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Changes in acetylcholinesterase, Na^+,K^+-ATPase, and Mg^{2+}-ATPase activities in the frontal cortex and the hippocampus of hyper- and hypothyroid adult rats

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Abstract

The thyroid hormones (THs) are crucial determinants of normal development and metabolism, especially in the central nervous system. The metabolic rate is known to increase in hyperthyroidism and decrease in hypothyroidism. The aim of this work was to investigate how changes in metabolism induced by THs could affect the activities of acetylcholinesterase (AChE), (Na^+,K^+)− and Mg^{2+}-adenosinetriphosphatase (ATPase) in the frontal cortex and the hippocampus of adult rats. Hyperthyroidism was induced by subcutaneous administration of thyroxine (25 μg/100 g body weight) once daily for 14 days, and hypothyroidism was induced by oral administration of propylthiouracil (0.05%) for 21 days. All enzyme activities were evaluated spectrophotometrically in the homogenated brain regions of 10 three-animal pools. A region-specific behavior was observed concerning the examined enzyme activities in hyper- and hypothyroidism. In hyperthyroidism, AChE activity was significantly increased only in the hippocampus (+22%), whereas Na^+,K^+-ATPase activity was significantly decreased in the hyperthyroid rat hippocampus (−47%) and remained unchanged in the frontal cortex. In hypothyroidism, AChE activity was significantly decreased in the frontal cortex (−23%) and increased in the hippocampus (+21%). Na^+,K^+-ATPase activity was significantly decreased in both the frontal cortex (−35%) and the hippocampus (−43%) of hypothyroid rats. Mg^{2+}-ATPase remained unchanged in the regions of both hyper- and hypothyroid rat brains. Our data revealed that THs affect the examined adult rat brain parameters in a region- and state-specific way. The TH-reduced Na^+,K^+-ATPase activity may increase the synaptic acetylcholine release and, thus, modulate AChE activity. Moreover, the above TH-induced changes may affect the monoamine neurotransmitter systems in the examined brain regions.

1. Introduction

Besides the critical role of thyroid hormones (THs) in brain development and metabolism, recent investigations have highlighted the involvement of these hormones in the adult mammalian brain neurotransmission [1-4]. In particular, a very close association exists between the THs and brain cholinergic function [5]. These effects are mainly observed in specific cholinergic nuclei and their pathways, such as the basal forebrain and the hippocampus [6].

Acetylcholine (ACh) is a very important neurotransmitter for central nervous system (CNS) function. Its action is dependent on its metabolizing enzyme, acetylcholinesterase (AChE, EC 3.1.1.7), which was found to be involved in the release of ACh [7] and to be co-released from the dopaminergic neurons [8]. Thyroid dysfunction has been shown to influence AChE activity in both developing and adult rats [9]. Moreover, Appleyard [10] has reported that AChE induces long-term potentiation in hippocampal pyramidal neurons, suggesting that AChE per se might enhance cognitive function.

Because THs mediate important effects within the CNS, thyroid dysfunction may cause structural, functional, and behavioral alterations [11-16]. Functional consequences of adult-onset hypothyroidism include an inability to produce

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long-term potentiation in rat hippocampus, as well as impaired learning and memory in rats and humans [17]. Moreover, the effect of hypothyroidism on the cerebral cortex resulted in an increase of AChE activity in both young and aged rats and was reflected in an increase of type 1 muscarinic ACh receptors (M1-AChRs) in young rats [9].

An involvement of L-triiodothyronine (T_3) in ACh metabolism in adult cerebrocortical synaptosomes has been suggested by Sarkar and Ray [4], who have reported increased AChE and Mg^{2+}-adenosinetriphosphatase (ATPase) activity in both hyper- and hypothyroidism (single-dose T_3 treatment and propylthiouracil administration, respectively). Mg^{2+}-ATPase is another important enzyme implicated in the maintenance of high intracellular Mg^{2+}, changes of which can control rates of protein synthesis and cell growth [18].

The synaptic plasma membrane Na^+,K^+-ATPase (EC 3.6.1.3) is an enzyme that regulates the action potential that in turn is responsible for the regulation of synaptic transmission by other neurotransmitters [19]. Moreover, Na^+,K^+-ATPase is implicated in metabolic energy production [20] as well as in the uptake, storage, and metabolism of catecholamines [21,22], serotonin [23], and glutamate [24]. Thyroxine (T_4) 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 [25].

In vitro studies conducted by Sarkar and Ray [26,27] have demonstrated a dose-dependent inhibition of Na^+,K^+-ATPase activity in adult rat cerebrocortical synaptosomes by T_3 administration, indicating the involvement of T_3 in the synaptosomal function of adult rats. It is therefore possible that the previously mentioned memory and cognitive dysfunctions may also be attributed (among other reasons) to TH-induced functional alterations in brain Na^+,K^+-ATPase activity.

Data concerning the effects induced by chronic thyroid state alterations on adult rat brain region enzyme activities (such as those of AChE, Na^+,K^+-ATPase, and Mg^{2+}-ATPase) are limited. In a previous in vivo study from our group, conducted on rat whole-brain tissues [28], we found a statistically significant reduction of AChE and Na^+,K^+-ATPase activities in hyperthyroid rats, whereas hypothyroid rats exhibited a reduction in AChE activity and an increase in Na^+,K^+- and Mg^{2+}-ATPase activities. Moreover, in a recent in vivo study of ours [29] that was conducted on adult rat cerebellar and hypothalamic tissues, neither hyper- nor hypothyroidism had any effect on the examined hypothalamic enzymes, whereas in the cerebellum, both hyper- and hypothyroidism induced a statistically significant decrease in the activities of both AChE and Na^+,K^+-ATPase.

The aim of the present work was to assess the activities of AChE, Na^+,K^+-ATPase, and Mg^{2+}-ATPase in the frontal cortex and the hippocampus of adult rats with experimental hyper- and hypothyroidism.

2. Methods

2.1. Animals

Adult male albino Wistar rats (6 months old) were used in all experiments. The rats were housed in groups of 4 in a cage, at a constant room temperature (22°C ± 1°C) under a 12-h light/dark cycle (lights on, 0800-2000 hours). Food and water were provided ad libitum. Animals were cared for in accordance with the principles of the *Guide to the Care and Use of Experimental Animals* [30].

2.2. Experimental hyper- and hypothyroidism

Hyperthyroidism was induced in rats by T_4 administration. L-Thyroxine (Sigma, St Louis, MO) was dissolved in 99% ethanol by adding 20 μL of 25% NaOH and diluted 33 times by adding 0.9% NaCl to obtain a stock solution of 1 mg/mL. Before each injection, a fresh solution was made in 0.9% NaCl to obtain a concentration of T_4 at 50 μg/mL. Thyroxine, 25 μg/100 g body weight, was given subcutaneously once daily for 14 days. On the other hand, hypothyroidism was induced in rats by administration of 6-*n*-propyl-2-thiouracil in drinking water to a final concentration of 0.05% for 21 days. Each treatment resulted in a long-term moderate hyperthyroidism [31] or hypothyroidism [32]. Two controls were used: (*a*) saline controls (SC) that were treated with subcutaneous injections of normal saline given once daily for 14 days (control of hyperthyroid rats) and (*b*) controls without any treatment (NTC) for 21 days (control for hypothyroid rats).

2.3. Tissue preparation

The animals were killed by decapitation and the brain regions (entire frontal cortex and entire hippocampus) were rapidly removed. The tissue was homogenized in 10 volumes ice-cold (0°C-4°C) medium containing 50 mmol/L tris (hydroxymethyl)aminomethane-HCl (Tris-HCl), pH 7.4, and 300 mmol/L sucrose, using an ice-chilled glass homogenizing vessel at 900 rpm (4-5 strokes). Then, the homogenate was centrifuged at 1000*g* for 10 minutes to remove nuclei and debris [33,34]. The protein content of the resulting supernatant was determined according to the method of Lowry et al [35] and the enzyme activities were measured.

2.4. Determination of brain AChE activity

Acetylcholinesterase activity was determined by following the hydrolysis of acetylthiocholine according to the method of Ellman et al [36] as described by Tsakiris [34]. The incubation mixture (1 mL) contained 50 mmol/L Tris-HCl, pH 8, 240 mmol/L sucrose, and 120 mmol/L NaCl. The protein concentration of the incubation mixture was 80 to 100 μg/mL. The reaction was initiated after addition of 0.03 mL of 5,5′-dithionitrobenzoic acid (DTNB) and 0.05 mL of acetylthiocholine iodide, which was used as substrate. The final concentration of DTNB and substrate were 0.125