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Effect of adult onset hypothyroidism on behavioral parameters and acetylcholinesterase isoforms activity in specific brain regions of male mice

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HIGHLIGHTS

- Adult male mice were rendered hypothyroid through 1% w/v $KClO_4$ administration
- Passive avoidance task supported learning and memory deficits in hypothyroid mice
- EPM and OF tests indicated anxiety-like behavior and reduced locomotor activity
- Hypothyroidism caused brain region-specific inhibition of AChE fractions activity

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ABSTRACT

Thyroid hormones (TH) are essential for normal development and function of mammalian central nervous system (CNS); TH dysregulation has been implicated in several cognitive and behavioral deficits related to dysfunctions of neurotransmitter systems. In the present study, we investigated the effects of adult onset hypothyroidism on the activity of acetylcholinesterase (AChE) and on related behavioral parameters. For this purpose we used adult male Balb/cJ mice that were divided randomly into euthyroid and hypothyroid animal groups. Animals were rendered hypothyroid through administration of 1% w/v $KClO_4$ in their drinking water for 8 weeks. At the end of the treatment, learning/memory procedures were examined through step-through passive avoidance task while fear/anxiety was assessed using elevated plus-maze (EPM) and open-field (OF) tests. AChE activity was determined colorimetrically in two different fractions, salt-soluble fraction (SS) (containing mainly the G1 isoform) and detergent-soluble fraction (DS) (containing mainly the G4 isoform) in cerebral cortex, cerebellum, midbrain, hippocampus and striatum. Our results indicate that adult onset hypothyroidism caused significant memory impairment and increased fear/anxiety. Moreover, the activity of both isoforms of AChE was reduced in all brain regions examined in a brain region- and isoform-specific manner.

1. Introduction

Thyroid hormones (TH) are essential for normal brain development and function by mediating important effects within the central nervous system (CNS) throughout life [1–3]. TH effects are predominantly mediated by their binding on nuclear thyroid hormone receptors (TR), which act as transcriptional factors regulating the expression of specific thyroid hormone responsive genes [4], while, non-genomic TH actions have also been studied [5,6].

It is well established that, during development, TH are involved in the regulation of myelination, neural cell proliferation and formation of synapses [7]. Although most studies focus on the defects of perinatal hypothyroidism on CNS [8–10], it has been shown that adult onset hypothyroidism also exerts adverse effects on neurotransmission and behavior [11–13]. Of particular interest is that, adult onset hypothyroidism is closely related to neuropsychiatric and cognitive disorders [14]. One of the most prominent features of neural action of TH in adulthood is subsensitivity to norepinephrine as a result of a hypothyroid state [15]. Moreover, thyroid hormone deficiency increases the probability of depressive illness [16], whereas thyroid excess increases the probability of mania in susceptible individuals [15]. In addition, increased fear/anxiety and depression-like behavior, decreased locomotor activity

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and loss of memory have been established also in hypothyroid rodents [12,17].

However, although the critical role of thyroid hormones in cognitive function [18] and anxiety [13] is well documented, the underlined mechanism remain elusive. Appleyard has reported that AChE induces long-term potentiation in hippocampal pyramidal neurons, suggesting that AChE per se might enhance cognitive function [19]. In this context, it is worth mentioning that the termination of the nerve impulse in cholinergic synapses is mediated by AChE which catalyses the rapid hydrolysis of acetylcholine (ACh) into acetylCoA and choline. AChE exists into different molecular forms which involve asymmetric forms (An) and globular forms (Gn) containing one, two or four catalytic subunits (monomeric G1, dimeric G2, and tetrameric G4, respectively). In the brain, AChE is present in G1 (cytosolic) and G4 (membrane bound) isoforms, that are differentially localized in brain regions [20,21]. G4 is the predominant isoform in the mature brain [22] and has been implicated in cognitive functions, strongly indicating a correlation between cholinergic system and behavior that has been emerged in many studies [23–28]. In addition, increasing experimental evidence support an extensive interrelationship between TH and brain cholinergic system [29–31]. However, a comprehensive study of the effects of adult on set hypothyroidism on both G1 and G4 AChE isoforms has never been performed so far and most studies focus on the investigation of developmental hypothyroidism on cholinergic system function with emphasis on total or G1 AChE isoform activity [8,32–34] To this end, recently, it has been reported that TH have a direct effect on total AChE activity and consequently on the metabolism of acetylcholine at least in the hippocampus of adult rodents [35].

In the light of current studies, revealing TH along with AChE inhibitors as a combined pharmacological treatment of hippocampus synaptic protein impairment induced by adult onset hypothyroidism [35], the aim of the present study was to comprehensively investigate the effects of adult onset hypothyroidism on learning/memory and fear/anxiety behavior and on the activity of two different AChE isoforms in five different brain regions of adult male Balb/cJ mice. Although most relevant reports focus on cerebral hemispheres and hippocampus, we expand the current knowledge by investigating concomitantly cerebellum, midbrain and striatum along with cerebral hemispheres and hippocampus since our previous publications strongly suggest that cholinergic system function of all these brain regions affects differentially learning/memory and fear/anxiety behavior [36]. In addition, our and others previous reports revealed that these brain regions respond differentially to adult onset hypothyroidism with regard to several molecular and biochemical parameters [37–39]. Based on our results we discuss the possible relevance of the behavioral changes observed in adult onset hypothyroid animals with the activity of the salt-soluble (SS) AChE fraction, containing mostly the G1 AChE isoform and the detergent-soluble (DS) AChE fraction, containing mostly the G4 AChE isoform.

2. Materials & methods

2.1. Chemicals

Acetylcholine iodide (AChI), albumin (BSA), Tris-HCL, $MgCl_2(H_20)_6$ and 5,5′-Dithiobis [2-nitrobenzoic acid] (DTNB) were obtained from Sigma-Aldrich. DTNB was protected from light.

2.2. Animals

Balb/cJ male mice, 4 month-old and weighted 22–25 g, were kept in polyacrylic cages (38 × 23 × 10 cm) and housed under controlled temperature (24–26 °C), relative humidity (50–60%) and a 12-h light-dark cycle. They had ad libitum access to liquid and food in form of dry pellets (feed composition: grain and grain by-products oil seed products, minerals, vitamins and trace elements from Altromin Spezialfutter GmbH& Co. KG. Lage, Germany). The mice were randomly divided into two groups: the control group (n = 16) (euthyroid mice) and the treated group (n = 16) (hypothyroid mice). Each animal group was further divided into two subgroups (n = 8) each of which were used for the assessment of different behavioral parameters (intensity of anxiety and learning/memory performance). The animal subgroup used for the study of anxiety was also used for the biochemical part of our work. The adult onset hypothyroidism was induced through daily administration of 1% w/v aqueous solution of potassium perchlorate ($KClO_4$) for a period of 8 weeks [40]. The $KClO_4$ was freshly prepared two times per week during the experimental period. The body weight, food and liquid intake were measured weekly in both animal groups throughout the treatment period. All animal studies were governed by the EU guidelines of the Protocol for the Protection and Welfare of Animals (EU Directive 2010/63/EU) and Greek National Laws (Animal Act, PD 160/91) and authorized by the Veterinary Authority of the Prefecture of Western Greece.

2.3. Behavioral testing

2.3.1. Learning/memory: Step-through passive avoidance task

The test is based on the negative reinforcement to examine long-term memory [41]. It was performed on two consecutive days according to previously described procedures, using a two-compartment passive avoidance apparatus (white/dark, separated by a black wall with a guillotine door in the middle part). In the present study we used this particular test because it is useful for evaluating the effect of chemical entities on learning and memory through studying fear conditioning emotional memory and the mechanisms involved in cognition that have been shown to be affected in hypothyroidism [18].

In more detail, on day-53 (week 8) of the treatment period, the animals were allowed to habituate in the experimental room for 1 h prior to experiments. One hour later, each mouse was placed in the illuminated chamber for the acquisition trial, the guillotine door was opened and the animal was allowed to enter the dark compartment. The Initial Latency (IL) with which the animal crossed into the dark compartment was recorded (maximum time allowed was 120 s). Once the animal crossed with all four paws to the next compartment, the guillotine door was closed and a foot shock (25 V, 3 mA, 5 s) was immediately delivered to the grid floor of the dark room. Thereafter, the mouse was immediately removed from the apparatus and returned to its home cage. Animals that waited >120 s to enter the dark compartment were eliminated from the experiments. 24 h after training, a retrieval test was performed to determine long-term memory. Each animal was placed in the light compartment, the door was opened, and the step-through latency (STL) was measured for entering into the dark compartment. The test session ended when the animal entered the dark compartment or remained in the light compartment for 300 s (criterion for retrieval). During these sessions, no electric shock was applied. All training and testing sessions were carried out during the light phase between 08:00 and 14:00 h.

2.3.2. Anxiety-like behavior

The effect of adult onset hypothyroidism on anxiety-like behavior was assessed using two behavioral tests, based on two different factors causing fear/anxiety, the height (Elevated plus-maze test — EPM) and the open field (OF test). All animals performed the EPM test 24 h after performing the OFT.

2.3.2.1. Elevated plus-maze test. Elevated plus-maze test (EPM) is a rapid and selective technique which is used for detecting anxiety-like behavior [42]. The apparatus is in a shape of cross and consists of two arms without walls (opened arms) (30 × 5 × 0.25 cm) and two arms with walls and an open roof (closed arms) (30 × 5 × 15 cm) emanating from a common central platform (5 × 5 cm). Two pairs of identical arms were opposite to each other offering the motivation of exploration and the safety, respectively. The entire apparatus was elevated to a