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Impact of experimental hypothyroidism on monoamines level in discrete brain regions and other peripheral tissues of young and adult male rats

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ABSTRACT

The levels of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in different brain regions as well as in blood plasma, cardiac muscle and adrenal gland of young and adult male albino rats were measured following experimentally induced hypothyroidism. Hypothyroidism induced by daily oral administration of propylthiouracil (PTU, 5 mg/kg body wt) caused a significant reduction in DA levels in most of the tissues examined of both young and adult rats after 21 and 28 days, in NE levels after all the time intervals studied in young rats, and after 21 and 28 days in adult rats. 5-HT exhibited a significant reduction in the selected brain regions and blood plasma after 21 and 28 days and in cardiac muscle after all the time intervals in the two age groups of animals. It may be suggested that the changes in monoamine levels induced by hypothyroidism may be due to disturbance in the synthesis and release of these amines through the neurons impairment or may be due to an alteration pattern of their synthesizing and/or degradative enzymes.

1. Introduction

Thyroid hormones, including tri-iodothyronine (T_3) and tetra-iodothyronine (T_4) have physiological actions and essentially regulate a variety of biochemical reactions in virtually all tissues. They have also been shown to play a crucial role in the development and physiological functioning of the central nervous system (CNS), not only during brain maturation but also in adult vertebrate brain (Ahmed et al., 2008; DiPaola et al., 2010; Sigrun and Heike, 2010; Wang et al., 2011; Artis et al., 2012). Thus any deficiency of thyroid hormones during the developmental period may result in an irreversible impairment, morphological and cytoarchitecture abnormalities, disorganization, maldevelopment and physical retardation that are permanent (Zoeller and Rovet, 2004; Berbel et al., 2007; Argumedo et al., 2012). These effects may be responsible for the loss of neuronal vital functions and may lead, in turn, to biochemical dysfunctions (Ahmed et al., 2012; Jena et al., 2012). Moreover, adult-onset thyroid dysfunction is also associated with neurological abnormalities (Pilhatsch et al., 2011; Cortes et al., 2012).

Besides the crucial role of thyroid hormones 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., 2007; Ahmed et al., 2010; Tousson et al., 2012). Several investigators showed that the ontogeny of numerous CNS neurotransmitter systems that include catecholamine and serotonin could be affected by neonatal thyroid deficiency (Ahmed et al., 2010 and Bhanja and Jena, 2012). It has been suggested that thyroid dysfunction may be linked with abnormalities in central noradrenergic neurotransmission (Saravanan et al., 2006). Several lines of evidence also support a relationship between thyroid hormones and serotonergic transmission in the brain (Bauer et al., 2002; Lifschytz et al., 2006).

As most of the previous publications deal with the effect of thyroid hormones on the structural development of the brain and neurons, detailed information on the neuronal pathways and the neurotransmitters involved is scare. Therefore, the present study aims to assess the effects of experimentally induced hypothyroidism on different neurotransmitters level in various brain regions as well as in blood plasma, cardiac muscle and adrenal gland of young and adult rats, besides elucidating the impact of the hypothyroidism on body weight.

2. Materials and methods

2.1. Experimental animals

The experimental animals used in this study were male albino rats of Wistar origin: 60 young rats aged 3 weeks (30–40 g) and 60 adult rats aged 18 weeks (130–160 g). The animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR). They were housed under normal

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environmental conditions of temperature and humidity and allowed to adjust to the new environment for two weeks before starting the experiment. Animal rooms were maintained on a 12-h light, 12-h dark photoperiods. Animals were provided with food and water ad libitum. All experiments were conducted in accordance with the NODCAR Guidelines for the Care and Use of Laboratory Animals.

2.2. Induction of hypothyroidism

Animals were daily given 5 mg/kg body wt of PTU (Sigma, St. Louis, MO) via oral administration for 28 consecutive days. PTU has been shown previously to inhibit thyroid hormones synthesis effectively in the rat (Kulikov et al., 2005). In addition to blocking synthesis, PTU also inhibits the peripheral deiodination of T_4 to T_3 (Yi et al., 1997). The control groups received sterile water for full period of experiment. PTU-treated groups and their controls were decapitated after 14, 21 and 28 days from daily oral administration.

2.3. Body weight measurement

Body weight was recorded daily beginning on zero time (the time prior to treatment) and continued until decapitation. The body weight was averaged for each week until the end of the treatment.

2.4. Handling of tissue samples

In all experiments done the conditions were adjusted to decapitate the animals between 3.00 and 4.00 pm. After decapitation, two blood samples were collected from each rat. The first was allowed to coagulate at room temperature (to obtain serum). The second blood sample was collected in a heparinized centrifuge tube (to obtain plasma). All tubes were centrifuged at 2000 × *g* for 15 min to separate serum and plasma. The brain, cardiac muscle and adrenal gland were rapidly excised. The brain was transferred to a dry ice-cold glass plate and dissected into the following regions: cerebral cortex, thalamus & hypothalamus, midbrain, cerebellum and pons-medulla. All tissues were plotted dry on filter paper to remove the excess fluid then weighed. Tissue samples were stored at −20 °C till taken for analysis.

2.5. Determination of thyroid hormones

T_3, T_4 and TSH were determined in blood serum by ELISA using commercial kits (Diagnostic Systems Laboratories INC.) according to the methods of Wenzel (1981), Midgeley (2001) and Bravermann (1996), respectively.

2.6. Estimation of the amine levels

The estimation of DA, NE and 5-HT levels in the selected rat tissues were carried out according to the fluorometric method described by Ciarlone (1978).

2.6.1. Extraction and separation

Homogenization of tissue and recovery of the three amines from tissue homogenates has been described previously (Chang, 1964; Maickel et al., 1968; Ciarlone, 1974). Each tissue sample was homogenized in 10 volumes of cold acidified n-butanol using a glass homogenizer. The sample weighing less than 300 mg was homogenized in 3 ml of acidified n-butanol. For plasma samples 3 ml of acidified n-butanol were added to 0.3 ml plasma.

Duplicate internal standard tubes were carried in parallel with the tissue homogenates. Internal standards were prepared by adding 0.3 ml of standard mixture (0.1 ml of each amine) to 9.7 ml of 0.2 N acetic acid. Aliquots of 0.2 ml of this solution were diluted to 0.3 ml with 0.2 N acetic acid then received 3 ml of acidified n-butanol. The homogenates, plasma and internal standard tubes were centrifuged at 1000 × *g* for 5 min then 2.5 ml of the supernatant fluid were transferred to tubes containing 1.6 ml of 0.2 N acetic acid and 5 ml of n-heptane. All tubes were placed on a vortex mixer for 30 s and the phases were separated by centrifugation at 1000 × *g* for 5 min. The organic supernatant phase was discarded. One milliliter of the aqueous phase was transferred to tubes (for the analysis of NE and DA) and 0.2 ml was transferred to tubes for continuation of 5-HT.

External standards for NE and DA were prepared in duplicate, in 0.2 N acetic acid and to a total volume of 1.6 ml per tube. Two and one-half milliliters of acidified n-butanol and 5 ml of heptane were added to the tubes. All tubes were placed on a vortex mixer for 30 s and centrifuged at 1000 × *g* for 5 min. The organic supernatant phase was discarded and 1 ml of the aqueous phase was transferred to clean, dry test tubes.

The remainder of the assay procedure (oxidation) should be followed according to the method described by Ciarlone (1976). To all tubes: samples, internal standard, external standard and reagent blank (1 ml of 0.2 N acetic acid), 0.2 ml EDTA reagent was added and mixed then 0.1 ml of 0.1 N iodine was added and mixed again. Two minutes later, 0.2 ml alkaline sulphite reagent was added and mixed. The tubes were allowed to stand exactly 2 min then 0.2 ml of 5 N acetic acid was added and mixed. All tubes were placed in a boiling water bath for 2 min, cooled in tap water and read for NE fluorescence at excitation and emission wavelengths of 380 nm and 480 nm, respectively.

Table 1
Effect of oral administration of propylthiouracil (5 mg/kg body wt, daily for 28 consecutive days) on T3 (pg/ml), T4 (ng/dl) and TSH (μIU/ml) concentrations in serum of young and adult male albino rats after 14, 21 and 28 days of administration. The number of animals was six in each group. Values given are Mean ± S.E.

Hormone	Treatment	Young						Adult					
		14 days		21 days		28 days		14 days		21 days		28 days	
		Mean ± S.E	% Difference	Mean ± S.E	% Difference	Mean ± S.E	% Difference	Mean ± S.E	% Difference	Mean ± S.E	% Difference	Mean ± S.E	% Difference
T3	Control	2.45 ± 0.035a+	−38.4	2.46 ± 0.040a+	−78.5	2.44 ± 0.037a+	−88.5	2.18 ± 0.071a	−14.2	2.05 ± 0.042a+	−66.8	2.19 ± 0.049a+	−80.4
	PTU	1.51 ± 0.151		b+	−64.9	b+ c+	−81.5	d+	+23.8	b+ d+	−63.6	b+ c+ d+	−77.0
				0.53 ± 0.028		0.28 ± 0.029	−47.2	1.87 ± 0.113		0.68 ± 0.039	+28.3	0.43 ± 0.034	−36.8
													+53.6
T4	Control	1.63 ± 0.038a+	−40.5	1.69 ± 0.073a+	−71.6	1.68 ± 0.080a+	−89.3	1.25 ± 0.076a+	−24.0	1.28 ± 0.042a+	−64.8	1.22 ± 0.053a+	−85.2
	PTU	0.97 ± 0.045		b+	−50.5	b+ c+	−81.4	0.95 ± 0.036	−02.1	b+	−52.6	b+ c+	−81.1
				0.48 ± 0.048		0.18 ± 0.018	−62.5			0.45 ± 0.044	−06.3	0.18 ± 0.015	−60.0
													00.0
TSH	Control	1.80 ± 0.077a+	+46.1	1.73 ± 0.076a+	+240.5	1.81 ± 0.081a+	+400.0	1.56 ± 0.093a+	+41.0	1.58 ± 0.058a+	+177.8	1.49 ± 0.068a+	+439.6
	PTU	2.63 ± 0.128		b+	+124.0	b+ c+	+244.1	d	−16.3	b+ d+	+99.5	b+ c+ d	+265.5
				5.89 ± 0.183		9.05 ± 0.190	+53.7	2.20 ± 0.095		4.39 ± 0.391	−25.5	8.04 ± 0.310	+83.1
													−11.2

a: significant at $p < 0.05$ vs control values; a+: highly significant at $p < 0.01$ vs control values. %: difference represents a comparison between control and treated values; b+: highly significant at $p < 0.01$ vs 14 days-treated values. %: difference represents a comparison between 14 days-treated and 21, 28 days-treated values; c+: highly significant at $p < 0.01$ vs 21 days-treated values. %: difference represents a comparison between 21 days-treated and 28 days-treated values; d: significant at: $p < 0.05$ vs young treated values; d+: highly significant at $p < 0.01$ vs young treated values. %: difference represents a comparison between young and adult values.