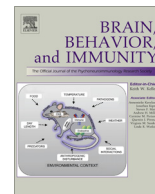




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Sickness behavior is delayed in hypothyroid mice

Vanessa Cardoso Silva^{a,b}, Alexandre Giusti-Paiva^{a,c,*}^a Programa Multicêntrico de Pós-Graduação em Ciências Fisiológicas, Sociedade Brasileira de Fisiologia (SBFis), Brazil^b Universidade Federal de Juiz de Fora, Campus Governador Valadares, Minas Gerais, Brazil^c Instituto de Ciências Biomédicas, Universidade Federal de Alfenas, Minas Gerais, Brazil

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ABSTRACT

Sickness behavior is an expression of a motivational state triggered by activation of the peripheral innate immune system, whereby an organism reprioritizes its functions to fight infection. The relationship between thyroid hormone and immune cells is complex, and additional insights are needed about the involvement of the cross-talk between thyroid hormone, the central nervous system and immune function, as demonstrated by the consequences to sickness behavior. The aim of this work was to evaluate sickness behavior in hypothyroid mice. Control mice and mice treated with propylthiouracil (PTU) for 30 days (0.05%; added to drinking water) received a single dose of LPS (200 µg/kg; i.p.) or saline, and the behavioral response was assessed for 24 h. We provide evidence that thyroid status acts a modulator for the development of depressive-like and exploratory behaviors in mice that are subjected to an immunological challenge because the PTU pretreatment delayed the LPS-induced behavioral changes observed in an open field test and in a forced swimming test. This response was observed concomitantly with a lower thermal index until 4 h after the LPS administration. This result demonstrates that thyroid status modifies behavioral responses to immune challenge and suggests that thyroid hormones are essential for the manifestation of sickness behavior during endotoxemia.

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1. Introduction

Thyroid hormones exert strong control over many functions involved in homeostasis and development. A proper thyroid status is crucial for various mechanisms, protein synthesis and the development of the central nervous system, as well as for regulating long-term energy consumption, storage and expenditure through different mechanisms (Gutiérrez-Mariscal et al., 2008; Warner and Beckett, 2010; Brix et al., 2011).

The neuroendocrine system and the immune system engage in complex cross-talk via hormones and immunomodulatory signaling molecules. Increasing evidence suggests that thyroid hormones, L-thyroxine (T4) and 3,3',5-triiodo-L-thyronine (T3), are modulators of the immune response (Warner and Beckett, 2010). In monocytes, macrophages, leukocytes, natural killer cells, and lymphocytes, a wide range of immune functions, such as chemotaxis, phagocytosis, the generation of reactive oxygen species (ROS), and cytokine synthesis and release, are altered under hypo- and hyperthyroid conditions (Warner and Beckett, 2010; De Vito

et al., 2011). However, systemic inflammation, such as during injury, or starvation, leads to the downregulation of hypothalamic thyrotropin-releasing hormone (TRH); lowered secretion of thyroid-stimulating hormone (TSH), free T4, and free T3; decreased levels of circulating free T3 due to decreased peripheral conversion of T4 to T3; and increased conversion of biologically active T3 to inactive reverse T3. All mechanisms lead to the inhibition of the hypothalamic–pituitary–thyroid gland axis (HPT) (Göthe et al., 1999; De Vito et al., 2011; Fonseca et al., 2013).

Thyroid hormones exert their regulation of the HPT axis by a feedback mechanism, and its reduction is associated with reduced TSH, leading to hypothyroidism. Hypothyroidism is characterized by a set of factors that reduce metabolism, including weight gain over long periods of time, fatigue, weakness, cold intolerance and mental slowness. The reduced basal metabolism in hypothyroidism produces central and peripheral effects on feeding behavior, thermogenesis, locomotor activation and autonomic regulation (López et al., 2013). Thyroid hormones influence specific immune responses, as well as several aspects of innate and adaptive immunity. The relationship between thyroid hormone and immune cells is complex, and additional insights are needed about the cross-talk between thyroid hormone and immune function. During acute systemic infectious disease, the precisely regulated release of energy-rich substrates (glucose, free fatty acids, and amino acids)

* Corresponding author at: Instituto de Ciências Biomédicas, Universidade Federal de Alfenas, UNIFAL, Rua Gabriel Monteiro da Silva, 700, Alfenas, 37130-000 MG, Brazil. Tel.: +55 (35) 3299 1303.

E-mail address: agiustipaiva@gmail.com (A. Giusti-Paiva).

and auxiliary elements, such as calcium/phosphorus from storage sites (fat tissue, muscle, liver, and bone) (Straub, 2014), are highly important because these factors are needed by the energy-consuming immune system in a situation with little or no food/water intake (sickness behavior) (Dantzer et al., 2007; Straub, 2014).

Sickness behaviors constitute an array of symptoms exhibited by animals during the course of an acute infection, including reduced activity, reduced food and water intake, and reduced social interactions (Straub, 2014). It is assumed that these symptoms enable the reallocation of finite energy resources to fighting infection.

In the present study, our aim was to explore the link between hypothyroidism and endotoxin-induced sickness behavior. To this end, the thyroid status was disrupted in mice using the antithyroid molecule propylthiouracil (PTU), and we explored the effects on behavior and thermoregulation during an immunological challenge.

2. Material and methods

2.1. Animals

Adult male Swiss mice (35–40 g) were obtained from the Central Animal Facility of the Federal University of Alfenas. The animals were housed under controlled light (12:12 h light–dark cycle; lights on at 07:00 a.m.) and temperature conditions ($23 \pm 1^\circ\text{C}$) with ad libitum access to water and food. The animals were allowed to habituate to the housing facilities for at least 1 week before the experiments began. Behavioral studies were performed in a quiet room between 08:00 a.m. and 12:00 p.m. to minimize circadian variation. All experiments were conducted with the approval of the Ethics Committee of the Federal University of Alfenas and in accordance with their guidelines (protocol 435/2012). To induce the hypothyroidism condition in the experimental groups, we administered 0.05% 6-propyl-2-thiouracil (PTU), which was diluted in the drinking water, for a period of 30 days, according to previous reports (Gordon, 1997). Control groups received filtered water. Lipopolysaccharide (LPS) from *Escherichia coli* serotype 026:B6 and 6-propyl-2-thiouracil were purchased from Sigma–Aldrich (USA).

2.2. Biometrical parameters

After the 30th day of the addition of PTU to the drinking water, the animals were weighed to determine the weight gain and were sacrificed by isoflurane inhalation. The naso-anal length was assessed by measuring the length from the snout to the beginning of the tail (cm) and by calculating the Lee index (Roggers and Webb, 1980). The perigonadal and retroperitoneal fat and the thyroid and parathyroid glands were excised and weighed.

2.3. Blood samples and hormonal measurements

After the 30th day of addition of PTU to the drinking water, another set of mice were decapitated, and blood samples were collected from the control ($n = 7$) and PTU groups ($n = 8$) and placed in tubes. The blood samples were centrifuged (3000 rpm for 20 min), and the sera were separated and stored at -20°C . Serum T3 and T4 levels were determined using specific Coated-Tube Radioimmunoassay (RIA) kits (MP Biomedicals, LLC, USA). The procedures were performed according to the recommendations of the kits. The sensitivity was 4.3 ng/dl for T3 and 0.4 $\mu\text{g/dl}$ for T4. The inter- and intra-assay coefficient of variation values varied from 4.2% to

6.0% and from 5% to 6.5% for T3 and 7.1% to 7.4% and from 2.9% to 5.1% respectively for T4.

2.4. Behavioral experimental procedures

In the animal room, the mice were pretreated with or without PTU in the water for 30 days before injections of lipopolysaccharide (LPS) serotype 026:B6 (200 $\mu\text{g/kg}$; i.p.) or sterile isotonic saline (0.9% NaCl). The behavioral tests were performed 2, 6 or 24 h after the LPS treatment. These time points were chosen on the basis of previous behavioral, endocrine and neurochemical studies (Yang and Gordon, 1997; Dunn and Swiergiel, 2005; de Paiva et al., 2010). The behavioral sections were video-recorded and analyzed by an experimenter blind to the testing condition.

2.4.1. Open field test (OFT)

Locomotor activity was quantified for 5 min in an open field, which was a cylindrical box with a floor that was divided into 12 squares. Furthermore, for tests that were longer than 5 min, the mice became habituated to the apparatus, thereby decreasing the differences between groups. Four squares were defined as the center, and the eight squares along the walls were considered the periphery. Each mouse ($n = 10$ per group) was gently placed exactly in the center of the box, and the activity was considered a line crossing when a mouse removed all four paws from one square and entered another, and rearing (frequency with which the mice stood on their hind legs in the apparatus). Line crossings among the central four squares of the open field were counted separately from line crossings among the peripheral squares (Dunn and Swiergiel, 2005; de Paiva et al., 2010).

2.4.2. Forced swimming test (FST)

This test was performed according to the method developed by Porsolt et al. (1977) for mice. Mice ($n = 12$ per group) were placed in a vertical glass cylinder (26 cm in high, 12 cm in diameter) filled with 25°C water to a depth of 16 cm. The water depth was chosen so that the animals must swim or float without their hind limbs or tail touching the bottom. For testing, each mouse was placed in the cylinder for 6 min, and the immobility (floating), swimming and climbing were assessed every 5 s. As suggested by Porsolt, only the data scored during the last 4 min were analyzed and presented.

2.5. Food intake and weight gain

Animals ($n = 10$ per group) were fasted for 12 h before receiving injections. The fasted animals exhibited increased food consumption during the first few hours after the refeeding compared with the animals fed ad libitum. Immediately after the injections, a fresh supply of pre-weighed food was provided. Food intake was calculated 2, 4, 6, 8, 12 and 24 h after the injection by measuring the difference between the pre-weighed standard chow available and the weight of chow and spilled crumbs (de Paiva et al., 2010).

2.6. Body temperature

One week prior to the immunological challenge, another set of animals were subjected to a surgical procedure for the implantation of a temperature transmitter in the peritoneal cavity. These procedures were performed under ketamine–xylazine (42.0 and 4.8 mg/kg, respectively, i.p.) anesthesia and antibiotic (pentabiotic, 3.75 mg/kg im) protection. For implantation of a miniature (25 mm) temperature transmitter (series 4000 E-Mitter; Mini Mitter, Bend, OR), a midline laparotomy was performed, the probe was inserted in the peritoneal cavity, and the surgical wound was sutured. After surgery, the animals were kept in individual cages under the same conditions as mentioned above. One week after

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