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Thyroid hormone regulation by stress and behavioral differences in adult male rats

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ABSTRACT

Thyroid hormones are essential regulators of growth, development and normal bodily function and their release is coordinated by the hypothalamic–pituitary–thyroid (HPT) axis. While the HPT axis has been established as an acutely stress-responsive neuroendocrine system, relatively little is known about the mechanisms of its stress regulation. The present study examined acute stress-induced changes in peripheral hormone levels [triiodothyronine (T3); thyroxine (T4), thyroid-stimulating hormone (TSH), reverse triiodothyronine (rT3)] and central mRNA levels of regulators of the HPT axis [thyrotropin-releasing hormone (TRH), somatostatin (SST), type II deiodinase (D2)] in response to an inescapable tail-shock, a rodent model of stress. Additionally, we examined whether individual differences in spontaneous exploratory behavior in an open field test predicted basal levels of TH or differential susceptibility to the effects of stress. The stress condition was associated with decreases in peripheral T3, T4 and TSH, but not rT3, when compared with controls. No changes were observed in TRH or SST mRNA levels, but there was a trend suggesting stress-related increases in D2 mRNA. We also found that an animal's exploratory behavior in an unfamiliar open field arena was positively related to peripheral thyroid hormone levels and predicted the magnitude of stress-induced changes.

In conclusion, we found suggestive evidence for stress-induced decrease in central drive HPT axis, but the central mechanisms of its stress regulation remain to be elucidated. Additionally, we found that individual differences in animals' exploratory behavior were correlated with peripheral TH levels.

Thyroid hormones [TH; both 3,5,3′,5′-tetraiodothyronine, thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3)] are classically considered to play an important role in growth, differentiation, and metabolism. Additionally, thyroid hormones have effects extending beyond development and maturation and are essential for normal bodily function in adults; in adults, thyroid hormones exert profound effects on metabolic regulation, including oxygen consumption and carbohydrate, lipid, and protein metabolism (Wrutniak-Cabello et al., 2001; Zoeller et al., 2007). Comparing endocrine systems across species, TH function as permissive hormones important for transitions and plasticity (Tata, 2006), including puberty (Mann and Plant, 2010), seasonal breeding (Ebling and Barrett, 2008), and metamorphosis in amphibians (Crespi and Denver, 2005). Moreover, the hypothalamic–pituitary–thyroid axis (HPT) is also a stress-responsive system (Langer et al., 1983; Armario et al., 1984; Turakulov et al., 1994; Cizza et al., 1996; Kondo et al., 1997; Servatius et al., 2000; Helmreich et al., 2005; Helmreich et al., 2006; Kilburn-Watt et al., 2010).

In mammals, the HPT axis is controlled by neurons located within the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) that synthesize and release thyrotropin-releasing-hormone (TRH) into the median eminence. TRH stimulates the release of thyroid-stimulating hormone (TSH) from the anterior pituitary, and then TSH travels through the peripheral vasculature to stimulate thyroid hormone (TH) release (both T_3 and T_4) from the thyroid gland. T_3 is considered the biologically active form of thyroid hormone because of its greater affinity for thyroid hormone receptors. T_4 is converted to T_3 by the activity of deiodinase enzymes located within most target tissues, including the central nervous system. Thyroid hormones exert negative feedback effects at the pituitary and hypothalamus to inhibit the release of TSH (Zoeller et al., 2007). In addition to TRH, other hypothalamic neuropeptides, such as somatostatin (SST), can modulate the release of TSH from the pituitary (Haugen, 2009). Furthermore, deiodinase enzymes, which can alter local or tissue specific levels of T_4 and T_3, have been shown to play a key role in thyroid hormone signaling (Gereben et al., 2008). Specifically, deiodinase II (D2) activity in the hypothalamus is important for the set-point of feedback regulation (Fekete and Lechan, 2007) and the decrease in TRH mRNA observed after LPS administration (Sanchez et al., 2008).

We have previously demonstrated that physical stress (foot-shock) causes a decrease in T_3 and T_4 in adult rats (Helmreich et al., 2005; Helmreich et al., 2006), but overall, stress regulation of the HPT axis has not been completely characterized. Interestingly, the HPT axis is sensitive to stress intensity. Mild stressors may cause a slight increase or no change in peripheral thyroid hormone levels (Armario et al., 1984; Turakulov et al., 1994) while more severe stressors cause a decrease in thyroid hormone levels (Langer et al., 1983; Cizza et al., 1996; Kondo et al., 1997; Kilburn-Watt et al., 2010). Changes in TH caused by fasting and acute stress-induced decreases in thyroid hormone, such as those

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caused by immobilization and lipopolysaccharide administration appear to be, at least in part, centrally mediated, as evidenced by decreases in TRH mRNA levels within the PVN (Cizza et al., 1996; Kondo et al., 1997; Legradi et al., 1997). In the current studies, we sought to characterize stress-induced changes of the HPT axis at multiple levels within the HPT axis after inescapable tail-shock stress. Functionally, the short-term benefits and advantages of a stress-induced decrease in thyroid hormone levels may reflect a conservation of energy and resources in an unpredictable environment (Engel and Schmale, 1972). However, stress-induced hormonal changes that are initially adaptive can become inappropriate or excessive and may lead to stress-related pathologies and disease (McEwen and Wingfield, 2003; Davis and Tremont, 2007).

In a second set of experiments, we began preliminary studies to ask whether individual differences in animals' behavior may predict the animals' basal TH set-point and any susceptibility to stress-induced TH decrease. Humans differ in TH set-point and maintain this set-point across time (Andersen et al., 2002). Individual differences in TH response to traumatic stimuli have been demonstrated in rats, which may have consequences for successful recovery (Kilburn-Watt et al., 2010). Individual differences in HPA (hypothalamic–pituitary–adrenal) axis function, based on baseline locomotor behavior have been characterized and, interestingly, these differences in baseline behavior not only predict HPA axis responsiveness, but they also predict propensity for drug seeking behavior (Kabbaj et al., 2000). In the current experiments, we sought to determine if an animal's behavior in an open field test correlates with that animal's thyroid hormone set-point and stress responsivity, employing a widely used out-bred strain of rat (Sprague–Dawley). The identification of individual differences within an experimental population may serve to decrease within experiment variability and also to inform experimental models to explore further links between behavior and endocrine systems (Whishaw and Kolb, 2005).

Materials and methods

Experiment 1

Animals

Seventy-five adult male Sprague–Dawley rats (Charles River Laboratories), body weight 250–310 g at the beginning of the stress sessions were used for this study. All animals were housed two per tub in 40×18×20 cm tubs. Lights were on 0730 to 1930 h and food and water were available ad libitum. All animals were acclimated to the housing facility for at least 5 days before testing. Within each tub, each animal was randomly assigned to either the inescapable tail-shock stress group or control group.

Stress paradigm

A single session of inescapable tail-shock was used for the experiments. Animals in the stress groups were subjected to 80 trials of 5 s 1.0 mA tail-shock. Each tail-shock chamber (Med Associates, St. Albans, VT) measured 11.75×7.6×14.4 cm, with floors composed of 7 grid rods measuring 4.8 mm in diameter spaced 1.6 cm apart. Extending out the back of each chamber was a tail holder; white athletic tape was used to hold the tail in place. Each tail-shock chamber was housed within a sound attenuating chamber (24.13 cm×41.9 cm×43.8 cm), with house light and fan. The shock generated by a 24 V regulated power supply (Med Associates) was delivered to the animals' tail through the adhesive electrode tapes. Med-PC IV software was used to control the onset of the tail-shock stimulus. One session consisted of 80 trials; the inter-trial interval was a variable schedule ranging from 5 to 115 s, with a mean duration of 60 s. Control animals were placed within a tail-shock chamber, but did not receive tail-shock. The stress session lasted approximately 90 min; animals were returned to their home cage immediately at the end of the session. The animals in both groups were rapidly decapitated at 0, 120, 180 min or 24 h post-stress initiation (n=8–9 for each group); animals in the 0 min stress groups were removed directly from their home cage. Stress sessions were staggered so all decapitations occurred at 1300 h to minimize variations caused by diurnal rhythm (Kalsbeek et al., 2000). Brains were quickly removed and frozen; trunk blood was collected in untreated tubes; serum was separated and frozen for later assay.

Experiment 2

Animals were used as described in Experiment 1, except that animals were housed with others from the same treatment condition. Additionally, in order to assess individual differences among animals, at 0900 h the day before the stress session, the animals' behavior was quantified in an open field arena. Individual subjects were placed in the center of the arena (81×81 cm) under dim lights (20 lx) and behavior was recorded for 10 min using a digital camcorder suspended above. Total distance traveled, time spent in the center of the arena and average velocity in the perimeter were analyzed using TopScan 2.0 Tracking Software (Clever Sys, Reston, VA) and manually inspected for accuracy. The amount of time spent rearing with both forepaws against the wall of the arena was scored manually by a condition-blinded observer using Annostar Software (Clever Sys, Reston, VA). On the same day as the open field test, a tail-nick sample was collected at 1245 h to assess basal T_4 levels.

On the following day, the stress session was as described in Experiment 1, except that 100 trials of 1.0 mA tail-shock were utilized in an attempt to elicit less ambiguous between-group differences in peripheral TH levels. Animals were decapitated at 1300 h, either at 0 or 180 min post-stress initiation. The 180 min time point was chosen based on the results of Experiment 1, in order to reduce animal use. Therefore, both groups experienced the open field test and tail-nicking on the previous day. On the experimental day the 180 min control animals were placed into the stress chamber, but received no tail-shocks; the 0 min animals were not handled before sacrifice, but were housed next to animals that were removed and transported to the experimental room.

The University Committee on Animal Resources of the University of Rochester Medical Center approved all experimental protocols and all animals were treated in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Radioimmunoassays

Peripheral levels of Total T_3 and Total T_4 were determined using radioimmunoassays kits from MP Biomedicals/Pharmaceuticals (Solon, OH; formerly ICN) using the included protocols (Servatius et al., 2001; Helmreich et al., 2005). Peripheral TSH levels and rT_3 were determined via radioimmunoassay kits from ALPCO Diagnostics (Salem, NH), again using included protocols. The rat TSH kit had <0.1% cross-reactivity for rat GH, rat PRL or rat FSH; the antibody had 5.1% cross-reactivity with rat LH. The rT_3 RIA had <0.01% cross-reactivity with T_3 or T4, and 1.8% cross-reactivity with 3,3′ diiodo-L-thyronine.

In-situ hybridization

PVN TRH mRNA, periventricular nucleus (PeVN) SST mRNA and medio-basal hypothalamus D2 mRNA levels were determined via in-situ hybridization. Brains were sectioned at 12 μm in the coronal plane throughout the PVN. Four sections per slide were collected onto Superfrost plus slides (Fisher Scientific, Pittsburgh, PA) and stored at −80 °C until further processing. In-situ hybridization was conducted using techniques previously described (Schafer et al., 1993). Briefly, sections were removed from the freezer and placed into cold 4% paraformaldehyde for 60 min. Following washes in 2× SSC, sections were placed in 0.1 M triethanolamine for 15 min, to which 0.25% acetic anhydride had