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Antidepressant behavior in thyroidectomized Wistar rats is induced by hippocampal hypothyroidism

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HIGHLIGHTS

- Thyroidectomy reduces all basic elements associated with the thyroid hormones metabolism in hippocampus.
- Thyroidectomized rats show antidepressant behavior.
- Antidepressant behavior in thyroidectomized rats is induced by hippocampal hypothyroidism.

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ABSTRACT

Thyroidectomy is a surgical procedure indicated in cases of several maligned or benign thyroid diseases, thus, the aim of our study was to verify how the hypothyroidism induced by thyroidectomy influences behavioral parameters and its relation to thyroid hormones metabolism and neurogenesis at hippocampus. For this purpose, Adult male Wistar rats underwent to thyroidectomy to induce hypothyroidism. Behavioral tests, the thyroid profile and hippocampal gene expression were evaluated in control and in thyroidectomized animals. It was observed that thyroidectomized group had a significant increasing in serum thyroid-stimulating hormone (TSH) and a decreasing in thyroxine (T4) levels as well as in triiodothyronine (T3) serum level. It was also observed reduction of the monocarboxylate transporter 8 (*Mct8*), thyroid hormone receptor alfa (*Trα1*), deiodinase type 2 (*Dio2*), ectonucleotide pyrophosphatase/phosphodiesterase 2 (Enpp2) and brain-derived neurotrophic factor (*Bdnf*) mRNA expression in hippocampus of thyroidectomized animals. In the forced swimming test, it was verified that thyroidectomy promotes a decrease in time of immobility and climbing when compared with the control group. In summary, we demonstrated that antidepressant behavior in thyroidectomized Wistar rats is induced by hippocampal hypothyroidism. This effect could be associated to an impaired neuronal activity in acute stress response as it is observed in forced swimming paradigm.

1. Introduction

Thyroid hormones (THs) are produced by the thyroid gland and play a very important role in the development and homeostasis of the central nervous system (CNS) [5, 48]. The actions of THs are mediated by gene expression after interaction with thyroid hormone receptor isoforms (TRs): TRα1, TRβ1 and TRβ2, members of the nuclear hormone receptor family, that are encoded by TRα and TRβ genes, respectively [11, 26]. Notably, TRα1 comprises 70% to 80% of all TR expression in the adult vertebrate brain and this receptor is present in nearly all neurons [43]. Besides, it is noteworthy that deiodinase type 2 (Dio2) and monocarboxylate transporter 8 (MCT8) expression is also indispensable for regulation of glia-neuron cell interaction in TH metabolism in the CNS [42].

One of the most important effects of THs is their participation in neurogenesis, especially in perinatal growth [42]. Nonetheless, it is well known that the hippocampus exhibits morphological plasticity throughout adult life [10, 17, 21]. In this limbic structure, hypothyroidism causes reduced growth, reduced number of cells in the dentate

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gyrus, as well as abnormal neuronal migration and maturation [24, 30]. Moreover, clinical findings include cognitive, attention and mood disorders such as depression [4, 8, 16, 20, 22].

In this context, in recent years, a large number of scientific articles has appeared on the subject of relationships between psychiatric disease and thyroid hormones [19, 23]. Both excess and insufficient thyroid hormones can cause mood abnormalities, including depression, which is generally reversible with proper thyroid treatment [19]. Although thyroid disease is rare in depression, 1% to 4% of patients with affective disorders are found to have overt hypothyroidism while subclinical hypothyroidism occurs in 4% to 40% of these patients [50].

Besides serum levels of THs, it has been suggested that some patients may be suffering from "central hypothyroidism". This clinical condition could be related to: the inability of the hormone to enter into neuronal cells, inappropriate deiodination, TR desensitization or down regulation [32, 37]. Based on this premise, more recently, a prevalent polymorphism in Dio2 has been associated with clinical syndromes. This polymorphism results in a single amino acid change within the Dio2 molecule where its susceptibility to ubiquitination and proteasomal degradation is regulated [32].

Although thyroidectomy is a surgical procedure indicated in certain cases of malignant or benign thyroid disease [27, 28, 47], up until now, no studies have been published on whether this surgical method triggers affective disorders in experimental models of hypothyroidism. Thus, the aim of our study was to determine how thyroidectomy influences behavioral parameters and its relation to metabolism and neurogenesis in the hippocampus.

2. Material and methods

2.1. Subjects

Sixty-day-old male Wistar rats (~250 g) derived from the Federal University of São Paulo (UNIFESP) colony were used in this protocol. All animals were housed in plastic cages (35 cm × 50 cm × 20 cm) at a controlled temperature (21 ± 2 °C) with daily exposure to a 12 h light-dark cycle and free access to water and standard rodent chow. This investigation was carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the institutional animal welfare committee in accordance with pertinent Brazilian legislation under Protocol number: "078 on p.59 of register 02."

2.2. Treatment

A hypothyroidism model was induced surgically by thyroidectomy. For this purpose, animals were anesthetized with ketamine (10 mg/kg) and xylazine 100 mg/kg). After the surgery, thyroidectomized rats underwent methimazole treatment (0.03% in tap water) for 20 days, keeping a normal diet. The sham group (euthyroid animals) underwent the same procedure without thyroid removal. After the treatment, the animals were subjected to behavioral tests and euthanized. The serum was collected to assess triiodothyronine (T3), thyroxine (T4) and thryroid-stimulating hormone (TSH) levels and the hippocampus was dissected from the whole brain under cold plate and kept at −70 °C for subsequent RNA extraction.

2.3. Serum hormone measurements

Serum TSH was measured by in-house competitive assay, using reagents from NIDDK (National Institute of Digestive and Kidney Diseases) with analytical sensitivity of 0.2 ng/ml, and T4, T3 were determined by electrochemiluminescence immunoassay method (Elecsys® Systems Roche Diagnostic Kit) following the recommendations of the manufacturer.

2.4. RNA isolation and quantitative real-time RT-PCR

Total RNA was isolated from frozen hippocampus (weighing 100 mg) with TRIzol Reagent® (Life Technologies Corporation, Carlsbad, CA) according to the manufacturer's protocol. RNA integrity was checked by agarose gel electrophoresis, and RNA purity reached the following criteria: A260/280 ≥ 1.8. The extracted total RNA concentration was measured using a Nanodrop spectrophotometer (ND-1000) (Bio-Rad, USA), and 1 μg of total RNA was subjected to reverse transcription procedure. Complementary DNA (cDNA) synthesis was generated using MML-V Reverse Transcriptase kit (Promega, Madison, WC) according to manufacturer's protocol. Quantitative real-time PCR (qPCR) was carried out using the QuantiTech SYBR green PCR kit (Qiagen, Valencia, CA) and ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) which 2 μl of cDNA was used to amplify specific primers sequences for *Dio2*, *Mct8* (*Slc16a2*), *TRα-1*, *Enpp2* and *Bdnf*. The forward and reverse primers sequences were, respectively: 5′-AGAAGCACCGGA ACCAAGAG-3′ and 5′-AGCCACAACTTGACACTGGG-3′ for *Dio2*; 5′-CCCA AGCAAGAGAGGCGCCC-3′ and 5′-CGGTAGGTGCGCTGGCGAAA-3′ for *Mct8*; 5′-ACCTCCGCATGATCGGGGC-3′ and 5′-CCTGATCCTCAAAGAC CTC-3′ for *TRα-1*; 5′-TCCTTCCTCACCGACCCGAC-3′ and 5′-GCACCCGA GCTGTGTGCATCT-3′ for *Enpp2*; 5′-TCCTTCCTCACCGACCCGAC-3′ and 5′-GCACCCGAGCTGTGTGCATCT-3′ for *Bdnf*; 5′-GTCAACCCCACCGTGTTC TTC-3′ and 5′-ACTTGCCACCAGTGCCATTATG-3′ for *Cyclophilin-A* used as internal control. The procedure consisted of an initial step 10 min at 95 °C followed by 45 cycles of 20 s each at 95 °C, 20 s at 58 °C, and 20 s at 72 °C. Gene expression was determined by *C*t, and all values were expressed using cyclophilin A mRNA as an internal control [29].

2.5. Behavioral tests

Rats underwent the following tests: open field test and forced swimming test. It should be emphasized that the tests were performed with an interval of two days and the order of each battery of tests was determined according to the degree of invasiveness. In all them, the number of animals used ranged from 7 to 9 individuals in each experimental group. All tests were carried out from 11:00 am to 2: 00 pm. During each test, the experimenter remained outside the testing room except between the trials. Each test was recorded from an overhead view and behavior parameters were analyzed later by at least two observers.

2.5.1. Open field test

The animals were placed individually in a white acrylic cage (80 cm × 80 cm × 30 cm). Each rat was placed in the center of the experimental apparatus immediately before the test and allowed to explore it for five minutes. During this time, the test was subsequently recorded and analyzed by the ANY-Maze data collection program (Stoelting Co., Wheat Dale, IL, USA). Total distance traveled, number of rearings (standing on hind legs with paws pressed against the wall of the arena) episodes and time of grooming, time in center zone, center distance (the distance traveled in the center of the arena), time in corner zone, inactivity time and center ratio (center distance: total distance ratio) were assessed. At the end of testing, the number of fecal pellets was also measured and the arena was cleaned with a 10% ethanol solution. In this test, locomotor activity is indicated as the total distance traveled in the apparatus while the vertical activity is assigned as number of rearings. Furthermore, the time in the corner zone and number of fecal pellets were used to assess emotional reactivity [3, 49]. Lastly, the anxiety-like responses are linked to the time spent in the central zone and central ratio.

2.5.2. Forced swimming test

This test is based on the observation that rodents, after performing initial escape-oriented movements, develop an immobile posture when placed in a stressful inevitable situation [40]. In this test, rats were placed individually in a polypropylene cylinder measuring 25 cm, containing water at 25 °C at a depth of 50 cm. Thus, the animals