Brain Research Bulletin 124 (2016) 198–205

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Lateral habenula as a link between thyroid and serotoninergic system modiates depressive symptoms in hypothyroidism rats

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 10 May 2016
Accepted 12 May 2016
Available online 13 May 2016

Keywords:
Hypothyroidism
Depression-like behavior
Lateral habenula
Dorsal raphe nucleus
Serotonin
Forced swimming test

ABSTRACT

Depression-like behavior is observed in both rats and people with hypothyroidism, which suggests that altered thyroid hormone levels are closely associated with mental illness. Furthermore, decreased serotonin (5-hydroxytryptamine, 5-HT) levels are found in some brain regions of hypothyroid rats with depression-like behavior. However, the mechanism underlying the effects of hypothyroidism on the central serotonin system is unclear. The lateral habenula (LHb) is related to both the serotonin and thyroid systems and also plays an important role in the pathogenesis of depression. Our study aimed to disclose the role of the LHb in the onset of depression-like behavior in thyroidectomy (TD) rats. Forced swimming (FST) and open-field tests (OFT) were performed to measure behavioral changes in TD rats. The expression of β calmodulin-dependent protein kinase type II (β CaMKII) in the LHb, cytochrome C oxidase (COX) activity in the LHb and dorsal raphe nucleus (DRN), and 5-HT levels in the DRN were assayed. We found that TD rats exhibited depression-like behavior in the FST and OFT. Compared with the sham group, neural activity and the expression of β CaMKII in TD rats were higher in the LHb, and neural activity and 5-HT levels were lower in the DRN. Depressive behavior and decreased 5-HT levels in the DRN in TD rats were reversed by LHb lesioning. Our study indicates that depression-like behavior in TD rats can be attributed to decreased 5-HT levels in the DRN resulting from inhibition by an overactive LHb. The LHb mediates the effect of the thyroid system on 5-HT function in the DRN.

1. Introduction

Thyroid hormone plays an important role in the development, metabolism, and function of most organs, and disturbances in thyroid function could result in the dysfunction of many organs. It has been reported that hypothyroidism has a close relationship with psychiatric disorders such as depression (Colin and Vijay, 2013). Patients with hypothyroidism usually display various types of depression-like behavior, such as cognitive dysfunction, apathy, dull reactions, and poor sociability. Decreased serum levels of triiodothyronine (T3) can be detected in most patients with depression (Kamble et al., 2013), thus thyroid hormone has also been used as an adjunct to clinical antidepressant therapy (Bauer and Whybrow, 2001; Bauer et al., 2002).

The pathogenesis of depression is complex, and might be closely related to disorders of monoamine systems, the social environment, individual characteristic, endocrine changes, and mental illnesses (Hamon and Blier, 2013; Studd, 2015). Many studies have focused on deficiencies of monoamine neurotransmitters, especially serotonin (5-hydroxytryptamine, 5-HT), because of its important role in the onset of depression (Stockmeier, 2003; Oquendo et al., 2007). It has been reported that 5-HT levels in the serum and cerebrospinal fluid are decreased in patients with depression (Gao et al., 2008) and serotonin reuptake inhibitors have been used clinically as antidepressants (Lanzenberger et al., 2012), which undoubtedly demonstrates that decreased 5-HT levels play a key role in depression. Other factors related to depression may also influence the serotonergic system. Interestingly, there is a close functional interaction between the hypothalamus-pituitary-thyroid (HPT) axis and the serotoninergic system (Sullo et al.,

Abbreviations: β CaMKIIβ, calmodulin-dependent protein kinase type II; COX, cytochrome C oxidase; DRN, dorsal raphe nucleus; FST, forced swimming test; HPT, hypothalamus-pituitary-thyroid; 5-HT, 5-hydroxytryptamine; LHb, lateral habenula; OFT, open-field test; T3, triiodothyronine; T4, thyroxine; TD, thyroidectomy; TSH, thyroid-stimulating hormone.

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http://dx.doi.org/10.1016/j.brainresbull.2016.05.007
0361-9230/

2011). It has been reported that the levels of monoamine neurotransmitters are lower in the serum and some brain region of rats with hypothyroidism induced by propylthiouracil, a thiourea antithyroid agent (Hassan et al., 2013), and decreased 5-HT levels in the serum have also be found in hypothyroidism patients with depression (Stipcevic et al., 2009). This indicates that depression-like behavior resulting from hypothyroidism is associated with decreased levels of central 5-HT, but the mechanism underlying the effects of thyroid disorders on the central serotoninergic system is unknown and the key region that connects the HPT axis and serotoninergic systems has yet not to be identified.

The dorsal raphe nuclei (DRN) is the principal region involved in 5-HT synthesis and release in the brain, and the DRN lesion could result in a significant decrease of central 5-HT levels (Yalcin et al., 2008). The lateral habenula (LHb) is the key region controlling the DRN, and neuronal activity in the DRN can be inhibited by electrical stimulation of the LHb (Wang and Aghajanian, 1977). A large body of evidence has shown that the LHb acts as a key area in the onset of depression both in experimental animals and clinical patients (Sartorius et al., 2010; Yang et al., 2014; Luo et al., 2015). Activity of the LHb was obviously elevated in four different animal models of depression (Caldecott-Hazard et al., 1988; Shumake et al., 2003). Yang's research has indicated that the LHb lesion could improve the depression-like behavior in rats via increasing 5HT levels in the DRN (Yang et al., 2008). Furthermore, an enlarged LHb and decreased serum thyroxine (T4) could be detected in rats after excision of the cervical neural ganglion (Tomalik et al., 1988). Other studies have shown that thyroid hormone receptors are expressed in LHb neurons (Nobrega et al., 1997; Alessandra et al., 2012). These studies together indicate that the LHb may be involved in the pathogenesis of depression resulting from hypothyroidism by mediating the effects of the HPT axis on the serotonergic system in the DRN.

In this study, we first investigated the behavioral changes in rats induced by thyroidectomy (TD) using forced swimming test. Then we detected the changes of activity in the LHb and DRN by cytochrome-c oxidase (COX) histochemistry assay in TD rats. The expression of βCaMKII, whose high expression was considered to be related to the increased activity of the LHb in depression model rats (Li et al., 2013), was also detected in LHb of TD rats. We finally elucidated the role of the LHb in the onset of depressive behavior induced by thyroidectomy and the underlying mechanism.

2. Materials and methods

2.1. Animals

Female Wistar rats, aged 8 weeks with body weights of 200–220 g, were used in the experiments. They were housed in standard cages with free access to food and water under a 12-h light/dark cycle (lights on at 7:00 am) in a room with constant temperature ($22 \pm 1\,^{\circ}C$) and humidity (50–60%). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Research committee at Jilin University.

A total of 70 rats were used in the experiment. Twenty-two rats, including 12 TD rats and 10 TD-sham rats, received behavior test and cytochrome-c oxidase (COX) histochemistry assay in LHb and DRN. Eighteen rats were divided into TD group($n = 10$) and TD-sham group($n = 8$) for detecting of β CaMKII in the LHb by Western blot. Twenty-three rats were divided into TD + LHb-lesion group($n = 8$), TD + LHb-sham-lesion group($n = 9$) and TD-sham group($n = 6$) for detecting the changes of depressive-like behavior and 5-HT level in DRN after bilateral LHb lesion. Five rats died during the procedure and two rats were not successfully induced for the LHb lesion, thus these rats were excluded from the experiment.

2.2. Animal model of hypothyroidism

Rats were divided randomly into two groups: thyroidectomy group and sham groups. After being anesthetized with 10% chloral hydrate (0.35 ml/100 g, intraperitoneal injection), the TD rats were subjected to total thyroidectomy; the thyroid glands in the sham group were totally mobilized but not excised, so the sham rats suffered the same relative level of pain after the operation. Supplementation with 10% calcium gluconate in the drinking water was provided for the TD rats after surgery in order to prevent the hypocalcemia that might possibly result from hypoparathyroidism.

2.3. Behavioral experiments

The open-field test (OFT) and forced swimming test (FST) were used to assess depression-like behavior in our study. All behavioral tests were conducted during the dark circadian period (19:00–23:00) and in the following order: OFT (day 42 after treatment) and FST (days 43–44 after treatment).

2.3.1. Open-field test

Rats were transported to the test room 2 h before the test. In accordance with a previous report (Katz et al., 1981), rats were placed in the center of the open-field chamber (100 cm × 100 cm × 50 cm) with black-painted inner walls, and allowed to explore the apparatus for 5 min. The behavior of the rats was videotaped during the procedure and subsequently analyzed using Noldus software (Noldus Information Technology, Wageningen, The Netherlands). After the test, the rats were returned to their home cages and the open field chamber was cleaned with 70% ethanol between tests.

2.3.2. Forced swimming test

The FST was performed in accordance with a previous report (Porsolt et al., 1978). Rats were placed in a cylinder (50 cm in height, 20 cm internal diameter) filled with water to a depth of 30 cm and maintained at $24 \pm 1\,^{\circ}C$. An initial 15-min pretest was performed 24 h before the 5-min test. The time spent immobile or climbing was recorded by a video camera mounted above the cylinder. Rats were removed from the water after each session, dried with paper towels, and return to their home cages. The water was changed after each test. The immobile condition was defined as an absence of any activity in the water. The climbing condition was defined as any attempt to climb out of the water using the forepaws. All the data were recorded by one member who was blind to the experiment.

2.4. Measurement of the serum concentrations of thyroid hormones

Blood was taken from the tail vein 24 h after the FST under general anesthesia using 10% chloral hydrate (0.35 ml/100 g, peritoneal injection). Thyroid-stimulating hormone (TSH), T3, and T4 concentrations were detected using ELISA kits (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions.

2.5. Quantitative cytochrome-c oxidase (COX) histochemistry assay

This experiment was conducted according to a previous method (Shumake et al., 2003). The brains of anesthetized rats were isolated and frozen quickly in isopentane at $-80\,^{\circ}C$ for 3 min, and embedded in optimal cutting-temperature compound. Coronal 30-μm cyrosections were prepared. Every fifth section was mounted on a glass