

LACTATING PTU EXPOSURE: I- ALTERS THYROID-NEURAL AXIS IN NEONATAL CEREBELLUM

R.G. Ahmed^{a*}, A.W. El-Gareib^b

^aDivision of Anatomy and Embryology, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

^bDivision of Anatomy and Embryology, Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

Corresponding author: R.G. Ahmed

Tel/Fax numbers: +02-010-9147-1828

ABSTRACT: *The purpose of this study was to determine the effects of lactating propylthiouracil (PTU) on the thyroid-neural development in newborns. PTU was administered to female rats in drinking water (0.1% w/v) from birth to lactation day (LD) 30. A hypothyroid state was recorded at LDs 20 and 30 in both dams and their offspring where a marked depression ($P < 0.01$) was observed in serum thyroxine (T4) and triiodothyronine (T3) levels, while a reverse pattern was noticed in serum thyrotropin (TSH) level as compared to a control group. Also, the maternal administration caused a highly significant decrease in the level of neonatal growth hormone (GH) at postnatal days (PNDs) 20 and 30. This hypothyroid condition produced inhibitory effects on 5'-monodeiodinase (5'-DI), and on cholinergic enzymes [butyrylcholinesterase (BuchE) and acetylcholinesterase (AchE)] in the neonatal cerebellum at the studied PNDs. This may also delay partially the development of the cerebellar Purkinje cells (PCs) via altering the dendritic morphology at both examined PNDs. All tested parameters in the control group followed a synchronized course of development, and their progress may depend, largely on thyroid state. Thus, PTU may act as a developmental thyroid-neural disruptor, causing dysmorphogenesis and cerebellum dysgenesis.*

KEYWORDS: PTU, Thyroid, Cerebellum, Purkinje cells, Rat newborns

INTRODUCTION

Thyroid hormones (THs) regulate growth, development, and critical biochemical functions in the developing brain (Ahmed et al., 2008; Horn and Heuer, 2010; Ahmed and Incerpi, 2013). A large proportion of the T3 produced in humans and in rats is generated by 5'-DI of T4 in peripheral tissues (Larsen et al., 1981). Also, THs have been shown to play a vital role in virtually all aspects of neuronal development from neuronal migration, neurite extension and guidance to the regulation of neurotransmitters (Bernal, 2002). These offer a wealth of opportunity to intervene pharmacologically in THs signaling at numerous steps (Moriyama et al., 2007). Propylthiouracil (PTU), an anti-thyroid thioureyline drug, is the U.S. Food and Drug Administration-approved thionamide that used clinically to treat Graves' hyperthyroidism (Cooper, 2005). It has intrathyroidal action (THs synthesis) and extrathyroidal action (deiodination) (Jena et al., 2012;

Tousson et al., 2012; Hassan et al., 2013; Bhanja and Jena, 2013). However, several studies reported that PTU during the development has been shown to disrupt the levels of THs and young growth, and induce long-term structural and functional brain deficits (Zoeller and Crofton, 2005; Koohestani et al., 2012), even at doses that produce only a relatively mild, transient hypothyroidism in the mothers (Chakraborty et al., 2012). Importantly, the cholinergic system (BuchE and AchE) (Carageorgiou et al., 2007; Tousson et al., 2012), and the neuronal proliferation and migration (Hadj-Sahraoui et al., 2000; Zhang et al., 2009) in various brain regions are perhaps the most profoundly affected by neonatal thyroid deficiency. This is probably due to the inhibition of thyroperoxidase (TPO) and peripheral deiodinase (Zoeller and Crofton, 2005). However, the studies focused on the development of the neuroendocrine system concurrently with transfer of PTU from dams to their pups during the whole suckling period and on postnatal examinations for neural functions have received only limited attention.

In the present study, PTU was added to the drinking water of maternal rats to reversibly block TH production in their offspring from birth until LD 30. This developmental stage in the rat model reflects the time period between the third trimester of gestation to the second postnatal year in the human (Ahmed et al., 2008). Therefore it is desirable to investigate the effects of suckling PTU on the thyroid markers of rat dams (serum T4, T3 and TSH) and their pups (serum T4, T3, TSH and GH). Also, the current study extended to estimate the changes in the cerebellar peripheral metabolism of THs (5'-DI), the changes in the cholinergic enzymes (BuchE and AchE) and the changes in the development of the PCs at PNDs 20 and 30. Moreover, we selected the PCs because they are central to the function of the cerebellum and are inhibitory neurons that relay information from the cerebellar cortex to the rest of the brain (Anderson et al., 2003).

MATERIALS AND METHODS

Chemicals

PTU and ethopropazine hydrochloride were purchased from Sigma- Aldrich, St. Louis, MO, USA. T4, T3, TSH and GH kits were obtained from Calbiotech INC (CBI), USA. All other reagents were of the purest grades commercially available.

Animals and treatments

Pregnant white albino rats at gestation day 15 were purchased from the National Institute of Ophthalmology, Giza, Egypt. They were housed singly in cages until giving birth, after which litters were culled to 8 pups per cage so that mothers could provide sufficient milk for the pups. The male litters only were randomly divided into control and PTU groups. The animals were 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 (El-bakry et al., 2010). Tap water was used for drinking *ad libitum* and these animals were exposed to constant daily light/dark periods of 12 h each (lights on at 06:00 h) and $50 \pm 5\%$ relative humidity (Ahmed and Incerpi, 2013). Generally, all the animal procedures were in accordance with the general guidelines of animal care and the recommendations of the Canadian Council on Animal Care (CCAC; Olfert et al., 1993). All efforts were made to minimize the number of animals used and their suffering.

0.1% w/v PTU (Koohestani et al., 2012) (Sigma- Aldrich, St. Louis, MO, USA) was administered to female rats in drinking water from birth to lactation day (LD) 30, which was replaced daily with fresh PTU solution. PTU enters the bloodstream, passing through the mother's breast milk, to the rat pups. For each time point, age-matched control rats received normal drinking water. At LDs 20 and 30, dams and their newborns were subsequently sacrificed under mild diethyl ether anaesthesia, and the blood samples from dams and their pups were taken and centrifuged at 1006.2 xg for 30 min. The neonate's brains (cerebellum) were placed immediately in ice-cold normal saline. The clear, non-hemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at -30 °C until use for hormonal examinations. The cerebellums 10% (w/v) were homogenized by using a Teflon homogenizer (Glas-Col, Terre Haute, USA) in two buffers: ice-cold PBS (0.15M, pH 7.4) containing 0.25 M sucrose and 2mM EDTA for 5'-DI assay; and saline (0.9% NaCl) for AchE and BuchE assay. On the other hand, the cerebellums of the offspring were removed immediately after a rapid anaesthesia, dropped into potassium dichromate-formalin mixture as a fixative before further processing and staining with Golgi-Copsch stain.

The radioimmunoassay (RIA) examination:

T4, T3 and TSH in serum of mothers and their offspring, as well as GH in serum of the newborns were estimated quantitatively in the Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive Medicine, New Children Hospital, Faculty of Medicine, Cairo University, Egypt, according to the method of Thakur et al. (1997), Maes et al. (1997), Mandel et al. (1993) and Reutens (1995), respectively.

THE DEVELOPMENTAL AND BIOCHEMICAL EXAMINATIONS IN NEONATAL CEREBELLUM

Determination of 5'-DI activity

5'-DI activity in homogenate supernatant was estimated according to the method of Kahl et al. (1987), Gupta and Kar (1998) and Ahmed et al. (2010). The method depended 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 the method of Maes et al. (1997).

Determination of cholinesterases activity

The activities of both AchE and BuchE were assessed by standard spectrophotometric Ellman's method. Acetylthiocholine iodide (ATCI) or butyrylthiocholine iodide (BuTCI) were used as an appropriate substrates and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was used as a chromogen (Ellman et al., 1961). To distinguish the activity of AchE from BuchE, ethopropazine hydrochloride (10^{-4} M) was used as a BuchE inhibitor. BuchE activity was calculated as the difference between total cholinesterase (chE) and AchE activity. The activities of AchE and BuchE were expressed as μ mole of ATCI/BuTCI hydrolyzed per min per milligram of protein. All spectrophotometric measurements were done in duplicate. The data were normalized to the amount of protein measured by the Lowry method, using the Bio-Rad DC protein assay and bovine serum albumin as the standard.

Golgi-Copsch stain

The Golgi-Copsch staining was performed according to Tömböl (1967). The cerebellum of each group was cut into slices. The slices were placed in a 4:1 mixture of 5% potassium dichromate and concentrated formaldehyde (40%) for 4 days. The slices were transferred to 3.6% potassium dichromate for 4 days. They were washed in 0.75% silver nitrate and then placed in the same solution for 4 days. The last two steps were repeated once. The slices were dehydrated and then placed in xylene for 20 min. Embedding process was made in paraffin wax. After that, serial sections were made at 50 µm, at Histology and Histopathology Unit, Faculty of Veterinary Medicine, Beni Suef University, Egypt, to show the PCs. The de-waxed process was carried out by using xylene. The sections were mounted in Canada balsam. All sections were evaluated for the degree of maldevelopment.

Statistical analysis:

Data were analyzed using one-way ANOVA (PC-STAT University of Georgia 1985) followed by LSD analysis to determine the main effects and compare the groups with each other. F-probability for each variable expresses the general effect between the groups. The data are presented as means \pm S.E.M., and values of $P < 0.01$ and $P < 0.001$ were considered statistically highly significant and very highly significant respectively.

RESULTS**Maternal-neonatal thyroid markers**

The present data in lactating rat dams and their offspring showed changes of T4, T3, TSH and GH, and the effect of PTU administration (Tables 1 and 2). The levels of T3, T4 and TSH in control dams and their newborns, and neonatal GH were increased profoundly and gradually from LD 20 to 30.

The administration of PTU led to disruption of this behavioral pattern in dams and their offspring. In dams, the administration caused a highly significant reduction in both T3 and T4 levels while a highly significant elevation in TSH level was recorded at both examined LDs relative to control group (LSD; $P < 0.01$; Table 1). Interestingly, the effect was more severe at LD 30 when the percentage changes were -26.51%, -61.84%, +91.30%, for T4, T3 and TSH, respectively.

Table 1. Effect of PTU in thyroid markers (T4, T3 and TSH, ng/100ml) of lactating rats. Data are expressed as mean \pm SE. Number of animals in each group is six. Values which share the same superscript symbols are not significantly different. ANOVA (F-probability) expresses the effect between groups and tested PNDs, where $P < 0.001$ is very highly significant.

Periods	PTU	Serum T4	Serum T3	Serum TSH
LD 20	0	6.75±0.111 ^b	3.15±0.067 ^b	2.50±0.134 ^d
	0.1% w/v	5.75±0.066 ^c -14.81%	2.10±0.089 ^c -33.33%	5.60±0.089 ^b +124.00%
LD 30	0	9.05±0.112 ^a	3.80±0.088 ^a	3.45±0.066 ^c
	0.1% w/v	6.65±0.101 ^b -26.51%	1.45±0.110 ^d -61.84%	6.60±0.044 ^a +91.30%
ANOVA		P<0.001	P<0.001	P<0.001
LSD 5%		0.302	0.268	0.266
LSD 1%		0.412	0.366	0.362

In table 2, the newborns exposed to PTU via their dams during the lactation period exhibited a highly significant ($P<0.01$) decrease in the levels of T4, T3 and GH at PND 20 in comparison with their corresponding control rats. This behavior was more deteriorated at PND 30 (-47.54%, -53.48% and -45% for T4, T3 and GH, respectively). Alternatively, at PNDs 20 and 30, a highly significant ($P<0.01$) increase due to the administration was observed in the level of TSH (+66.66% and +46.15%, respectively) compared to the control group (Table 2). Regarding one way ANOVA, the general effect between both groups at PNDs 20 and 30 was very highly significant ($P<0.001$) for all tested hormones in both dams and their pups (Tables 1 and 2).

Table 2. Effect of lactating PTU in thyroid markers (T4, T3, TSH and GH, ng/100ml) of their newborns during the postnatal period. Data are expressed as mean \pm SE. Number of animals in each group is six. Values which share the same superscript symbols are not significantly different. ANOVA (F-probability) expresses the effect between groups and tested PNDs, where $P<0.001$ is very highly significant.

Periods	PTU	Serum T4	Serum T3	Serum TSH	Serum GH
LD 20	0	2.15±0.111 ^b	1.35±0.066 ^b	1.65±0.101 ^c	1.30±0.089 ^b
	0.1% w/v	0.99±0.046 ^d -53.95%	0.5±0.044 ^d -62.96%	2.75±0.064 ^b +66.66%	0.55±0.067 ^c -57.69%
LD 30	0	3.05±0.071 ^a	2.15±0.111 ^a	2.60±0.089 ^b	2.00±0.041 ^a
	0.1% w/v	1.60±0.087 ^c -47.54%	1.00±0.043 ^c -53.48%	3.80±0.133 ^a +46.15%	1.10±0.084 ^b -45.00%
ANOVA		P<0.001	P<0.001	P<0.001	P<0.001
LSD 5%		0.242	0.213	0.306	0.221
LSD 1%		0.330	0.291	0.418	0.301

DEVELOPMENTAL AND BIOCHEMICAL MARKERS IN NEONATAL CEREBELLUM:

Figure 1 illustrated the activity of neonatal 5'-DI in all experimental groups, control and PTU at PNDs 20 and 30. During the experimental period, the data showed that the 5'-DI activity of the control offspring was gradually increased. LSD test analysis indicated that the 5'-DI activity of the PTU group was severely decreased (LSD; $P < 0.01$) as the age progressed from PND 20 to 30 as compared with the control group (Figure 1). As one-way ANOVA test was applied on 5'-DI of control and PTU groups, the general effect was very highly significant ($P < 0.001$).

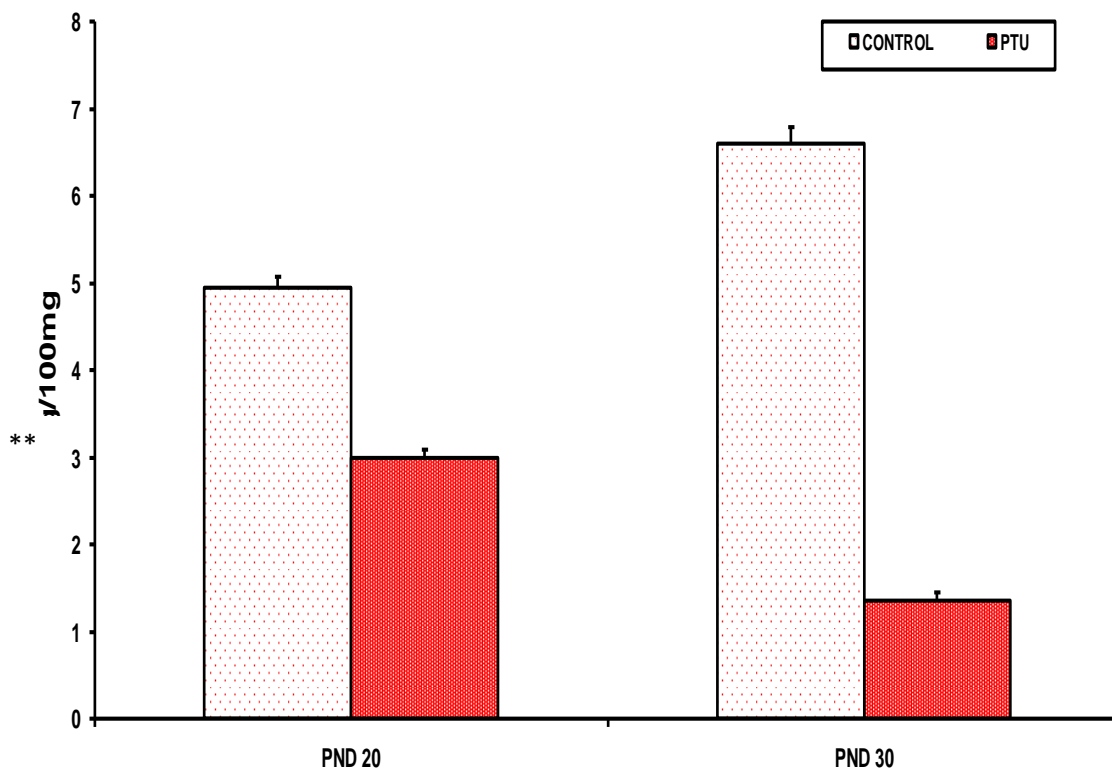


Figure 1. Effect of lactating PTU in 5'-DI (expressed in ng/100mg) activity of neonatal cerebellum during the postnatal period. Bars represent mean \pm SE of six animals/group, where the change between both groups/PND is highly significant (** $P < 0.01$) as determined by LSD test. ANOVA (F-probability) expresses the effect between groups and tested PNDs, where $P < 0.001$ is very highly significant.

Figure 2 demonstrated the effect of maternal PTU on the activities of neonatal BuchE and AchE at PNDs 20 and 30. In control newborns, the activities of BuchE and AchE exhibited enormous and gradual increase from PND 20 to 30. More importantly, during the tested PNDs, the activity of AchE was higher than BuchE in the control group. The administration induced a pronounced decrease (LSD; $P < 0.01$) in the activities of these enzymes as compared with the corresponding

control during both examined PNDs, particularly at PND 30 where the attenuation reached its lowest level. Concerning the ANOVA test of BuchE and AchE, the general effect between both groups was very highly significant ($P < 0.001$) throughout the experiment.

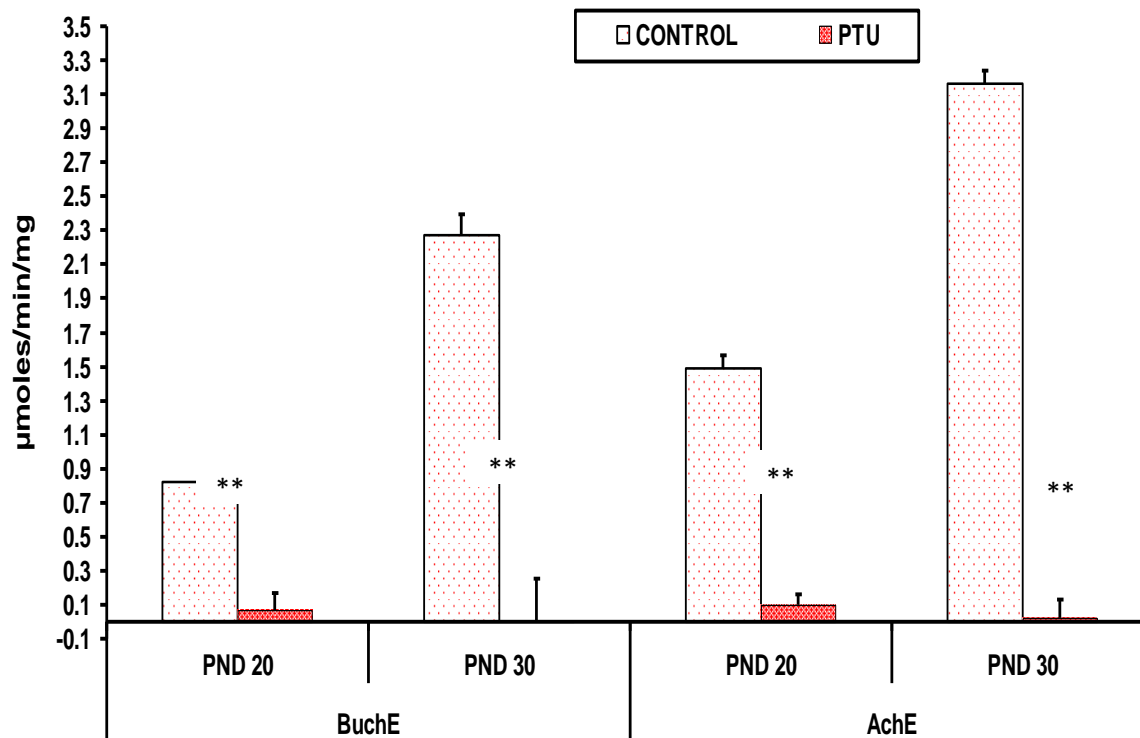


Figure 2. Effect of lactating PTU in BuchE and AchE (expressed in $\mu\text{moles}/\text{min}/\text{mg}$) activities of neonatal cerebellum during the postnatal period. Bars represent mean \pm SE of six animals/group, where the change between both groups/PND is highly significant (** $P < 0.01$) as determined by LSD test. ANOVA (F-probability) expresses the effect between groups and tested PNDs, where $P < 0.001$ is very highly significant.

Neurogenesis as shown by Golgi-Copsch stain

Purkinje cells (PCs) in the control and treated groups appeared possessing one main dendrite that arose from the upper end of the cell body and extended through the molecular layer (ML) at PNDs 20 and 30 (Figures 3A-D). These primary dendrites branched repeatedly through the ML into secondary and tertiary dendrites, giving a rich dendritic tree with innumerable tiny spines at PND 30 in the control group (Figure 3C). In addition, these arborizations were confined to a plane perpendicular to the pia matter in the control group at PND 20 (Figure 3A) and reached their maximum length at PND 30 (Figure 3C). At both PNDs, these cells were large in size and had long dendrites in the control group when compared with treated ones (Figures 3A-D). These dendrites had more spines in the control group with respect to PTU ones. On the other hand, in the treated group there was some degeneration in the dendrites of these cells at PNDs 20 and 30

(Figures 3B and 3D). Other important points at PND 30 can be summarized (Figures 3A-D): 1) PCs of the control group increased in number as compared with treated ones, and 2) the density of the dendritic network in the treated group was lesser than that seen in the control ones.

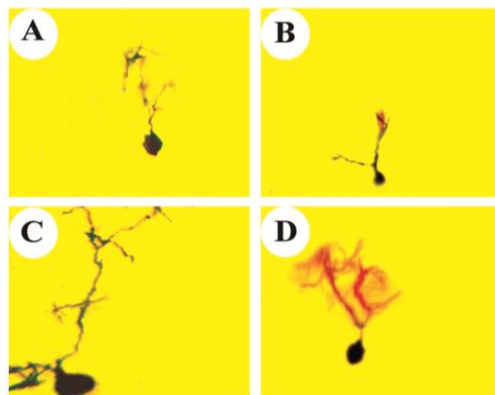


Figure 3. Sagittal sections in the cerebellar cortex of rat newborns showing the Purkinje cells at PND 20 (A: Control; B: PTU) and at PND 30 (C: Control; D: PTU). (Golgi-Copsch stain, X400)

DISCUSSION

In control dams and their pups, the levels of serum T4, T3 and TSH were obviously progressed from LD 20 to 30, a known period of THs action. The same pattern was recorded in the level of neonatal GH. Concomitantly, the levels of serum THs and TSH in dams and neonates were correlated positively (El-bakry et al., 2010; Ahmed et al., 2010 & 2012; Ahmed, 2011). After birth, TSH through its receptor (Tshr) is the major regulator of growth, differentiation and function of the thyrocytes (Milenkovic et al., 2007). Also, THs influence growth in part by altering the secretion and effects of pituitary GH via mRNA or growth factors (Akin et al., 2009; Saranac et al., 2013), while GH in turn mediates the growth and function of the thyroid as well as TH metabolism (Ahmed et al., 2008; Ahmed, 2013; Ahmed and Incerpi, 2013; Saranac et al., 2013).

In PTU-treated group, a decrease in serum T4, T3 and increase in TSH levels were observed during the tested days in dams and offspring, as well as GH level was decreased in offspring with respect to control group. Concurrently, PTU induced hypothyroidism during the development (Gilbert and Lasley, 2013; Hassan et al., 2013; Khalawi et al., 2013). This can be caused by reducing the binding of T3 to nuclear thyroid receptors (TRs) (Moriyama et al., 2007), inhibiting thyroperoxidase activity (which normally oxidizes anion iodide (I^-) to iodine (I^0) during thyroxine synthesis in the thyroid gland) within the thyroid gland, blocking the organification of thyroglobulin, and thus blocking synthesis of THs (Jena et al., 2012). Furthermore, Scott-Moncrieff et al. (1998) reported that the stimulating effect of PTU on TSH level may be due to the lack of negative feedback from the THs. This elevation increased thyroid weight and stimulated the thyroid follicular cell proliferations (Hood et al., 1999), and this can alter the

thyroid growth. With regard to the GH deficiency due to maternal PTU, the present data are in agreement with those of Tamasy et al. (1986) and Shibutani et al. (2009) showing that exposure to PTU resulted in the growth suppression in rat offspring of both sexes at weaning. Altogether, it is legitimate to propose that the imbalance in the hypothalamic–pituitary–thyroid axis (HPTA) and TH synthesis or degradation due to maternal PTU during lactation period may seriously compromise normal TH actions in dams and their litters.

Reference to the present work, the activity of 5'-DI in neonatal cerebellum of the control group was increased tremendously during the experimental period. Similarly, Ahmed et al. (2010) recorded that the elevation in the activity of 5'-DI from the 1st to 3rd week after birth was noticed in neonatal rat cerebellum, cerebrum and medulla oblongata. This elevation may be critical in the regulations of serum and tissue concentrations of THs (Galton, 2005; St Germain et al., 2005) and tissue/cellular levels of T3 (Lechan and Fekete, 2005; Takeuchi et al., 2006), and may be regulated in a complex manner that is related to both T4 and T3 availability in the serum (Ahmed et al., 2008 & 2010). These findings led to infer that the maintenance of THs and 5'-DI may be necessary to convert the available T4 to T3 in developing cerebellum during the suckling period.

Regarding the current results, the activity of neonatal 5'-DI in the developing cerebellum of maternal PTU-hypothyroid group showed noticeable and highly significant decreases with ageing from PND 20 to 30 in comparison with their respective controls. It was reported that administration of PTU to the lactating (Tamasy et al., 1984) or pregnant (Sigrun and Heike, 2010) rat caused hypothyroidism via blocking TH synthesis by decreasing the activity of DI (suppresses the conversion of T4 to T3). Generally, PTU inhibits DI in rat (Nguyen et al., 1998; Axelstad et al., 2008), mice (Dong et al., 2009) and human (Saber et al., 1975; Ahmed, 2012a). Similarly, maternal methimazole (MMI), antithyroid drug, reduced the activity of neonatal 5'-DI in rat cerebellum (Ahmed et al., 2010). It is conceivable that the drop in neonatal 5'-DI can be explained by the induction of a hypothyroid state by maternal PTU, and the variations in serum T3 level may arise from alterations in the monodeiodination pathway of T4. This may delay the development of neonatal cerebellum.

In this study, the activities of BuchE and AchE in neonatal cerebellum of the control group were elevated at PNDs 20 and 30 in an age-dependent manner. Muller et al. (1985) postulated that the activities of BuchE and AchE were increased mostly postnatally in rat brain and prenatally (before 11 weeks) in human brain. Recently, our group reported that the activity of AchE was gradually increased in cerebellum of the control rat offspring during the postnatal period (Ahmed et al., 2010; Ahmed, 2011 & 2012a,b). Also, the AchE activity in the control group was higher than BuchE during the examined PNDs. This observation was confirmed in the spinal cord of rat neonates by Koohestani et al. (2012 & 2013) who noticed that the postnatal AchE increase was a part of the postnatal proliferation of the synapses of the cholinergic neurons, while the behavior of BuChE reflected an earlier burst in formation of the non-neuronal cells containing this enzyme, and a slower postnatal rise in them. Taken together, THs may regulate the cholinergic transmission in various brain regions of rat (Evans et al., 1999; Ahmed et al., 2008; Ahmed, 2011) through their action on nerve growth factor (NGF) (Munoz et al., 1993). Based on these

results, the viability and normal biological reactions during the postnatal development of rat newborns may depend, at least in part, on the functional stimulus exerted by THs.

In the current experimental work, the reduction in the activities of BuchE and AchE in the neonatal cerebellum, from PND 20 to 30, was more marked in hypothyroid pups, whose mothers were administrated PTU from LD 1 to 30. Similarly, hypothyroidism due to PTU significantly reduced total AchE and total BuChE activities in neonatal spinal cord at PNDs 10 and 21 (Koohestani et al., 2012). Legrand et al. (1983) found that total BuChE activity declined at PND 10 and in older pups due to a PTU-hypothyroid state in developing rat cerebellum, while Ahmed (2012a) suggested that hypothyroidism by MMI could disturb the transformation and maturation of AchE in developing rat cerebellum at PNDs 7, 14 and 21. These disturbances may be associated with neurodevelopmental deficits via inhibiting T3 level (Sawin et al., 1998) by recruitment of its transcriptional corepressors and/or dissociation of coactivators (Moriyama et al., 2007). Generally, the development of the cerebellar cholinergic system or function was delayed in neonatal hypothyroid rat (Virgili et al. 1991; Ahmed et al., 2010; Ahmed, 2011). These observations strengthen the possibility that the maternal administration of PTU affects the development of the cholinergic system; it may reduce the postnatal development of cholinergic synapses, thereby influencing the functional development of this brain region. This action may be mediated by the thyroid axis.

Further, the developmental rate of PCs was increased in its density and complexity with the age progress from PND 20 to 30 in the control newborns. This increase was associated with the elevation in the levels of THs, and with the activities of the 5'-DI and cholinergic enzymes. This explanation was supported by several different lines of evidence. Firstly, THs regulate the neuronal cytoarchitecture, neuronal growth, and myelination and synaptogenesis, where their receptors are widely distributed in the CNS (Bernal, 2007; Ahmed et al., 2008; Leonard, 2008). Secondly, the 5'-DI is the main source of T3 for the developing rat neurons (Ahmed, 2012a). Thirdly, the cholinergic systems are essential for normal brain development as a modulator of neuronal proliferation, migration and differentiation processes (Coccini et al., 2007; Ahmed, 2011).

In the current study, the neonatal PCs of the maternal PTU-treated group were decreased in their numbers and dendrites with degenerative changes as compared to the control ones at both investigated PNDs. Similarly, the developmental hypothyroidism induced by PTU decreases the number of PCs, delays the dendritic arborization and synaptogenesis in PCs, and in general impairs the cerebellar development (Koibuchi, 2009; Wang et al., 2011). Other results showed that the different models of developmental hypothyroidism in rats can cause retardation in the morphological maturation of PCs (Shibutani et al., 2009; Gong et al., 2010; Ahmed, 2012a; Ahmed et al., 2012). In hypothyroid mice, a defect of TH levels provoked a decrease of cerebellum protein concentrations, and this confirmed the histological aspects of cerebellum with PCs incompletely differentiated (Ben Hamida et al., 2003).

In another instance, the abnormal neuronal development observed during hypothyroidism in rats may partially result from absence of GH (Savard et al., 1984; Ahmed, 2012a), alter the activity of 5'-DI (Ausó et al., 2004; Kester et al., 2004), or change the cholinergic enzymes (Ahmed et al., 2008 & 2010). These alterations may be mediated by a PTU-hypothyroid state during the lactating period and may be responsible for the loss of neuronal vital functions. Finally, PTU has a developmental thyroid-neural disrupting action via dyshormonogenesis and cerebellum dysgenesis (Figure 4). These adverse effects depend on the dose, experimental duration, developmental period and type of biological fraction studied. Future studies to find region-specific alteration of gene expression should shed light on details of molecular change in developmental hypothyroidism.

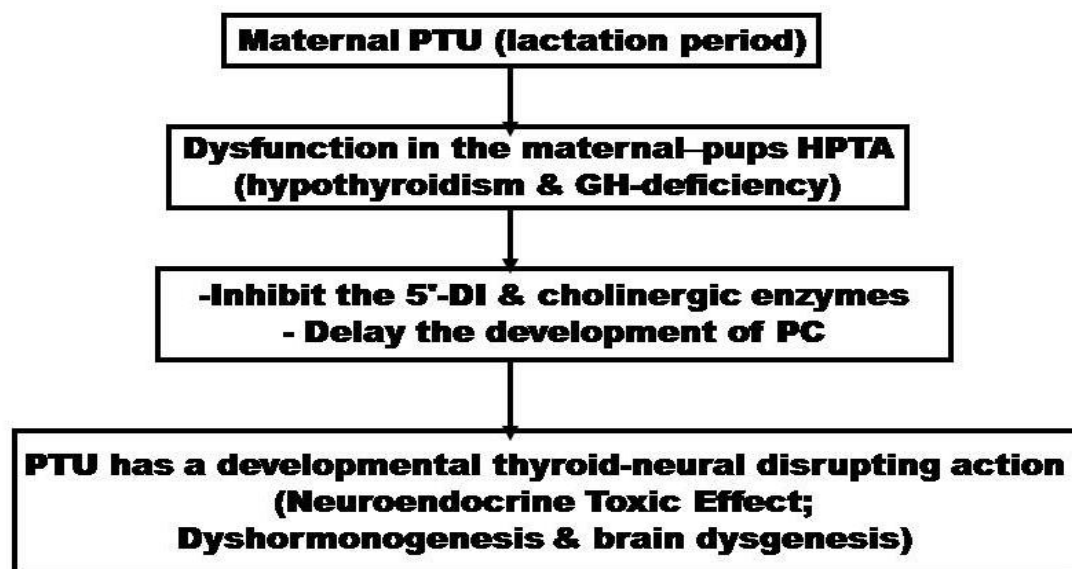


Figure 4. The developmental neuroendocrine toxic effect of PTU.

Conflict of interest

- The authors declare that no competing financial interests exist.

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REFERENCES

Ahmed, O.M., Abd El-Tawab, S.M., Ahmed, R.G., 2010. 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. *Int. J. Dev. Neurosci.* 28, 437-454.
- Ahmed, O.M., Ahmed, R.G., El-Gareib, A.W., El-Bakry, A.M., Abd El-Tawaba, S.M., 2012. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: II-The developmental pattern of neurons in relation to oxidative stress and antioxidant defense system. *Int. J. Dev. Neurosci.* 30, 517–537.
- 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., 2013. Early weaning PCB 95 exposure alters the neonatal endocrine system: thyroid adipokine dysfunction. *Journal of Endocrinology* 219(3), 205-215.
- Ahmed, R.G., 2011. Perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure alters developmental neuroendocrine system. *Food and Chemical Toxicology* 49, 1276–1284.
- Ahmed, R.G., 2012a. Maternal-newborn thyroid dysfunction. In the *Developmental Neuroendocrinology*, pp. 1-369. Ed R.G. Ahmed. Germany: LAP LAMBERT Academic Publishing GmbH & Co KG.
- Ahmed, R.G., 2012b. Postnatal heat stress and CNS maldevelopment. In the *Histopathology and Pathophysiology*, pp. 1-381. Ed R.G. Ahmed. Germany: LAP LAMBERT Academic Publishing GmbH & Co KG.
- Ahmed, R.G., Incerpi, S., 2013. Gestational doxorubicin alters fetal thyroid–brain axis. *Int. J. Devl. Neuroscience* 31, 96–104.
- Akin, F., Yaylali, G.F., Turgut, S., Kaptanoglu, B., 2009. Growth hormone/insulin-like growth factor axis in patients with subclinical thyroid dysfunction. *Growth Hormone & IGF Res.* 19, 252–255.
- Anderson, G.W., Schoonover, C.M., Jones, S.A., 2003. Control of thyroid hormone action in the developing rat brain. *Thyroid* 13(11), 1039-1056.
- 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 neocortico-genesis alters neuronal migration. *Endocrinol.* 145, 4037-4047.
- Axelstad, M., Hansen, P.R., Boberg, J., Bonnichsen, M., Nellemann, C., Lund, S.P., Hougaard, K.S., Hass, U., 2008. Developmental neurotoxicity of propylthiouracil (PTU) in rats: Relationship between transient hypothyroxinemia during development and long-lasting behavioural and functional changes. *Toxicol. and Applied Pharmacol.* 232, 1–13.
- Ben Hamida, F., Soussia, L., Guerhazi, F., Zeghal, N., 2003. Thiocyanate and central nervous system maturation of young mice. *Ann. Endocrinol. (Paris)* 64(4), 268-276.
- Bernal, J., 2007. Thyroid hormone receptors in brain development and function. *Nat. Clin. Pract. Endocrinol. Metab.* 3(3), 249-259.
- Bernal, J., 2002. Action of thyroid hormone in brain. *J. Endocrinol. Invest.* 25, 268–288.
- Bhanja, S., Jena, S., 2013. Modulation of antioxidant enzyme expression by PTU-induced hypothyroidism in cerebral cortex of postnatal rat brain. *Neurochem. Res.* 38(1), 42-49.
- Carageorgiou, H., Pantos, C., Zarros, A., Stolakis, V., Mourouzis, I., Cokkinos, D., Tsakiris, S., 2007. Changes in acetylcholinesterase, Na⁺,K⁺-ATPase, and Mg²⁺-ATPase activities in the

- frontal cortex and the hippocampus of hyper- and hypothyroid adult rats. *Metab.* 56(8), 1104-1110.
- Chakraborty, G., Magagna-Poveda, A., Parratt, C., Umans, J.G., MacLusky, N.J., Scharfman, H.E., 2012. Reduced hippocampal brain-derived neurotrophic factor (BDNF) in neonatal rats after prenatal exposure to propylthiouracil (PTU). *Journal of Endocrinology* 153(3), 1311–1316.
- Coccini, T., Roda, E., Castoldi, A.F., Goldoni, M., Poli, D., Bernocchi, G., Manzo, L., 2007. Perinatal co-exposure to methylmercury and PCB153 or PCB126 in rats alters the cerebral cholinergic muscarinic receptors at weaning and puberty. *Toxicology* 238, 34-48.
- Cooper, D.S., 2005. Antithyroid drugs. *N. Engl. J. Med.* 352, 905–917.
- Dong, H., Yauk, C.L., Rowan-Carroll, A., You, S.H., Zoeller, R.T., Lambert, I., Wade, M.G., 2009. Identification of thyroid hormone receptor binding sites and target genes using chip-on-chip in developing mouse cerebellum. *PLoS ONE.* 4(2), e4610.
- El-bakry, A.M., El-Ghareeb, A-W.A., Ahmed, R.G., 2010. Comparative study of the effects of experimentally-induced hypothyroidism and hyperthyroidism in some brain regions in albino rats. *Int. J. of Dev. Neurosci.* 28, 371–389.
- Ellman, G.L., Courtney, K.D., Andres, V.Jr., Feather-Stone, 1961. A new and rapid colorimetric determination of acetyl cholinesterase activity. *Biochem Pharmacol.* 7, 88-95.
- 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. *Endocrinol.* 161(2), 273-279.
- Galton, V.A., 2005. The roles of the iodothyronine deiodinases in mammalian development. *Thyroid* 15, 823-834.
- Gilbert, M.E., Lasley, S.M., 2013. Developmental thyroid hormone insufficiency and brain development: a role for brain-derived neurotrophic factor (BDNF)? *Neurosci.*, <http://dx.doi.org/10.1016/j.neuroscience.2012.11.022>
- Gong, J., Liu, W., Dong, J., Wang, Y., Xu, H., Wei, W., Zhong, J., Xi, Q., Chen, J., 2010. Developmental iodine deficiency and hypothyroidism impair neural development in rat hippocampus: involvement of doublecortin and NCAM-180. *BMC Neuroscience* 11, 50–59.
- Gupta, P., Kar, A., 1998. Role of ascorbic acid in cadmium-induced thyroid dysfunction and lipid peroxidation. *J. Appl. Toxicol.* 18, 317–320.
- Hadj-Sahraoui, N., Seugnet, I., Ghorbel, M.T., Demeneix, B., 2000. Hypothyroidism prolongs mitotic activity in the postnatal mouse brain. *Neurosci. Lett.* 280(2), 79–82.
- Hassan, W.A., Aly, M.S., Abdel Rahman, T., Shahat, A.S., 2013. Impact of experimental hypothyroidism on monoamines level in discrete brain regions and other peripheral tissues of young and adult male rats. *Int. J. Devl Neuroscience* 31, 225–233.
- Hood, A., Liu, Y.P., Gattone II, V.H., Klaasen, C.D., 1999. Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. *Toxicol. Sci.* 49, 263-271.
- Horn, S., Heuer, H., 2010. Thyroid hormone action during brain development: more questions than answers. *Mol. Cell. Endocrinol.* 315(1–2), 19–26.

- Jena, S., Chainy, G.B.N., Dandapat, J., 2012. Hypothyroidism modulates renal antioxidant gene expression during postnatal development and maturation in rat. *Gen. Comp. Endocrinol.* 178, 8–18.
- 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.
- 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.
- Khalawi, A.A., Al-Robai, A.A., Khoja, S.M., Ali, S.S., 2013. Can Nigella Sativa oil (NSO) reverse hypothyroid status induced by PTU in rat? biochemical and histological studies. *Life Sci.* 10(2), 802-811.
- Koibuchi, N., 2009. Animal models to study thyroid hormone action in cerebellum. *Cerebellum* 8, 89-97.
- Koohestani, F., Brown, C.M., Meisami, E., 2012. Differential effects of developmental hypo- and hyperthyroidism on acetylcholinesterase and butyrylcholinesterase activity in the spinal cord of developing postnatal rat pups. *Int. J. Devl Neuroscience* 30, 570–577.
- Koohestani, F., Brown, C.M., Meisami, E., 2013. Postnatal growth hormone deficiency in growing rats causes marked decline in the activity of spinal cord acetylcholinesterase but not butyrylcholinesterase. *Int. J. of Dev. Neurosci.*, in press.
- Larsen, P.R., Silva, J.E., Kaplan, M.M., 1981. Relationships between circulating and intracellular thyroid hormones: physiological and clinical implications. *Endocr. Rev.* 2, 87-102.
- Lechan, R.M., Fekete, C., 2005. Role of thyroid hormone deiodination in the hypothalamus. *Thyroid* 15(8), 883-897.
- Legrand, C., Ghandour, M.S., Clos, J., 1983. Histochemical and biochemical studies of butyrylcholinesterase activity in adult and developing cerebellum. Effects of abnormal thyroid state and undernutrition. *Neuropathol. and Applied Neurobiol.* 9, 433-453.
- Leonard, J.L., 2008. Non-genomic actions of thyroid hormone in brain development. *Steroids* 73(9-10), 1008-1012.
- Maes, M., Mommen, K., Hendrickx, D., Peeters, D., D'Hondt, P., Ranjan, R., De Meyer, F., Scharpe, 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. (http://www.nacb.org/lmpg/thyroid_LMPG_Word.stm).
- Milenkovic, M., De Deken, X., Jin, L., De Felice, M., Di Lauro, R., Dumont, J.E., Corvilain, B., Miot, F., 2007. Duox expression and related H₂O₂ measurement in mouse thyroid: onset in embryonic development and regulation by TSH in adult. *Journal of Endocrinology* 192, 615-626.

- Moriyama, K., Tagami, T., Usui, T., Naruse, M., Nambu, T., Hataya, Y., Kanamoto, N., Li, Y.S., Yasoda, A., Arai, H., Nakao, K., 2007. Antithyroid drugs inhibit thyroid hormone receptor-mediated transcription. *J. Clin. Endocrinol. Metab.* 92(3), 1066-1072.
- Muller, F., Dumez, Y., Massoulié, J., 1985. Molecular forms and solubility of acetylcholinesterase during the embryonic development of rat and human brain. *Brain Res.* 331, 295-302.
- Munoz, A., Wrighton, C., Seliger, B., Bernal, J., Beug, H., 1993. Thyroid hormone receptor/c-erbA: Control of commitment and differentiation in the neuronal/chromaffin progenitor line PC12. *J. Cell. Biol.* 121, 423-438.
- Nguyen, T.T., Chapa, F., DiStefano, J.J., 1998. Direct measurement of the contributions of type I and type II 5'-deiodinases to whole body steady state 3,5,3'-triiodothyronine production from thyroxine in the rat. *Endocrinol.* 139(11), 4626-4633.
- Olfert, E.D., Cross, B.M., McWilliam, A.A. (Eds), 1993. In *Guide to the care and use of experimental animals*. vol 1. Ottawa, Ontario, Canada: CCAC.
- 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.
- 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.
- Saberi, M., Sterling, F.H., Utiger, R.D., 1975. Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. *J. Clin. Invest.* 55, 218-223.
- Saranac, L., Stamenkovic, H., Stankovic, T., Markovic, I., Zivanovic, S., Djuric, Z., 2013. Growth in children with thyroid dysfunction. In *The Current Topics in Hypothyroidism with Focus on Development*, chapter 5, pp. 119-134. Ed E. Potluková. Croatia: Intech Open Access Publisher.
- Savard, P., Blanchard, L.M., Mérand, Y., Dupont, A., 1984. Serotonin, 5-hydroxyindoleacetic acid and substance P content of discrete brain nuclei in rats made hypo- or hyperthyroid in the neonatal period: Effect of growth hormone treatment. *Brain Res.* 317(2), 239-245.
- Sawin, S., Brodish, P., Carter, C.S., Stanton, M.E., Lau, C., 1998. Development of cholinergic neurons in rat brain regions: dose-dependent effects of propylthiouracil-induced hypothyroidism. *Neurotoxicol. and Teratol.* 20(6), 627-635.
- Scott-Moncrieff, J.C.R., Nelson, R.W., Bruner, J.M., 1998. Comparison of serum concentrations of thyroid-stimulating hormone in healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. *J. of the Amer. Vet. Med. Association* 212, 387-391.
- 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, 297-307.
- Sigrun, H., Heike, H., 2010. Review: thyroid hormone action during brain development: more questions than answers. *Mol. Cell. Endocrinol.* 315, 19-26.
- St Germain, D.L., Hernandez, A., Schneider, M.J., Galton, V.A., 2005. Insights into the role of deiodinases from studies of genetically modified animals. *Thyroid* 15, 905-916.
- Takeuchi, Y., Suzuki, D., Oda, S-I., Refetoff, S., Seki, K., Tsunekawa, K., Kasahara, T., Murakami, 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 prolactin levels in hypothyroid rats. *Horm. Behav.* 18, 457-464.
- Tamasy, V., Meisami, E., Vallerga, A., Timiras, P.S., 1986. Rehabilitation from neonatal hypothyroidism: Spontaneous motor activity, exploratory behavior, avoidance learning and responses of pituitary-thyroid axis to stress in male rats. *Psychoneuroendocrinol.* 11, 91-103.
- 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.
- Tömböl, T., 1967. Short neurons and their synaptic relations in the specific thalamic nuclei. *Brain Res.* 3(4), 309-326.
- Tousson, E., Ibrahim, W., Arafa, N., Akela, M.A., 2012. Monoamine concentrations changes in the PTU-induced hypothyroid rat brain and the ameliorating role of folic acid. *Human and Exp. Toxicol.* 31 (3), 282–289.
- 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.
- Wang, Y., Zhong, J., Wei, W., Gong, J., Dong, J., Yu, F., Wang, Y., Chen, J., 2011. Developmental iodine deficiency and hypothyroidism impair neural development, upregulate caveolin-1, and downregulate synaptotagmin-1 in the rat cerebellum. *Biol Trace Elem. Res.* 144(1-3), 1039-1049.
- Zhang, L., Blomgren, K., Kuhn, H.G., Cooper-Kuhn, C.M., 2009. Effects of postnatal thyroid hormone deficiency on neurogenesis in the juvenile and adult rat. *Neurobiol. of Disease* 34, 366–374.
- Zoeller, R.T., Crofton, K.M., 2005. Mode of action: Developmental thyroid hormone insufficiency-neurological abnormalities resulting from exposure to propylthiouracil. *Crit. Rev. Toxicol.* 35, 771-781.