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Peripheral triiodothyronine (T_3) levels during escapable and inescapable footshock

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Abstract

Changes in peripheral thyroid hormone levels are associated with changes in human affective disorders, particularly depression. In the current study we used an animal stress paradigm, proposed to be an animal model of depression, to examine peripheral T_3 levels during and after escapable or inescapable stress in adult male rats. In this model, one animal can control the termination of foot-shock stress by performing a lever press, and therefore experiences escapable stress. His lever press also terminates the shock for his yoked partner, who has no control over the stressor, and therefore experiences inescapable stress. In three separate experiments, blood samples were collected during and after one or two sessions of escapable/inescapable stress. We found that exposure to inescapable stress, but not escapable stress, caused a decrease in T_3 levels 120 min post-stress initiation. Peripheral T_3 levels were not significantly altered in animals exposed to escapable stress. In sum, these results add to a large body of previous data indicating that psychological coping can prevent the effects of physical stress on many diverse systems.

Keywords: Thyroid; Coping; Inescapable; T_3; Stress; Rat

Thyroid hormones (triiodothyronine-T_3, thyroxine-T_4) have been linked to human depression through studies demonstrating that alterations in thyroid hormones are associated with alterations in affective state in humans. Because both hypo- and hyperthyroid states have been linked with depression, discerning the causes and effects of altered thyroid hormone secretion in affective states is a complex task [1].

A complementary approach to understanding situations or stimuli that influence thyroid hormone secretion in humans is to use animal studies. In animal studies, the clinical condition of depression is often modeled by paradigms involving stress. One model extensively utilized is the escapable/inescapable (ES/IS) stress paradigm. In this paradigm, one animal (the executive) experiences *escapable* stress by performing a behavior, such as lever pressing or wheel turning, to terminate the stressor (usually shock). This behavior also terminates the shock for the yoked partner who does not have control over the termination of the shock and therefore experiences *inescapable* stress. Both animals receive the same physical stressor but differ in the psychological aspect of coping. Many previous studies have shown that deleterious effects of stress, such as ulcers and learned helplessness, are only observed in animals experiencing inescapable, but not escapable, stress [2]. These results suggest that psychological factors associated with a physical stressor can dramatically alter the impact of the stressor. Moreover, many of these deleterious effects of stress mimic symptoms observed in depressed patients, therefore the ES/IS model, in particular the yoked animal, has been proposed to be an animal model for depression [3].

Thyroid hormones, while classically associated with growth, differentiation, and metabolism, are also stress-responsive. Previous studies have shown that restraint and tail-shock cause a decrease in peripheral T_3 and T_4 levels in male rats [4,5]. As the aforementioned stress stimuli include both physical and psychological components, one cannot determine the salient features of the stressor that cause a decrease in thyroid hormone levels. Results from studies examining the regulation of the hypothalamic-pituitary-adrenal axis suggest that stressor characteristics are important in determining neuronal circuits activated by the stressor [6]. The use of the ES/IS model would allow partitioning of the physical and psychological effects of stress on T_3 levels.

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In addition to the above-mentioned endocrine data, there is behavioral data suggesting that the function of the HPT axis may be altered by IS. Specifically, treatment of rats with either T_3 or tri-iodothyroacetic acid, a metabolite of T_3, prevents the development of learned helplessness in rats exposed to IS [7,8].

Previous studies [9,10] examining the effects of ES/IS on peripheral thyroid hormone levels have yielded inconsistent results. Friedman et al. [9] reported that neither escapable or inescapable foot-shock stress altered peripheral T_3 levels in male or female rats; in this study the samples were collected 3 h post-stress. Conversely, Jośko [10] reported that plasma T_3 levels were decreased in animals exposed to inescapable stress, but not escapable stress; in that study samples were collected immediately after the stress session. In the current study, peripheral thyroid hormone levels will be determined at multiple time points after ES/IS stress, using a stress paradigm similar to paradigms used to demonstrate differences between ES/IS in learned helplessness, 5-HT release [11], and dopamine metabolism [12].

1. Material and methods

1.1. Experiment 1

Thirty-three male Sprague-Dawley rats, from Harlan, Indianapolis, IN, body weight 265–385 g at time of testing were used. Animals were housed 3/cage in $40 \times 18 \times 20$ cm tubs. Lights were on 08:00–20:00 h, and food and water were available ad libitum. All animals were acclimated to the housing facility for at least a week before testing. Within each tub, an animal was randomly assigned to either the executive, yoked, or control treatment. To reduce the influence of a novel environment on hormonal responses, animals were habituated to the appropriate stress chamber for 10 min/day 5 times before the stress testing.

For the stress session the animals were placed in operant testing chambers (30.5×29.2 cm) with stainless steel grid floors, which were connected to scrambled shock generators (Med Associates, St. Albans, VT). Med-PC software (Med-Associates) was used to control the onset of the foot shock stimulus (0.8 mA), concurrent light stimulus, and to record data. Within the executive and yoked boxes, a 6.3 cm lever was 7.6 cm above the floor and 1.3 cm away from the back wall, and a light was 9 cm from the floor, above the lever. A lever press by the executive animal terminated the shock and light in both the executive and yoked boxes. One session consisted of 80 trials; the intertrial interval was a variable schedule ranging from 5 to 115 s, with a mean duration of 60 s. If the executive animal made no response, the shock was terminated after 30 s. The stress sessions lasted approximately 80 min. The control animal was placed in a stress chamber neighboring the executive and yoked chambers, but it did not receive foot-shock. After the end of the stress session and the 80-min sample, animals were returned to their home cages in the homeroom.

Blood samples were collected (via tail nick) at 0, 40, 80, 120, and 180 min post-stress initiation. The 40 and 80 min samples were collected in the stress room, and the 0, 120, and 180 samples were collected in the homeroom. All experiments were conducted between 09:00 and 13:00 h.

1.2. Experiment 2

Forty-two adult male rats, similar to Experiment 1, were randomly assigned to the executive, yoked, and control groups. Stress session parameters similar to Experiment 1 were used except that animals were quickly decapitated at 120 and 240 min post-stress initiation ($n = 5–6$ group/time point). All animals were decapitated between 13:00 and 13:30 h.

1.3. Experiment 3

Animals ($n = 24$; BWT 250–360 g) from Charles River Canada were housed as described for Experiment 1 except animals were not habituated to the stress chambers prior to the experiment. Unpublished data from our laboratory and the current results indicate that habituation does not impact changes in thyroid hormones in this stress paradigm. Stress sessions were conducted on two consecutive days, to ensure that the executive animal performed the proper operant behavior for the entire stress session on Day 2, after learning it on Day 1. Samples were collected, via tail nick, at 0 and 120 min on Day 1, and 0, 120, 180, and 240 min on Day 2. Also, to reduce the space [13] within the chamber and to eliminate interesting corners to explore, a white opaque PVC cylinder (20 cm I.D., 23 cm h) was placed within the executive and yoked stress chambers, on top of the grid floor. A lever was 7.62 cm from the floor, and a light was 15 cm from the floor, above the lever. The stress sessions were similar to those described for Experiment 1. A PVC cylinder was not placed in the control cage, but it is important to note that the PVC cylinders were not restraining nor did they prevent movement by the rats.

For all experiments, results from triads in which the executive animal failed to lever press (i.e. shock terminated after 30 s for all trials) are not reported. The Middlebury College Institutional Animal Care and Use Committee approved all experiments, and all animals were treated in accordance with the NIH Guide for Care and Use of Laboratory Animals.

1.4. Radioimmunoassays

Samples from Experiments 1 and 3 were analyzed using Total T_3 kits from ICN Diagnostics/MP Pharmaceuticals (Irvine, CA), and the laboratory of Dr. Charles Emerson at the University of Massachusetts Medical Center analyzed samples from Experiment 2, as previously described [14].

1.5. Statistics

Results from Experiments 1 and 3 were analyzed with a two-way repeated measures ANOVA, and followed by further post-hoc analysis via one-way ANOVAs at each time point and Fisher's PLSD for Experiment 3. Results from Experiment 2

were analyzed by a two-way ANOVA, followed by Fisher's PLSD (Statview 5.01, SAS Institute, Cary, NC). Since post-hoc analyses were used to determine the sources of variation, no multiple comparison procedure was used for controlling the type I error.

2. Results

2.1. Experiment 1

Animals were subjected to a single day of either escapable or inescapable stress. Average bar press latencies for the executive animals during the 80 trial stress session are shown in Fig. 1; the executive animals did learn to press the bar, as evidenced by the decrease in bar press latency from the first block of ten trials to the eighth block.

Baseline blood samples collected via tail-nick at the 0 min time point revealed variability among the groups in initial T_3 levels, although they were not significantly different from each other. Therefore, subsequent values were calculated as a percentage of the value at each animals' own 0 min time point. A repeated-measures two-way ANOVA showed a trend for a significant interaction between stress condition and time of sample (Fig. 2; $F=1.87$; $p=0.07$). Visual inspection of the data suggested that groups could differ at the 120-min time point; this time point, in addition to 240 min, was chosen for further study.

2.2. Experiment 2

Animals were again subjected to a single day of either escapable or inescapable stress; bar press latency for the first 10 trials was 11.6±1.1 s (mean±S.E.M.) and 2.3±0.2 s (mean±S.E.M.) for the last 10 trials. T_3 and T_4 levels were measured in trunk-blood samples collected at 120 or 240 min post stress initiation. For T_3, the two-way ANOVA demonstrated a significant interaction between stress conditions and time ($F=5.86$, $p\leq 0.01$). Post-hoc tests demonstrated that T_3 levels in yoked animals, not executive, were significantly lower than control animals at 120 min ($p\leq 0.05$; Fig. 3). At 240 min, serum T_3 levels were significantly greater in executive animals compared to control animals ($p\leq 0.05$). At no time point were T_3 levels in executive animals lower than control animals.

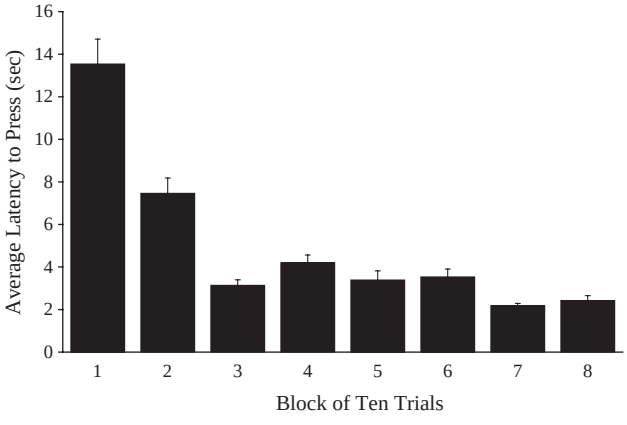

Fig. 1. Average (mean±S.E.M.) bar press latencies for the executive animals ($n=11$) in Experiment 1. Averages were calculated for 8 blocks of 10 trials, encompassing the 80 trial stress session.

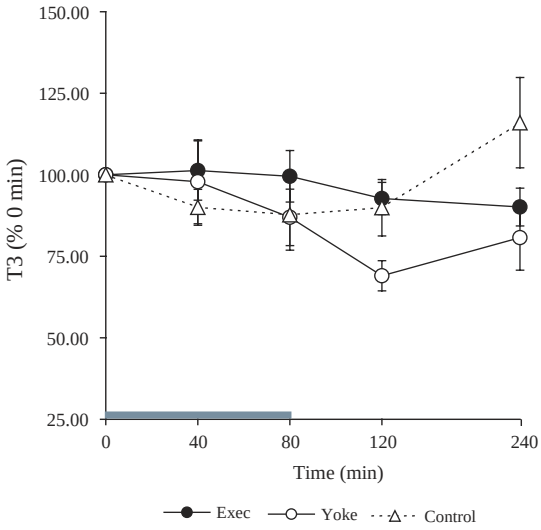

Fig. 2. Shown are circulating levels of T_3, plotted as a percentage of values at the 0 min time point, collected via tail-nick during one stress session (indicated by heavy line) from executive ($n=11$), yoked ($n=11$) or control ($n=11$) animals.

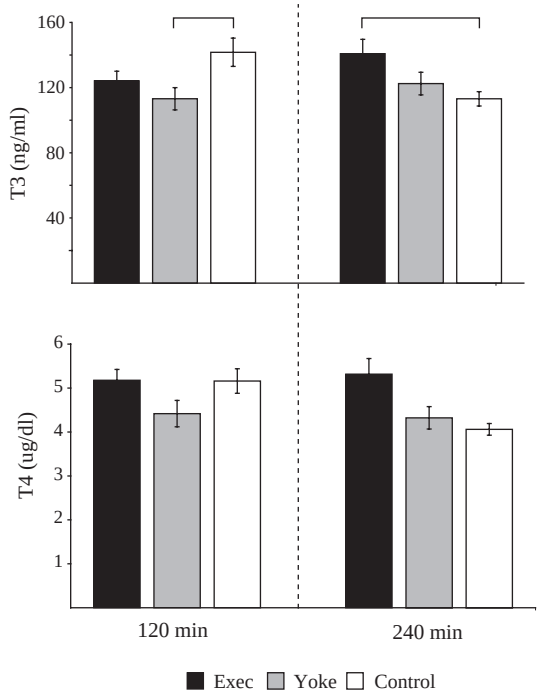

Fig. 3. Top panel: Circulating T_3 levels measured in trunk blood samples collected at 120 min ($n=5$ for each stress condition) and 240 min ($n=6$ for each stress condition) post-stress initiation. There is a significant interaction between stress conditions and time ($F=5.86$, $p\leq 0.01$); horizontal lines indicate significant differences ($p\leq 0.05$) between groups determined by post-hoc tests. Bottom panel: Circulating T_4 levels measured in trunk blood samples collected at 120 min ($n=5$ for each stress condition) and 240 min ($n=6$ for each stress condition) post-stress initiation. A two-way ANOVA revealed a main effect of stress condition ($F=5.64$, $p<0.01$); executive animals were significantly greater than yoked or control animals.

Changes circulating T_4 levels were also analyzed via two-way ANOVA, which revealed a main effect of stress condition ($F=5.64$, $p<0.01$) and a trend for a significant interaction between stress and time ($F=2.98$, $p=0.06$; Fig. 3). Post-hoc tests indicated that, for the main effect of stress condition, serum T_4 levels were significantly elevated in the executive animals compared to both yoked and control; yoked animals were not significantly different from control.

2.3. Experiment 3

Animals were subjected to two consecutive days of either escapable or inescapable stress in modified stress chambers. On Day 1, bar press latency for the first 10 trials was 9.7±0.8 s (mean±S.E.M.) and 3.8±0.3 s (mean±S.E.M.) for the last 10 trials. On Day 2, bar press latency for the first 10 trials was 3.8±0.7 s (mean±S.E.M.) and 2.2±0.3 s (mean±S.E.M.) for the last 10 trials.

As in Experiment 1, blood samples collected via tail-nick at the 0 min time point revealed variability among the groups in baseline T_3 levels, although they were not significantly different from each other. Therefore, subsequent values are reported as a percentage of T_3 values at the 0 min time point, Day 1. A repeated measure two-way ANOVA revealed a significant interaction between stress condition and time of sample ($F=2.14$; $p\leq0.05$; Fig. 4). Further analysis via ANOVA indicated there was a strong trend for plasma T_3 levels at Day 1 120 min post stress-initiation to be lower in yoked animals, as compared to executive and control animals ($F=3.06$; $p=0.07$). On the second day of stress, plasma T_3 levels in yoked animals were significantly lower, determined by ANOVA and Fisher PLSD, than executive and control animals at 120 min, $p\leq0.05$, as shown in Fig. 4. No significant differences among groups were observed at the 240-min time point.

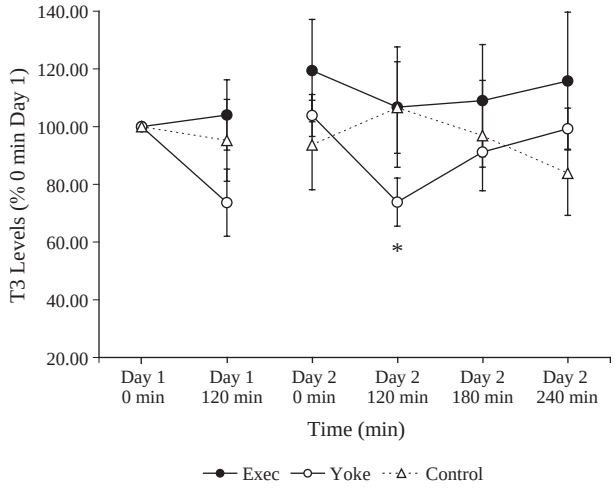

Fig. 4. Shown are circulating levels of T_3, plotted as a percentage of values at the 0 min Day 1 time point, collected via tail-nick during two consecutive daily stress sessions, $n=8$ for each stress condition. A repeated measure two-way ANOVA revealed a significant interaction between stress condition and time of sample ($F=2.14$; $p\leq0.05$) and * indicates a significant difference of yoked animals from executive and control groups at the same time point, via ANOVA and Fisher's PLSD ($p\leq0.05$).

3. Discussion

The goal of these experiments was to determine if circulating T_3 levels are sensitive to physical and/or psychological components of stress. We found, in three separate experiments, that exposure to inescapable, but not escapable, foot-shock tended to cause a decrease in circulating T_3 levels at 120-min post-stress initiation. Other excursions in hormone levels were not significant or consistent. In all experiments, the animals subjected to escapable and inescapable stress received the same physical stressor; the only difference between the groups was the psychological factor of coping. These results add to previous work indicating that psychological, rather than physical, components of the stressor are important in determining the ultimate impact of a stressor on an organism.

The magnitude of the change in T_3 levels in yoked animals at 120-min post stress was not large, not reaching significance in certain circumstances (Day 1 in Experiments 1 and 3). However, the overall trend, observed in all 3 experiments, suggests that inescapable stress, rather than escapable stress, is associated with a decrease in peripheral T_3 levels. An accompanying conclusion is that this is not an easy phenomenon to capture or study. With regard to methodology, it appears that decapitation is preferable to tail-nicking for sample collection, and that more reliable changes in hormone secretion are observed after 2 days of stress.

With these caveats, we believe our data complement and extend previous results reported by Jośko [10]. That author reported a decrease in TSH (thyroid-stimulating hormone), T_4, and T_3 after exposure to non-controllable, but not controllable, stress (3 days of daily 10-min sessions). However, only one time point was examined in that study and blood samples were collected under light ether anesthesia, which may have confounded results. Friedman et al. [9] did not observe any alterations in peripheral thyroid hormone levels in male rats 3 h after exposure to very brief sessions (15 shocks) of escapable or inescapable stress; these results are similar to the current data collected at 240 min post-stress initiation, although our stress session was much longer.

Although not measured in the current study, other studies from our laboratory have demonstrated that foot-shock stress, in addition to decreases in total T_3, also causes significant decreases in free T_3, and total and free T_4. In that study, animals were subjected to one daily session of inescapable foot-shock stress for 14 days, and samples were collected on Day 15 [15]. These results indicate that if extended for 14 days, the current inescapable shock stimulus will also cause changes in free hormone levels in addition to total hormone levels.

Besides the decreases in circulating T_3 levels measured in yoked animals, we also observed an increase in circulating T_4 levels in the executive animals in Experiment 2. This increase in T_4 could result from either an increased release of T_4 from the thyroid gland in executive animals, or a decrease in the peripheral conversion rats of T_4 to T_3. As peripheral T_3 levels did not decrease in executive animals, which may be expected if the T_4 to T_3 conversion rate was altered in these animals, our current data support the initial hypothesis that the increase in

T_4 is a result of increased secretory activity of the thyroid gland. Future experiments measuring both TSH and reverse T_3 are needed to fully address this issue.

The observed change in T_3 levels at 120 min is currently not easy to explain, given the half-life of T_3 (approximately 12 h [16]). However, the percentage change in hormone levels at 120 min observed in the current study is similar to the rate and magnitude of stress-induced decreases in thyroid hormone previously reported [17,18]. Future experiments measuring TSH levels during the initial period of the stress session would indicate if the observed decrease in T_3 is a result of neuroendocrine regulation, via the pituitary and/or hypothalamus. Alternatively, changes in plasma/serum T_3 levels may be a result of alterations in sequestration and compartmentalization of T_3 [19].

Interestingly, rapid changes in peripheral steroid hormone concentrations have been previously reported. Levine et al. [20] demonstrated that performance of a consummatory behavior (such as drinking or fighting) during stress causes a rapid decrease in plasma corticosterone levels. As the decline they observed was similarly faster than what would have been predicted based on the half-life of the hormone, they postulated the presence of an "active inhibitory process which suppresses adrenocortical activity" [20].

Support for the functional significance of rapid changes in T_3 levels comes from studies demonstrating rapid effects of T_3 administration, i.e. within 30 min, on measures such as oxygen consumption and mitochondrial gene transcription [21]. Furthermore, Scanlan et al. [22] have identified a novel metabolite of T_4 that does not act via traditional nuclear receptors, but rather through G-protein coupled receptors. This type of signal transduction could convey rapid changes in thyroid hormone levels to vital organs and cells.

On a longer time scale, the functional significance of a decrease in T_3 levels caused by inescapable stress may be gleaned from studies showing similarities between hypothyroidism and consequences of inescapable stress. For example, stress-induced gastric ulceration occurs at a higher frequency in yoked animals than in executive animals [2]. Likewise, hypothyroidism is also associated with an increased prevalence of stress-induced gastric ulcers [23]. Also, administration of T_3 after uncontrollable stress prevents the development of learned helplessness, suggesting that a decrease in thyroid hormone levels caused by the shock session maybe partly responsible for the development of learned helplessness [7]. The stress conditions used in most previously mentioned studies [2] are more intense than those used in the current studies; use of a more intense stressor in the current paradigm, such as tail-shock or higher amperage foot-shock, may cause greater decreases in peripheral T_3 levels.

Other interesting future directions, in addition to addressing shock magnitude, include determining if other ethologically relevant inescapable stressors, such as social defeat, might cause changes in peripheral T_3 levels. Also, as there are known interactions between the efficacy of antidepressants and thyroid hormone levels [24–26], it would be of interest to determine if antidepressant treatment could prevent the inescapable stress induced decrease in T_3 levels. We have previously demonstrated that antidepressant treatment prevents the decrease in mating behavior caused by exposure to inescapable stress [27].

In sum, we report that psychological components of stimuli, particularly inescapability or non-controllability, appear to be important in causing stress-induced decreases in peripheral T_3 levels. A decrease in T_3 levels after inescapable stress may reflect a conservation and protection of available resources in an unpredictable environment [28].

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