-T4 treated rat group. Hyperthyroidism, during the developing rat brain, results in a shift in the balance of inhibitory and excitatory modulation after T4 treatment (Bruno et al., 2003). The increased levels of the neurotransmitter ATP together with decreased adenosine levels in a synaptic fraction originated mainly from neuronal cells could explain the predominantly excitatory status found in rat's hyperthyroidism (Bruno et al., 2005). T4 administration to neonatal rats stimulated the activity of Na^+,K^+-ATPase in the brain cortex of euthyroid and hypothyroid animals, whereas it did not affect the synaptic membrane Na^+,K^+-ATPase of adult (30 days old) rats (Lindholm, 1984). However, Carageorgiou et al. (2007) revealed that Na^+,K^+-ATPase activity was significantly decreased in the hyperthyroid rat hippocampus and remained unchanged in the frontal cortex; this change is not concomitant with the current study. Therefore, from the current results, it is obvious that, in hyperthyroid group, the increased activities of Na^+,K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase may be secondary to increased synthesis of THs. It is worth mentioning that (1) Na^+,K^+-ATPase is essential to maintain the high concentration of Na^+ outside the nerve cell membrane and K^+ inside, (2) Ca^{++}-ATPase maintains a

high extracellular concentration in face of a low intracellular concentration to allow entry of Ca^{++} through voltage-dependent Ca^{++} channels active by action potential of presynaptic membrane (Mata and Fink, 1989; Kaplan, 2002; Ahmed et al., 2008). Based on these hypotheses, the decrease or increase of these cations ATPases in brain in hypothyroidism and hyperthyroidism may lead to disturbances in resting membrane potential and in neurotransmitter release from synaptic vesicles. These effects in turn perturb the neurons excitability and synaptic transmission within CNS.

In conclusion, the methimazole-induced hypothyroidism and exogenous T4-induced hyperthyroidism in rat dams affect the serum THs level and thyroid gland histological architecture of their offspring. The maternal hypothyroidism induced decreases in monoamine levels as well as AchE activity and increase in GABA content concomitant with suppression of Na^+,K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase activity in different brain regions of the offspring. The maternal hyperthyroidism produced reverse effects in offspring. Thus, the maternal hypothyroidism and hyperthyroidism may respectively induce inhibitory and stimulatory effects on the excitability and synaptic neurotransmissions in cerebrum, cerebellum and medulla oblongata of their offspring.

References

Abdel-Moneim, A.A., 2005. Effects of repaglinide administration on some biochemical and oxidative stress parameters of diabetic and hyperthyroid albino rats. J. Egypt. Ger. Soc. Zool. Comp. Physiol. 46 (A), 1–29.

Ahmed, O.M., 2006. Evaluation of the antihyperglycemic, antihyperlipidemic and myocardial enhancing properties of pioglitazone in diabetic and hyperthyroid rats. J. Egypt. Ger. Soc. Zool. Comp. Physiol. 51 (A), 253–278.

Ahmed, O.M., Bahgat, M., Ahmed, R.G., 2007. Age and heat stress related changes in monoamine contents and cholinesterase activity in some central nervous system regions of albino rat offspring. Int. J. Zool. Res. 3 (2), 65–76.

Ahmed, O.M., El-Gareib, A.W., El-bakry, A.M., Abd El-Tawab, S.M., Ahmed, R.G., 2008. Thyroid hormones states and brain development interactions. Int. J. Dev. Neurosci. 26 (2), 147–209, Review.

Ahmed, R.G., 2004. Effect of heat Stress on the development of the nervous system in albino rats. M.Sc. Thesis, Fac. of Sci., Beni-Suef Branch, Cairo Univ., Egypt.

Aszalós, Z., 2007. Some neurologic and psychiatric complications in endocrine disorders: the thyroid gland. Orv. Hetil. 148 (7), 303–310.

Atterwill, C.K., 1981. Effects of acute and chronic triiodothyronine (T3) administration to rats on central 5-HT and dopamine-mediated behavioural responses and related brain biochemistry. Neuropharmacology 20, 131–144.

Atterwill, C.K., Reid, J., Athayde, C.M., 1985. Effect of thyroid status on the development of the different molecular forms of Na^+, K^+-ATPase in rat brain. Mol. Cell. Endocrinol. 40 (2–3), 149–158.

Ausó, E., Lavado-Autric, R., Cuevas, E., Escobar del Rey, F., Morreale de Escobar, G., Berbel, P., 2004. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 145, 4037–4047.

Awad, M.F.I., 2002. Increased risk of fetal anomalies following maternally induced hypothyroidism in female albino rats. Ph.D. Thesis, Fac. of Sci., Cairo Univ., Egypt.

Bancroft, J.D., Stevens, A., 1982. Theory and Practical Techniques, 2nd edn. Churchill-Livingstone Edinburg, London, Melbourne, New York.

Bauer, M., Whybrow, P.C., 2001. Thyroid hormone, neural tissue and mood modulation. World J. Biol. Psychiatry 2, 59–69.

Bertoni, J.M., Siegel, G.J., 1978. Development of Na^+, K^+-ATPase in rat cerebrum: correlation with Na^+-dependent phosphorylation and K^+-paranitrophenylphosphatase. Neurochemistry 31 (6), 1501–1511.

Bianco, A.C., Kim, B.W., 2006. Deiodinases: implications of the local control of thyroid hormone action. J. Clin. Invest. 116, 2571–2579.

Bianco, A.C., Larsen, P.R., 2005. Cellular and structural biology of the deiodinases. Thyroid 15, 777–786.

Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J., Larsen, P.R., 2002. Biochemistry, cellular and molecular biology and physiological roles of the iodothyronine selenodeiodinases. Endocr. Rev. 23, 38–89.

Billimoria, F.R., Dave, B.N., Katyare, S.S., 2006. Neonatal hypothyroidism alters the kinetic properties of Na^+, K^+-ATPase in synaptic plasma membranes from rat brain. Brain Res. Bull. 70, 55–61.

Brimijoin, S., Koenigsberger, C., 1999. Cholinesterases in neural development: new findings and toxicologic implications. Environ. Health Perspect. 107 (Suppl. 1), 59–64.

Bruno, A.N., Da Silva, R.S., Bonan, C.D., Battastini, A.M., Barreto-chaves, M.L., Sarkis, J.J., 2003. Hyperthyroidism modifies ecto-nucleotidase activities in synaptosomes from hippocampus and cerebral cortex of rats in different phases of development. Int. J. Dev. Neurosci. 21 (7), 401–408.

Bruno, A.N., Ricachenevsky, F.K., Pochmann, D., Bonan, C.D., Battastini, A.M., Barreto-Chaves, M.L., Sarkis, J.J., 2005. Hypothyroidism changes adenine nucleotide hydrolysis in synaptosomes from hippocampus and cerebral cortex of rats in different phases of development. Int. J. Dev. Neurosci. 23 (1), 37–44.

Canadian Council on Animal Care (CCAC), 1993. Guide to the Care and Use of Experimental Animals, vol. 2. CCAC, Ottawa, Ontario, Canada, pp. 1–298.

Carageorgiou, H., Pantos, C., Zarros, A., Mourouzis, I., Varonos, D., Cokkinos, D., Tsakiris, S., 2005. Changes in antioxidant status, protein concentration, acetylcholinesterase, (Na^+,K^+)-, and Mg^{2+}-ATPase activities in the brain of hyper- and hypothyroid adult rats. Metab. Brain Dis. 20 (2), 129–139.

Carageorgiou, H., Pantos, C., Zarros, A., Stolakis, V., Mourouzis, I., Cokkinos, D., Tsakiris, S., 2007. Changes in acetylcholinesterase, Na^+, K^+-ATPase, and Mg^{2+}-ATPase activities in the frontal cortex and the hippocampus of hyper- and hypothyroid adult rats. Metabolism 56 (8), 1104–1110.

Chakrabarti, S., Poddar, M.K., 1989. Long-term electroacupuncture-induced changes in mammalian central GABA system. Biogenic Amines 6, 417–426.

Chatterjee, A.K., Dalal, B.B., Sadhu, U., 1989. Hormone mediated changes of brain glutamic acid system in amino acid imbalance. Horm. Metab. Res. 21, 116–119.

Cheluja, M.G., Scolari, M.J., Coelho, T.M., Blake, M.G., Boccia, M.M., Baratti, C.M., Acosta, G.B., 2007. L-Serine and GABA uptake by synaptosomes during postnatal development of rat. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 146 (4), 499–505.

Cooper, D.S., 1984. Antithyroid drugs. N. Engl. J. Med. 311, 1353–1362.

Cristovao, F.C., Bisi, H., Mendonca, B.B., Bianco, A.C., Bloise, W., 2002. Severe and mild neonatal hypothyroidism mediate opposite effects on Leydig cells of rats. Thyroid 12, 13–18.

Cutler, R.W.P., Dudzinski, D.S., 1974. Regional changes in amino acid content in developing rat brain. Neurochemistry 23, 1005–1009.

Davis, P.J., Zhou, M., Davis, F.B., Lansing, L., Mousa, S.A., Lin, H.-Y., 2010. Mini-review: cell surface receptor for thyroid hormone and nongenomic regulation of ion fluxes in excitable cells. Physiol. Behav. 99, 237–239.

DeVito, M., Biegel, L., Brouwer, A., Brown, S., Brucker-Davis, F., Cheek, A.O., Christensen, R., Colborn, T., Cooke, P., Crissman, J., Crofton, K., Doerge, D., Gray, E., Hauser, P., Hurley, P., Kohn, M., Lazar, J., McMaster, S., McClain, M., McConnell, E., Meier, C., Miller, R., Tietge, J., Tyl, R., 1999. Screening methods for thyroid hormone disruptors. Environ. Health Perspect. 107 (5), 407–415, Review.

Di Paola, M., Luders, E., Di Iulio, F., Cherubini, A., Passafiume, D., Thompson, P.M., Caltagirone, C., Toga, A.W., Spalletta, G., 2010. Callosal atrophy in mild cognitive impairment and Alzheimer's disease: different effects in different stages. NeuroImage 49, 141–149.

dos Reis-Lunardelli, E.A., Ramirez, M.R., Castro, C.C., Coitinho, A.S., Bavaresco, C., da Trindade, L.S., Perrenoud, M.F., Wyse, A.T., Sarkis, J.J., Izquierdo, I., 2007. Effects of an acute treatment with L-thyroxine on memory, habituation, danger avoidance, and on Na^+, K^+-ATPase activity in rat brain. Curr. Neurovasc. Res. 4 (4), 259–267.

Elekwa, I., Monanu, M.O., Anosike, E.O., 2005. *In vitro* effects of aqueous extracts of zanthoxylum macrophylla roots on adenosine triphosphatases in erythrocytes of different genotypes. Biokemistri 17 (1), 19–25.

Ellman, G.L., 1978. Modified Ellman method procedure for assay of cholinesterase in crude preparation. Anal. Biochem. 89, 324–326.

Evans, I.M., Sinha, A.K., Pickard, M.R., Edwards, P.R., Leonard, A.J., Ekins, R.P., 1999. Maternal hypothyroxinemia disrupts neurotransmitter metabolic enzymes in developing brain. Endocrinology 161 (2), 273–279.

Fisher, D.A., Klein, A.H., 1981. Thyroid development and disorders of thyroid function in the newborn. N. Engl. J. Med. 304, 702–712.

Fisher, D.A., Nelson, J.C., Carlton, E.I., Wilcox, R.B., 2000. Maturation of human hypothalamic-pituitary–thyroid function and control. Thyroid 10, 229–234.

Gilbert, M.E., Paczkowski, C., 2003. Propylthiouracil (PTU)-induced hypothyroidism in the developing rat impairs synaptic transmission and plasticity in the dentate gyrus of the adult hippocampus. Brain Res. Dev. Brain Res. 145, 19–29.

Guerrero, A., Palmpona, R., Portero-Otín, M., Baja, G., López-Torres, M., 1999. Effect of thyroid status on lipid composition and peroxidation in the mouse liver. Free Radic. Biol. Med. 26 (1–2), 73–80.

Gupta, P., Kar, A., 1998. Role of ascorbic acid in cadmium-induced thyroid dysfunction and lipid peroxidation. J. Appl. Toxicol. 18, 317–320.

Gur, E., Lerer, B., Newman, M.E., 1999. Chronic clomipramine and triiodothyronine increase serotonin levels in rat frontal cortex *in vivo*: relationship to serotonin autoreceptor activity. J. Pharmacol. Exp. Ther. 288, 81–87.

Hasebe, M., Matsumoto, I., Imagawa, T., Uehara, M., 2008. Effects of an anti-thyroid drug, methimazole, administration to rat dams on the cerebellar cortex development in their pups. Int. J. Dev. Neurosci. 26 (5), 409–414.

Hashimoto, H., Maruyama, M., Koshida, R., Okuda, N., Sato, T., 1995. Central hypothyroidism resulting from pituitary suppression and peripheral thyrotoxicosis in a premature infant born to a mother with Graves' disease. J. Pediatr. 127, 809–811.

Hashimoto, H., Sato, T., Horita, S., Kubo, M., Ohki, T., 1991a. Maturation of the pituitary–thyroid axis during the perinatal period. Endocrinol. Jpn. 38 (2), 151–157.

Hashimoto, H., Walker, C.H., Prange, A.J., Mason, G.A., 1991b. The effects of thyroid hormones on potassium stimulated release of ^{3}H-GABA by synaptosomes of rat cerebral cortex. Neuropsychopharmacology 5, 49–54.

Hesketh, J.E., London, J.B., Reading, H.W., Glen, A.I., 1978. The effect of lithium treatment on erythrocyte membrane ATPase activities and erythrocyte ion content. Br. J. Clin. Pharmacol. 5, 323–329.

Higuchi, R., Kumagai, T., Kobayashi, M., Minami, T., Koyama, H., Ishii, Y., 2001. Short-term hyperthyroidism followed by transient pituitary hypothyroidism in a very low birth weight infant born to a mother with uncontrolled Graves' disease. J. Pediatr. 107 (4), E57, Available at: www.pediatrics.org/cgi/content/full/107/4/e57.

Higuchi, R., Miyawaki, M., Kumagai, T., Okutani, T., Shima, Y., Yoshiyama, M., Ban, H., Yoshikawa, N., 2005. Central hypothyroidism in infants who were born to mothers with thyrotoxicosis before 32 weeks gestation: 3 cases. J. Pediatr. 115 (5), e623–e625.
Hisao, F., Tamura, S., Tanakano, T., Ishibashi, S., Tanaka, T., 1980. Fine structural changes in the ageing mouse thyroid. J. Gerontol. 35 (1), 3–15.
Ito, J.M., Valcana, T., Timiras, P.S., 1977. Effect of hypo- and hyperthyroidism on regional monoamine metabolism in the adult rat brain. Neuroendocrinology 24 (1), 55–64.
Jacoby, J.H., Mueller, G., Wurtman, R.J., 1975. Thyroid state and brain monoamine metabolism. Endocrinology 97 (5), 1332–1335.
Jahnke, G.D., Choksi, N.Y., Moore, J.A., Shelby, M.D., 2004. Thyroid toxicants: assessing reproductive health effects. Environ. Health Perspect. 112 (3), 363–368.
Kahl, S., Capuco, V., Bitman, J., 1987. Serum concentrations of thyroid hormones and extrathyroidal 5′-monodeiodinase activity during lactation in the rat (42458). Proc. Soc. Exp. Biol. Med. 184, 144–150.
Kalaria, R.N., Prince, A.K., 1985. Effects of thyroid deficiency on the development of cholinergic, GABA, dopaminergic and glutamate neuron markers and DNA concentrations in the rat corpus striatum. Int. J. Dev. Neurosci. 3 (6), 655–666.
Kaplan, J.H., 2002. Biochemistry of Na,K-ATPase. Annual Rev. Biochem. 71, 511–535.
Kaplan, M.M., 1986. Regulatory influences on iodothyronine deiodination in animal tissues. In: Hennemann, G. (Ed.), Thyroid Hormone Metabolism. Marcel Dekker, New York, pp. 231–253.
Kaplan, M.M., McCann, U.D., Yaskoski, K.A., Larsen, P.R., Leonard, J.L., 1981. Anatomical distribution of phenolic and tyrosyl ring deiodinases in the nervous system of normal and hypothyroid rats. Endocrinology 109, 397–402.
Katyare, S.S., Billimoria, F.R., Dave, B.N., 2006. Effect of neonatal hypothyroidism on the kinetic properties of Na^+, K^+-ATPase from rat brain microsomes. Neuroendocrinology 18, 361–366.
Katyare, S.S., Rajan, R.R., 2005. Influence of thyroid hormone treatment on respiratory activity of cerebral mitochondria from hypothyroid rats. A critical re-assessment. Exp. Neurol. 195, 416–422.
Kester, M.H., Martinez de Mena, R., Obregón, M.J., Marinkovic, D., Howatson, A., Visser, T.J., Hume, R., Morreale de Escobar, G., 2004. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J. Clin. Endocrinol. Metab. 89 (7,), 3114–3116.
Kirkegaard, C., Faber, J., 1998. The role of thyroid hormones in depression. Eur. J. Endocrinol. 138, 1–9.
Köhrle, J., Jakob, F., Contempre, B., Dumont, J.E., 2005. Selenium, the thyroid and the endocrine system. Endocr. Rev. 26, 944–984.
Koibuchi, N., 2008. The role of thyroid hormone on cerebellar development. Cerebellum 7 (4), 530–533.
Krause, W.J., Cutts, J.H., 1983. Postnatal development of the thyroid gland in the opossum (*Didelphis virginana*). Acta Anat. 116, 322–338.
Kuiper, G.G., Kester, M.H., Peeters, R.P., Visser, T.J., 2005. Biochemical mechanisms of thyroid hormone deiodination. Thyroid 15, 787–798.
Kumar, V., Prasad, R., 2003. Thyroid hormones stimulate calcium transport systems in rat intestine. Biochim. Biophys. Acta 1639, 185–194.
Larsen, P.R., 1982. Thyroid–pituitary interaction: feedback regulation of thyrotropin secretion by thyroid hormones. N. Engl. J. Med. 306, 23–32.
Legrand, J., 1986. Thyroid hormone effects on growth and development. In: Henneman, G. (Ed.), Thyroid Hormone Metabolism. Dekker, M., Inc., New York, pp. 503–534.
Leonard, J.L., 2008. Non-genomic actions of thyroid hormone in brain development. Steroids 73 (9–10), 1008–1012.
Lindholm, D.B., 1984. Thyroxine regulates the activity and the concentration of synaptic plasma membrane Na^+, K^+-ATPase in the developing rat brain cortex. Brain Res. 317, 83–88.
MacNabb, C., O'Hare, E., Cleary, J., Georgopoulos, A.P., 2000. Varied duration of congenital hypothyroidism potentiates preservation in a response alteration discrimination task. Neurosci. Res. 36 (2), 121–127.
Maes, M., Mommen, K., Hendrickx, D., Peeters, D., D'Hondt, P., Ranjan, R., De Meyer, F., Scharpé, S., 1997. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin. Endocrinol. (Oxford) 46 (5), 587–598.
Mandel, S.J., Brent, G.A., Larsen, P.R., 1993. Levothyroxine therapy in patients with thyroid disease. Ann. Intern. Med. 119, 492–502, Also available at http://www.nacb.org/lmpg/thyroid_LMPG_Word.stm (accessed 01.05).
Mason, G.A., Bondy, S.C., Nemeroff, C.B., Walker, C.H., Prange, A.J., 1987. The effects of thyroid state on beta-adrenergic and serotonergic receptors in rat brain. Psychoneuroendocrinology 12, 261–270.
Mata, M., Fink, D.J., 1989. Ca^{++}-ATPase in the central nervous system: an EM cytochemical study. J. Histochem. Cytochem. 37, 971–980.
Matsuzaki, S., 1976. Reduction of 5′-nucleotidase activity in rat thyroid and adenohypophysis following methylthiouracil treatment. Endocrinol. Jpn. 23 (1), 49–54.
Mazurkiewicz, D., Saggerson, D., 1989. Changes in the activities of adenosine-metabolizing enzymes in six regions of the rat brain on chemical induction of hypothyroidism. Biochem. J. 261, 667–672.
Messer, A., Eisenberg, B., Martin, D.L., 1989. Effects of mild hyperthyroidism on levels of amino acids in the developing Lurcher cerebellum. J. Neurogenet. 5, 77–85.
Mookadam, M., Leske, D.A., Fautsch, M.P., Lanier, W.L., Holmes, J.M., 2004. The anti-thyroid drug methimazole induces neo-vascularization in the neonatal rat analogous to ROP. Invest. Ophthalmol. Vis. Sci. 45, 4145–4150.
Morley, J.E., 1981. Neuroendocrine control of thyrotropin secretion. Endocr. Rev. 2, 396–436.
Morreale de Escobar, G., Calvo, R., Escobar Del Rey, F., Obregón, M.J., 1993. Differential effects of thyroid hormones on growth and thyrotrophic hormones in rat fetuses near term. Endocrinology 132 (5), 2056–2064.
Morreale de Escobar, G., Pastor, R., Obregón, M.J., Escobar del Rey, F., 1985. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues before and after onset of fetal thyroid function. Endocrinology 117 (5), 1890–1901.
Muller, F., Dumez, Y., Massoulié, J., 1985. Molecular forms and solubility of acetylcholinestrase during the embryonic development of rat and human brain. Brain Res. 331, 295–302.
Obregón, M.J., Mallol, J., Pastor, R., Morreale de Escobar, G., Escobar del Rey, F., 1984. L-Thyroxine and 3,5,3′-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. Endocrinology 114, 305–308.
Oppenheimer, J.H., Schwartz, H.L., 1997. Molecular basis of thyroid hormone-dependent brain development. Endocr. Rev. 18, 462–475.
Ornellas, D.S., Grozovsky, R., Goldenberg, R.C., Carvalho, D.P., Fong, P., Guggino, W.B., Morales, M., 2003. Thyroid hormone modulates ClC-2 chloride channel gene expression in rat renal proximal tubules. Endocrinology 178, 503–511.
Pacheco-Rosado, J., Arias-Citalán, G., Ortiz-Butrón, R., Rodríquez-Páez, L., 2005. Selective decrease of Na^+/K^+-ATPase activity in the brain of hypothyroid rats. Proc. West. Pharmacol. Soc. 48, 52–54.
Pagel, P., Blome, J., Wolf, H.U., 2000. High-performance liquid chromatographic separation and measurement of various biogenic compound possibly involved in the pathomechanism of Parkinson's disease. J. Chromatogr. B 746, 297–304.
PC-STAT, 1985. One way analysis of variance. Version 1A(c) copyright. Programs coded by Roa, M., Blane, K., Zonneberg, M. Univ. of Georgia, USA.
Porterfield, S.P., Hendrich, C.E., 1993. The role of thyroid hormones in prenatal and neonatal neurological development-current perspectives. Endocr. Rev. 14, 94–106.
Potter, B.J., Mano, M.T., Belling, G.B., McIntosh, G.H., Hua, C., Cragg, B.G., Marshall, J., Wellby, M.L., Hetzel, B.S., 1982. Retarded fetal brain development resulting from severe dietary iodine deficiency in sheep. Neuropathol. Appl. Neurobiol. 8, 303–313.
Puymirat, J., 1985. Effects of dysthyroidism on central catecholaminergic neurons. Neurochem. Int. 7, 969–977.
Pyer, A.F., Battle, C.L., Peake, R.L., 1981. Ultrastructural and cytochemical effects of trypan blue on TSH stimulation of thyroid follicular cells. Cell Tissue Res. 218 (3), 547–556.
Quirin-Stricker, C., Nappey, V., Simoni, P., Toussaint, J.L., Schmitt, M., 1994. Transactivation by thyroid hormone receptor the 5′ flanking region of the human ChAT gene. Mol. Brain Res. 23, 253–265.
Ramos, S., Goya, L., Martin, M.A., Escriva, F., Pascual-Leone, A.M., 2002. Influence of hypothyroidism on circulating concentrations and liver expression of IGF-binding proteins mRNA from neonatal and adult rats. Endocrinology 172, 363–373.
Rao, G.S., Abraham, V., Fink, B.A., Margulies, N., Ziskin, M.C., 1990. Biochemical changes in the developing rat CNS due to hyperthermia. Teratology 41 (3), 327–332.
Represa, A., Ben-Ari, Y., 2005. Trophic actions of GABA on neuronal development. Trends Neurosci. 28 (5), 278–283.
Reutens, A.T., 1995. Evaluation and application of a highly sensitive assay for serum growth hormone (GH) in the study of adult GH deficiency. J. Clin. Endocrinol. Metab. 80 (2), 480–485.
Saleh, A.M., Abd El-Tawab, S.M., Bashir, S.R., El-Maghraby, M., Aly, F.A., 1986. Histological and histochemical studies on the postnatal growth of thyroid gland of albino rats. Egypt. J. Histol. 9 (1), 151–175.
Salvati, S., Attorri, L., Campeggi, L.M., 1994. Effect of propylthiouracil induced hypothyroidism on cerebral cortex of young and aged rats: lipid composition of synaptosomes, muscarinic receptor sites, and acetylcholinesterase activity. Neurochem. Res. 19, 1181–1186.
Sandrini, M., Marrama, D., Vergoni, A.V., Bertolini, A., 1991. Effects of thyroid status on the characteristics of alpha1-, alpha2-, beta, imipramine and GABA receptors in the rat brain. Life Sci. 48, 659–666.
Sanui, H., Rubin, H., 1982. The role of magnesium in cell proliferation and transformation. In: Boynton, A.L., McKochan, W.L., Whitfield, J.P. (Eds.), Ions, Cell Proliferation and Cancer. Academic Press, New York, pp. 517–537.
Schwartz, H.L., Ross, M., Oppenheimer, J.H., 1997. Lack of effects of thyroid hormone on late fetal rat brain development. Endocrinology 138, 3119–3124.
Segni, M., Gorman, C.A., 2001. The aftermath of childhood hyperthyroidism. J. Pediatr. Endocrinol. Metab. 14 (Suppl. 5), 1277–12782.
Sharifi, J., St Germain, D.L., 1992. The cDNA for type 1 iodothyronine 5′-deiodinase encodes an enzyme manifesting both high Km and low Km activity. J. Biol. Chem. 267, 12539–12544.
Shibutani, M., Woo, G.H., Fujimoto, H., Saegusa, Y., Takahashi, M., Inoue, K., Hirose, M., Nishikawa, A., 2009. Assessment of developmental effects of hypothyroidism in rats from in utero and lactation exposure to anti-thyroid agents. Reprod. Toxicol. 28 (3), 297–307.
Sigrun, H., Heike, H., 2010. Review: thyroid hormone action during brain development: more questions than answers. Mol. Cell. Endocrinol. 315, 19–26.
Silva, J.E., Matthews, P.S., 1984. Production rates and turnover of triiodothyronine in rat-developing cerebral cortex and cerebellum. Responses to hypothyroidism. J. Clin. Invest. 74 (3), 1035–1049.

Singhal, R.L., Rastogi, R.B., Hrdina, P.D., 1975. Brain biogenic amines and altered thyroid function. Life Sci. 17 (11), 1617–1626.
Skopec, F., Bajgar, J., Patoka, J., 1981. Changes of acetylcholinesterase activity in different brain areas of the rat following head irradiation. Radiobiol. Radiother. 22 (5), 631–634.
Smals, A.G.H., Ross, A.H., Kloppenborg, P.W.C., 1981. Dichotomy between serum free triiodothyronine and free thyroxine concentrations in familial thyroxine-binding globulin deficiency. J. Clin. Endocrinol. Metab. 53, 917–922.
Takeuchia, Y., Suzukia, D., Odab, S.-I., Refetoffc, S., Sekid, K., Tsunekawad, K., Kasaharad, T., Murakamid, M., Murata, Y., 2006. Unique regulation of thyroid hormone metabolism during fasting in the house musk shrew (*Suncus murinus*, Insectivora: Soricidae). Gen. Comp. Endocrinol. 146 (3), 236–241.
Tamasy, V., Du, J.-Z., Vallerga, A., Meisami, E., Timiras, P.S., 1984. Suckling ability and maternal prolactine levels in hypothyroid rats. Horm. Behav. 18, 457–464.
Thakur, C., Saikia, T.C., Yadav, R.N., 1997. Total serum levels of triiodothyronine (T3), thyroxine (T4) and thyrotropin (TSH) in school going children of Dibrugarh district: an endemic goiter region of Assam. Indian J. Physiol. Pharmacol. 41 (2), 167–170.
Upadhyaya, L., Agrawal, J.K., 1993. Effect of L-thyroxine and carbimazole on brain biogenic amines and amino acids in rats. Endocr. Res. 19 (2–3), 87–99.
Upadhyaya, L., Agrawal, J.K., Dubey, G.P., Udupa, K.N., 1992. Biogenic amines and thyrotoxicosis. Acta Endocrinol. (Copenh.) 126 (4), 315–318.
Vaccari, A., Rossetti, Z.L., De Montis, G., Stefanini, E., Martino, E., Gessa, G.L., 1990. Neonatal hypothyroidism induces striatal dopaminergic dysfunction. Neuroscience 35 (3), 699–706.
Valcana, T., Timiras, P.S., 1969. Effect of hypothyroidism on ionic metabolism and Na^+–K^+ activated ATP phosphohydrolase activity in the developing rat brain. Neurochemistry 16 (3), 935–943.
Valenzuela, I.M., Hume, R.I., Krejci, E., Akaaboune, M., 2005. *In vivo* Regulation of Acetylcholinesterase Insertion at the Neuromuscular Junction. The American Society for Biochemistry and Molecular Biology, Manuscript, M502874200, 1-18.
Varas, S.M., Muñoz, E.M., Hapon, M.B., Aguilera Merlo, C.I., Giménez, M.S., 2002. Hyperthyroidism and production of precocious involution in the mammary glands of lactating rats. Reproduction 124, 691–702.
Varma, S.K., Crawford, J.D., 1979. Long-term perspectives of thyroxine administration in neonatal rats. Horm. Res. 10 (6), 327–335.
Venditti, P., Balestrieri, M., Di Meo, S., De Leo, T.J., 1997. Effect of thyroid state on lipid peroxidation, antioxidant defences and susceptibility to oxidative stress in rat tissues. Endocrinology 155, 151–157.
Virgili, M., Saverino, O., Vaccari, M., Barnabei, O., Contestabile, A., 1991. Temporal, regional and cellular selectivity of neonatal alteration of the thyroid state on neurochemical maturation in the rat. Exp. Brain Res. 83, 555–561.
Wasniewska, M., De Luca, F., Cassio, A., Oggiaro, N., Gianino, P., Delvecchio, M., Aiazzi, R., Stoppioni, V., Lombardo, F., Messina, M.F., Valenzise, M., Arrigo, T., 2003. In congenital hypothyroidism bone maturation at birth may be a predictive factor of psychomotor development during the first year of life irrespective of other variables related to treatment. Eur. J. Endocrinol. 149, 1–6.
Wiens, S.C., Trudeau, V.L., 2006. Thyroid hormone and γ-aminobutyric acid (GABA) interactions in neuroendocrine systems. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 144 (3), 332–344.
Wong, A.O., Zhou, H., Jiang, Y., Ko, W.K., 2006. Feedback regulation of growth hormone synthesis and secretion in fish and the emerging concept of intrapituitary feedback loop. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 144 (3), 284–305.
York, R.G., Barnett J.Jr., Brown, W.R., Garman, R.H., Mattie, D.R., Dodd, D., 2004. A rat neurodevelopmental evaluation of offspring, including evaluation of adult and neonatal thyroid, from mothers treated with ammonium perchlorate in drinking water. Int. J. Toxicol. 23 (3), 191–214.
Zamoner, A., Funchal, C., Heimfarth, L., Silva, F.R.M.B., Pureur, R.P., 2006. Short-term effects of thyroid hormones on cytoskeletal proteins are mediated by GABAergic mechanisms in slices of cerebral cortex from young rats. Cell. Mol. Neurobiol. 26, 209–224.
Zhou, Y., Wang, X., Hadley, J., Corey, S.J., Vasilatos-Younken, R., 2005. Regulation of JAK2 protein expression by chronic, pulsatile GH administration *in vivo*: a possible mechanism for ligand enhancement of signal transduction. Gen. Comp. Endocrinol. 144, 128–139.