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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

and 0.5 mmol/L, respectively. The reaction was followed spectrophotometrically by measuring the increase in absorbance ($\Delta\overline{OD}$) at 412 nm.

2.5. Determination of Na^+,K^+-ATPase and Mg^{2+}-ATPace activities

Na^+,K^+-ATPase activity was calculated from the difference between total ATPase activity (Na^+,K^+,Mg^{2+}-dependent ATPase) and Mg^{2+}-dependent ATPase activity (as determined in different samples). Total ATPase activity was assayed in an incubation medium consisting of 50 mmol/L Tris-HCl, pH 7.4, 120 mmol/L NaCl, 20 mmol/L KCl, 4 mmol/L $MgCl_2$, 240 mmol/L sucrose, 1 mmol/L EDTA K_2 salt (K^+-EDTA), 3 mmol/L disodium ATP, and 80 to 100 μg protein of the homogenate in a final volume of 1 mL. Ouabain (1 mmol/L) was added to determine the activity of Mg^{2+}-ATPase. The reaction was started by adding ATP and stopped after an incubation period of 20 minutes by addition of 2 mL of a mixture of 1% lubrol and 1% ammonium molybdate in 0.9 mol/L H_2SO_4 [34,37]. The yellow color that developed was read at 390 nm.

2.6. Statistical analysis

Data were analyzed by 1-way analysis of variance followed by Bonferroni post hoc test. P values <.05 were considered statistically significant.

3. Results

The effects of hyper- and hypothyroidism on the activities of AChE, Na^+,K^+-ATPase, and Mg^{2+}-ATPase in the frontal cortex of adult rats are presented in Table 1. Hyperthyroidism did not seem to affect the examined enzyme activities, whereas hypothyroidism induced a significant decrease in both the AChE (−23%, $P < .001$) and the Na^+,K^+-ATPase (−35%, $P < .001$) activities. The effects of hyper- and hypothyroidism on the hippocampal AChE and Na^+,K^+- and Mg^{2+}-ATPase activities in adult rats are shown in Table 2. In the hippocampus, both hyper- and hypothyroidism provoked a significant increase of the AChE activity (about 22%, $P < .001$) and a significant decrease of the Na^+,K^+-ATPase activity (about −45%, $P < .001$). Mg^{2+}-ATPase activity was found unchanged in both the hyper- and hypothyroid brain regions.

4. Discussion

There is a lack of and inconsistency with(in) the information regarding the effect of the THs on the activity of the enzymes, AChE and Na^+,K^+- and Mg^{2+}-ATPase, which are important for the neurotransmission in the adult rat brain. The inconsistency seems to be attributed to a variety of reasons, such as the experimental use of different animal strains and the use of different experimental protocols (being either in vivo or in vitro, examining different and various brain areas, including variations in dose, way of administration, and type of TH dysfunctions) [4,9,26,38,39].

The present study was carried out to investigate the effect of chronic TH dysfunction on the above enzyme activities in the frontal cortex and the hippocampus of adult rats. The overall analysis of our data revealed that THs affected in various ways the examined parameters in those brain regions. In the case of hypothyroidism, a reduction of AChE activity was observed in the frontal cortex. This is in accordance with the findings of Virgili et al [40], who reported decreased AChE activity in the prefrontal cortex and the striatum of hypothyroid rats. A decrease in AChE activity was also found in a previous work of ours, which concerned the whole brain of hypothyroid rats [28]. On the contrary, hypothyroidism induced a significant increase in hippocampal AChE activity. Why was such a discrepancy traced?

To begin with, there seems to be a regional variation in the rat brain enzyme activity [17,41-44] that could be attributed to the histologically different innervation and functioning of these regions. Different AChE activities among various brain regions have also been confirmed by us [29] in hypothyroid rats. Kundu et al [45] produced hypothyroidism by propylthiouracil administration for 30 consecutive days and measured TH levels in both serum and cerebrocortical synaptosomes, as well as the activities of AChE and Na^+,K^+-ATPase in the cerebral cortex of adult Sprague-Dawley rats.

Table 1
Effects of hyper- and hypothyroidism on AChE, Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in adult rat frontal cortex

Treatment	Activities		
	AChE ($\Delta\overline{OD}$/[min · mg protein])	Na^+,K^+-ATPase (μmol Pi/[h · mg protein])	Mg^{2+}-ATPase (μmol Pi/[h · mg protein])
SC, N = 10	0.358 ± 0.014	4.34 ± 0.26	8.77 ± 0.70
Hyperthyroid, N = 10	0.373 ± 0.016 (NS) (+4%)	4.40 ± 0.22 (NS) (+1%)	8.30 ± 0.75 (NS) (−5%)
NTC, N = 10	0.368 ± 0.018	4.50 ± 0.30	8.48 ± 0.60
Hypothyroid, N = 10	0.284 ± 0.016 * (−23%)	2.91 ± 0.28 * (−35%)	8.23 ± 0.62 (NS) (−3%)

Each value indicates the mean ± SD of 10 independent experiments (10 pools of 3 rat regions). The average value of each experiment was obtained from 4 evaluations in the homogenated brain region tissue of 3 animals. NS indicates not significant. There is no statistically significant difference between the 2 control groups.

* $P < .001$, compared with the respective control values (for details see Methods).

Table 2
Effects of hyper- and hypothyroidism on AChE, Na^+,K^+-ATPase, and Mg^{2+}-ATPase activities in adult rat hippocampus

Treatment	Activities		
	AChE ($\Delta\overline{OD}$/[min · mg protein])	Na^+,K^+-ATPase (μmol Pi/[h · mg protein])	Mg^{2+}-ATPase (μmol Pi/[h · mg protein])
SC, N = 10	0.326 ± 0.014	5.50 ± 0.28	7.19 ± 0.58
Hyperthyroid, N = 10	0.398 ± 0.016 * (+22%)	2.92 ± 0.26 * (−47%)	6.64 ± 0.55 (NS) (−8%)
NTC, N = 10	0.330 ± 0.017	5.65 ± 0.24	6.90 ± 0.62
Hypothyroid, N = 10	0.399 ± 0.019 * (+21%)	3.22 ± 0.22 * (−43%)	6.69 ± 0.60 (NS) (−3%)

Each value indicates the mean ± SD of 10 independent experiments (10 pools of 3 rat regions). The average value of each experiment was obtained from 4 evaluations in the homogenated brain region tissue of 3 animals. NS indicates not significant. There is no statistically significant difference between the 2 control groups.

* $P < .001$, compared with the respective control values (for details see Methods).

They observed that although serum T_4 level increased initially on the second day (compared with controls), serum T_3 decreased in a triphasic pattern (the first phase lasted from the 2nd day to the 6th day, the second phase ended on the 14th day, and the last phase continued till the 30th day). Cerebrocortical synaptosomal T_3 levels increased on the 2nd day (from the control), attained a peak on the 4th day, remained stable until the 18th day, and abruptly declined on the 20th day (it should be noted that synaptosomal T_4 content remained negligible or undetected throughout). This study suggests a central T_3 homeostasis that starts between the 1st and the 2nd day and terminates between the 18th and the 20th day. Moreover, in the same study [45], synaptosomal membrane AChE and Na^+,K^+-ATPase activities exhibited an inverse relationship during the experimental regimen. This relationship was much more prominent on the 2nd, the 18th, and the 20th day, coinciding with the variations in brain T_3 levels.

In our study, we measured the AChE activity only on the 21st day of the experimentally induced hypothyroidism in rat frontal cortex and hippocampus. Taking into account the data from the experiment of Kundu et al [45], we find that there is a time- and, probably, a strain-dependent behavior of these enzyme activities due to variations in brain TH levels. Moreover, it has been reported that T_3 administration in single doses (0.025-4 μg/g) increased AChE activity maximally at 24 hours, in a dose-dependent manner, in both euthyroid and hypothyroid rats [4].

The same argument could be applied for the decrease in Na^+,K^+-ATPase activity, which was observed in our experiment in both of the examined regions, due to hypothyroidism. In addition, this finding is in accordance with those of Pacheco-Rosado et al [46] (decreased Na^+,K^+-ATPase activity in both hypothyroid rat cortex and hippocampus) and those of Kundu et al [45] (but toward the end of their previously described experiment). Nevertheless, in our previous whole-brain study [28], hypothyroidism had induced a significant Na^+,K^+-ATPase increase in its activity.

It is known that Na^+,K^+-ATPase is involved in the maintenance of the polarized condition [19]. It should also be noted that the decrease in Na^+,K^+-ATPase activity can enhance (at least in part) the release of ACh [47], and thus affect in a similar way the AChE and vice versa. Although this seems to be happening in the hippocampus under both hyper- and hypothyroidism, the frontal cortex does not seem to follow.

Hyperthyroidism appears to significantly affect the Na^+,K^+-ATPase activity of the hippocampus (Table 2), but not that of the frontal cortex (which was found unaltered, as shown in Table 1). In our previous whole-brain findings [28], Na^+,K^+-ATPase activity was found significantly decreased. According to Schmitt and McDonough [48], brain Na^+,K^+-ATPase is sensitive to T_3 by as late as 15 days of age, and the period of TH responsiveness is over by 22 days of age. However, a dose-dependent inhibition of Na^+,K^+-ATPase activity in the adult rat cerebral cortex was observed after a single T_3 injection [26].

We are dealing here with chronic moderate hyperthyroidism. Taking under consideration an autoregulatory mechanism that functions to keep brain T_3 concentrations in a homeostatic or stable condition as long as possible (in spite of peripheral TH disturbances) [45,49,50], and also the previously discussed data for hypothyroidism, one can support the idea that this mechanism (or homeostatic phenomenon) might as well exist in the induction of hyperthyroidism after T_4 administration. Furthermore, recent functional brain imaging studies using positron emission tomography with [^{18}F]fluorodeoxyglucose demonstrated that TH treatment with levothyroxine affects regional brain metabolism in patients with hypothyroidism and bipolar disorder [51]. These studies offer corroborative evidence that THs are active in modulating metabolic function in the mature adult brain [51].

A different response to chronic T_4 administration was observed in AChE activity between frontal cortex (unchanged) and hippocampus (increased). No change in the whole-brain AChE activity of T_3-treated rats was reported by Almeida and Santos [52], whereas in our whole-brain previous findings [28], a significant decrease in AChE activity was recorded under T_4-induced hyperthyroidism. By using a different protocol of T_4 administration (2.5 mg/kg for 4 days and 5 or 10 mg/kg every third day for 28 days intraperitoneally), Smith et al [5] found increased AChE activity in both rat frontal cortex and hippocampus in the first dosage regimen. However, this enzyme activity was

shown to be increased only in the hippocampus in the second dosage regimen (28 days). It is likely that among other reasons, the time duration of T_4 administration influences the examined enzyme activities. According to Smith et al, the increased AChE activity by T_4 refers to the increased cholinergic activity and supports their data on the improved (by T_4 administration) rat cognitive performance.

Two questions are important to ask: (*a*) why do these different responses in the activities of AChE and Na^+,K^+-ATPase appear between the 2 examined brain regions under the same thyroid state, and (b) why does the hippocampus seem to react in a similar pattern under hyper- and hypothyroidism (with regard, of course, to the examined parameters)?

It has been observed that the same agent can affect differently the various brain regions [29,46] as well as the different parts of the same brain region [3,53,54]. Furthermore, a different expression of deiodinases in the various brain regions [55], along with the functional heterogeneity of the CNS neurotransmission complex, may be a reflection of the lack of uniformity in the profile of the examined enzyme activities [43,56-59]. In addition, in another field of our investigation involving the THs, we concluded that hyper- and hypothyroidism seem to be 2 examples of states that cannot be considered as opposing in terms of the response of the (hyper- or hypothyroid) heart to ischemic stress, but are 2 different states that induce distinct forms of adaptation to ischemia (possibly through different mechanisms) [32,60,61]. Furthermore, it has been shown that THs regulate differently the expression of certain proteins in the heart in a chamber-specific manner. For instance, T_4 administration has been reported to increase the voltage-gated K^+ channel 1.5 (Kv1.5) messenger RNA levels [62] as well as the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2) protein expression [63] in the left ventricle but not in atrial tissue.

The unchanged Mg^{2+}-ATPase activity in both hyperthyroid examined brain regions is in accordance with our earlier rat findings on whole-brain [28], whereas the hypothyroid enzyme activities were not found increased as expected. Sarkar and Ray [4], having observed an increase of both AChE and Mg^{2+}-ATPase activities after single doses of T_3 in euthyroid and hypothyroid rats, suggested a stimulation of ACh metabolism by increasing AChE activity and ACh uptake, through an increase in synaptosomal Mg^{2+}-ATPase activity. Again, our results could be indicative of a different (state- and region-dependent) Mg^{2+}-ATPase behavior.

In conclusion, our data revealed that THs affect the examined adult rat brain parameters in a region- and state-specific way. It is not possible, however, at the moment, to identify a definite factor in order to explain the observed enzymatic alterations due to hyper- or hypothyroidism. Nevertheless, one should not leave out the possibility that the TH-reduced Na^+,K^+-ATPase activity may increase the synaptic ACh release and, thus, could modulate AChE activity (at least in the hippocampus). It appears likely that the above TH-induced changes may affect in a different way the implicated monoamine neurotransmitter systems as well as other enzymatic parameters of the examined brain regions. The matter requires further investigation.

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