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Research report

A behavioral and micro positron emission tomography imaging study in a rat model of hypothyroidism

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HIGHLIGHTS

- The mechanism underlying the depressive behavior in hypothyroid rats is detected.
- Behavioral abnormalities are observed in rats with adult onset hypothyroidism.
- Behavioral changes are related to reduce glucose metabolism in several brain areas.
- Decreased sucrose preference in hypothyroid rats may be attributed to anhedonia.

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ABSTRACT

Hypothyroidism leads to somatic, neuropsychological, and psychiatric changes that are similar to depression. The mechanisms underlying the behavioral abnormalities in adult onset hypothyroidism remain ambiguous. Hypothyroidism was induced in adult male Wistar rats by the maintenance of 0.05% propylthiouracil (PTU) in drinking water for 5 weeks (hypothyroid group; HP group); control rats (CON group) received an equivalent amount of water. The open field and sucrose preference tests were employed, and the link between behavioral changes and brain glucose metabolism was evaluated using micro positron emission tomography imaging. The open field test revealed slightly decreased locomotor activity and significantly reduced rearing and defecation in the hypothyroid group. Hypothyroid rats were also characterized by decreased body weight, sucrose preference, and relative sucrose intake compared to control rats. Hypothyroidism induced reduced brain glucose metabolism in the bilateral motor cortex, the caudate putamen, the cortex cingulate, the nucleus accumbens, and the frontal association cortex. A decreased sucrose preference was positively correlated with metabolic glucose changes in the caudate putamen and the nucleus accumbens. The results indicate that the activity pattern in adult onset hypothyroidism is different from the activity pattern when hypothyroidism is induced in the developmental period of the central nervous system. Decreased sucrose preference in hypothyroid rats may be attributed to anhedonia. Furthermore, these findings suggest there may be a common mechanism underlying adult onset hypothyroidism and depression.

1. Introduction

Thyroid hormones are essential for the normal development of the central nervous system and the maintenance of its proper function throughout life. It has been well documented that maternal hypothyroidism can retard a child's neuropsychological development because the fetal thyroid is unable to synthesize thyroxine (T_4) until 12–14 weeks of gestation. Adult onset hypothyroidism may lead to a variety of somatic, neuropsychological, and psychiatric symptoms, including psychomotor slowing, fatigue, an inability to concentrate, deficits in memory and learning, depression, and anxiety [3,2]. The mechanisms underlying adult onset thyroid dysfunction and these clinical phenomena remain unclear.

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Among the complications experienced in hypothyroid patients, the most common symptoms include cognitive impairment and depression, which are typically accompanied by cerebral blood flow and metabolic changes [3]. The first study that evaluated cerebral metabolism in hypothyroidism was performed in 1950 by Scheinberg et al. [25] who revealed a 38% reduction in global cerebral blood flow (CBF) and a 27% reduction in cerebraloxygen and glucose consumption using a non-radionuclide technique. These findings were interpreted as primarily resulting from changes in cerebral vascular resistance, which showed a 91% increase in that study. Recent studies have employed brain imaging techniques in an attempt to identify the relationships between clinical symptoms and metabolic changes in specific brain regions. In a positron emission tomography (PET) study performed in newly diagnosed hypothyroid patients [7], there was a generalized decrease in regional CBF and glucose metabolism without local deficits, indicating that brain function was globally affected in severe hypothyroidism of short duration. In contrast, Bauer et al. [4] reported lower regional metabolism in hypothyroid patients in the bilateral amygdala, the hippocampus, the perigenual anterior cingulate cortex (ACC), the left subgenual ACC, and the right posterior cingulate cortex. Moreover, the metabolic activities in the bilateral middle frontal gyrus and the right subgenual and dorsal ACC covaried negatively with the severity of depressive symptoms in this study. Thus, the authors proposed that a long duration of hypothyroidism induces regional metabolic brain changes, while a rapid decrease in circulating thyroid hormones after thyroidectomy induces global effects.

In animal studies, various behavioral tests have been employed in the evaluation of hypothyroid models. Behavioral defects have been demonstrated, including locomotor abnormalities in the open field test, immobility behavior in the forced swim test, and spatial learning and memory deficits in the Morris water maze. To the best of our knowledge, brain imaging techniques have not been applied in the evaluation of adult onset hypothyroidism in rats. In the present study, we used micro PET imaging to evaluate the glucose metabolic state of hypothyroid rat brains to identify the links between metabolic abnormalities and behavioral deficits.

2. Material and methods

2.1. Animals

This study was approved by the Animal Research Committee of Dalian Medical University. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twenty healthy adult male Wistar rats (approximately 8 weeks of age) were used in this study. All animals were randomly divided into two groups and housed two per cage at a room temperature of $22 \pm 1\,^{\circ}C$ and a humidity of 50–60%. Animals were housed on a 12 h/12 h light/dark cycle, with lights on from 8 AM to 8 PM daily. During the treatment period, rats in the hypothyroid group (HT group) were maintained on ad libitum access to 0.05% propylthiouracil (PTU) dissolved in water for 5 weeks, and control rats (CON group) received an equivalent amount of water. PTU is an anti-thyroid drug that inhibits the deiodination of T_4 in peripheral tissues. The dose of PTU was chosen based on several studies which showed 0.05% PTU induced a significant reduction in serum 3,5,3′-triiodothyronine (T_3) concentration and decreased cellular thyroid hormone activity in both peripheral tissues and the brain after 5 weeks [12,8,18]. Laboratory chow was provided ad libitum for both groups unless otherwise noted. All animals were acclimatized to the experimental environment for 1 week prior to testing.

2.2. Verification of hypothyroidism

All rats were euthanized at the end of the experiment. Blood samples were collected by cardiac puncture and then centrifuged at 4000 rpm for 7 min. The plasma was collected and stored at $-20\,^{\circ}C$ until the total T_4 (TT_4) and total T_3 (TT_3) levels were analyzed via a radioimmunoassay procedure.

2.3. Open-field test

The open field arena consisted of a 100 cm × 100 cm × 40 cm wooden box with a black square base and black walls. The box was divided by 1 cm white lines into 25 squares, including nine central squares and sixteen peripheral squares. The procedure was performed under dim light (approximately 80 lux) in a quiet room in the morning, and the animals were not disturbed for at least 12 h prior to the test. Rats were placed one by one in the center of the arena and allowed to explore freely for 5 min. During the procedure, the rat's behavior was recorded with a video camera for further analysis, which was completed separately by two experienced technicians who were blind to the animal's status. Behaviors were classified and counted as follows: the overall activity during the 5 min test, the number of lines crossed in the peripheral section, the number of lines crossed in the central section, the first min of activity, the number of lines crossed with the body inclined vertically ("rearing"), the number of feces boli excreted, and the grooming behaviors (e.g., combing, licking, and washing). The arena was thoroughly cleaned with a 5% ethanol solution before each test. The open-field test was performed before and after the experiment for both groups.

2.4. Sucrose preference test and body weight

The sucrose preference test was employed to determine anhedonia. Animals were food and water deprived for a period of 20 h and then given free access to two drinking bottles (water or 1% sucrose) placed side-by-side at the rear of the cage for 1 h. The relative position of the two bottles was varied randomly from trial to trial and interchanged in the middle of each trial. Fluid consumption was measured by weighing the drinking bottles before and after testing. During the sucrose preference test, the pair-housed animals were separated, and each animal was tested in one cage for 1 h. Sucrose preference was calculated by the following formula: sucrose preference = sucrose solution intake (g)/total fluid intake (g). The sucrose preference test and animal weighing were performed before PTU administration and then weekly throughout the experiment under identical conditions.

2.5. [F-18] FDG micro PET scan and data analysis

The Siemens Inveon micro PET/CT system was used for rat brain imaging. Prior to the [F-18] FDG injection, the animals were fasted for approximately 12 h to enhance tracer uptake into the brain. Each rat was placed on a heating pad at $30\,^{\circ}C$ for at least 30 min prior to tracer injection and until the end of the image acquisition. The rats were injected i.v. with 0.5 mCi FDG through the tail vein and were then returned to the home cage. To immobilize the animals, all scans were performed under isoflurane anesthesia (5% induction, 2–2.5% maintenance), and the head was fixed with tape to prevent slight movements during acquisition. Anesthesia began at the end of the 40 min uptake period to ensure it did not interfere with brain glucose metabolism. Functional static acquisition was performed for 15 min in a three-dimensional mode. Brain data were analyzed with Pmod 3.16, and the brain regions were manually extracted from the micro PET images. These images were then normalized to a [F-18] FDG rat brain template. The standard uptake value (SUV)