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Neuroendocrine and Behavioral Responses to Stress in Hypothyroidism

Gabrielle Hayden
Winona State University

Richard Deyo
Winona State University

Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project
Neuroendocrine and Behavioral Responses to Stress in Hyopthyroidism

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Student Name Gabrielle Hayden

Faculty Sponsor Dr. Richard Deyo

Department Psychology

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Abstract
A positive relationship has been discovered linking heightened ACTH levels in the pituitary to increased hypothyroidism rates, suggesting that stress negatively impacts thyroid functioning. Hypothyroidism is frequently misdiagnosed and treated as depression, yet little is known about this relationship. Nine C57BL/6J subjects were given 0.25% 6-propyl-2-thiouracil per gram for two weeks and twenty-nine were kept on a standard laboratory diet prior to tail suspension testing. The control group was further divided into six-day, single day, and no tail suspension testing. Following the final trail all groups were scored behaviorally in on the open field test. Serum samples were then acquired and T4 levels were calculated. Thyroid status was significantly different between euthyroid (9.87 μg/dL) and hypothyroid (5.50 μg/dL) treatment groups ($t(18)=4.57$, $p< .01$). The single day-TST group had significantly greater total activity ($t(18) = 2.721$, $p < .05$) and activity >2 volts ($t(18) = 2.886$, $p < .05$) than the six day-TST group. Also, open field behavioral testing showed that single day-TST and hypothyroid groups had less overall movement and more depressed behaviors. These results indicate that low stress and hypothyroidism tend to trigger the symptoms of major depression, but as the stress level increases the symptoms of depression tend to develop into an anxiety disorder. These data have important implications for the treatment of stress-induced affective disorders.

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The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature ____________________ Date ____________

Faculty Sponsor Signature ____________________ Date ____________

Neuroendocrine and Behavioral Responses to Stress in Hypothyroidism

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Winona State University

Abstract

A positive relationship has been discovered linking heightened ACTH levels in the pituitary to increased hypothyroidism rates, suggesting that stress negatively impacts thyroid functioning. Hypothyroidism is frequently misdiagnosed and treated as depression, yet little is known about this relationship. Nine C57BL/6J subjects were given 0.25% 6-propyl-2-thiouracil per gram for two weeks and twenty-nine were kept on a standard laboratory diet prior to tail suspension testing. The control group was further divided into six-day, single day, and no tail suspension testing. Following the final trail all groups were scored behaviorally in on the open field test. Serum samples were then acquired and T4 levels were calculated. Thyroid status was significantly different between euthyroid (9.87 µg/dL) and hypothyroid (5.50 µg/dL) treatment groups ($t(18)=4.57$, $p< .01$). The single day-TST group had significantly greater total activity ($t(18) = 2.721$, $p < .05$) and activity >2 volts ($t(18) = 2.886$, $p < .05$) than the six day-TST group. Also, open field behavioral testing showed that single day-TST and hypothyroid groups had less overall movement and more depressed behaviors. These results indicate that low stress and hypothyroidism tend to trigger the symptoms of major depression, but as the stress level increases the symptoms of depression tend to develop into an anxiety disorder. These data have important implications for the treatment of stress-induced affective disorders.

Neuroendocrine and Behavioral Responses to Stress in Hypothyroidism

It is well known that the symptoms of hypothyroidism are nearly identical to those of major depression and as a result are often misdiagnosed and treated as major depression (Pilhatsch, Marxen, Winter, Smolka and Bauer, 2011). Some of the symptoms shared by both conditions include: fatigue, weakness, weight gain, sleep dysfunction, memory impairments and feelings of sadness. It is generally accepted that abnormally low levels of thyroxine are responsible for these symptoms. Levothyroxine has been shown to be a sufficient treatment with remarkable outcomes to not only increase thyroid levels, but also in improving memory, somatic complaints, and depressive symptoms (Monzanil et al., 1993). However, some studies have revealed that in some people with apparent hypothyroidism Levothyroxine is effective in restoring thyroid hormone levels but without successfully eliminating the depressive symptoms (Demartini, Masu, Scarone, Pontiroli, and Gambini, 2010). Pae et al. (2009) have found that euthyroid patients with low but subclinical levels of thyroxine had less severe forms of depression but were also less responsive to antidepressants while patients with higher levels of thyroxine tended to respond better to antidepressants. It has also been found that the serotonin-selective reuptake inhibitor class or antidepressants (SSRIs) significantly decrease thyroxine levels in both hypothyroid and euthyroid patients with major depression without actually inducing hypothyroidism (de Carvalho, Bahis, Boeving and Graf, 2009). Even more perplexing is the observation that some persons with hypothyroidism also develop anxiety disorders (Constant et al., 2005). These observations suggest a complex interaction between mood, serotonergic pathways and thyroid function that is currently complicating treatment and is not well understood.

Although there have been few experimental studies that have explored this connection, a recent study found that social stress-induced hypothyroidism in rats treated with the SSRI fluoxetine produced an improvement in depression and total thyroxine (T4) circulating hormone

levels. However, there was no alteration of the T3 levels (active form of thyroxine) (Olivares et al., 2012). These data are not consistent with the decreased thyroxine levels found in depressed and hypothyroid patients treated with other SSRIs. Collectively, the results of these studies imply a link between depression, serotonin and hypothyroidism, yet little is understood about this relationship, leading to the current controversy in the appropriate treatment for both major depression and hypothyroidism (i.e., antidepressants or Levothyroxine).

Hypothyroidism is an endocrine disorder where the thyroid gland fails to produce enough thyroxin (T4) leading to an inability to fuel neuronal physiology (Hadley and Levine, 2006). One phase of the neuroendocrine response to stress results in Adrenocorticotropic Hormone (ACTH) being released by the pituitary (Miller and O'Callaghan, 2002). This release of ACTH impacts hippocampal and cortical neurogenesis, lessening the mass of these two anatomical regions, which is correlated with and believed to be a cause of depression (Julien, Advokat, and Comaty, 2011). Recent studies show that a positive relationship has been found between the presence of ACTH secreting microadenomas in the pituitary and increased hypothyroidism rates (Mathioudakis, Thapa, Wand, and Salvatori, 2012). These results suggest that there may be a potential connection between stress and thyroid performance.

The proposed relationship between major depression, increased ACTH levels, and hypothyroidism suggests that stress may be a mechanism of inducing thyroid dysfunction and depression and that there is a possible unexplored connection between hypothyroidism and serotonergic pathways. Porsolt's mouse tail-suspension test of learned helplessness will be used to test this hypothesis in this study. Porsolt's test leads to a stress-induced neurochemical change in the rodent brain that is extraordinarily similar to that of human depression (Castagne, Moser, Roux, and Porsolt, 2011). In addition, we also plan to study the effects of hypothyroidism on

anxiety since it has also been observed that some patients may show anxiety as opposed to depression (Constant et al., 2005).

Method

Subjects:

Thirty-eight C57BL/6J mice age 8 weeks at start and 14 at testing were be housed in groups of 4-5 during all phases of the study. Twenty-nine subjects were placed on a standard laboratory diet. The remaining nine subjects were given 0.25% 6-propyl-2-thiouracil (St. Louis, MO) alongside a standard laboratory diet for two weeks prior to testing.

Tail Suspension Testing

Apparatus*:*

The tail suspension model used consists of a chamber with a metal rod suspended by two supporting rods. An infrared photodiode placed in front of subject monitors movement for six consecutive minutes and observations are sent to and recorded on a computer program.

Procedure:

Subjects in control diet group were further divided into three test groups: six-day testing, single trial testing, and no-test controls for groups. Tail-suspension testing preceded testing in the open-field. Six-day and single trial subjects were placed in the dark chamber and taped to the tail suspension rod one at a time. The infrared sensor was then placed in front of each subject and the computer program was started. The program recorded each spurt of movement for six minutes and proceed to graph them accordingly. It measured movements based on size of motion and graphed them in real time. Immediately following the final trial serum samples were taken.

Hormone Analysis:

Using an ELISA kit testing for total thyroxin (T4) (Phoenix Pharmaceuticals, Phoenix, AZ), wells were filled with 25ml of serum from each of the 48 subjects and 100ml of working conjugate reagent and mixed for 30 seconds. Following a 60-minute incubation at 18-25C the incubation mixture was removed by flicking the microtiter wells with distilled water. 100ml of TMB Reagent were then added to each well and incubated in darkness for 20 minutes. Following the second incubation 100ml of stop solution were added to the mixture and absorbance values were calculated using a microplate well reader.

Open Field Test for Emotion, Motor Systems, and Learning

Apparatus:

The apparatus consisted of a 43.5 x 43.5 cm Plexiglas chamber. The chamber is kept in a sound resistant test room. A video camera was positioned parallel to the chamber and level with subjects.

Procedure:

Mice were individually placed in the chamber for a total of five minutes. The amount of spontaneous activity, and measures of emotionality including were recorded digitally and then recoded on DVD prior to scoring.

Results

Thyroid status was significantly different between euthyroid (9.87 µg/dL) and hypothyroid (5.50 µg/dL) treatment groups ($t(18)=4.57$, $p< .01$). There was a significant impact in total groom time between treatment groups ($F(3, 34) = 5.851$, $p < .01$). 1D-TST group spent significantly more time grooming than both euthyroid ($t_{q.05}(4,34)=4.349$, $p < .05$) and hypothyroid groups, ($t_{q.05}(4,34)=4.582$, $p< .05$). There was a significant difference in number of boli between treatment

groups ($F(3, 34) = 7.482$, $p < .01$). The hypothyroid treatment group made a significantly greater number of boli than both the 1D-TST ($t_{q.05}(4,34)=12.868$, $p< .01$) and the 6D-TST groups ($t_{q.05}(4,34)=15.531$, $p< .01$). There was a significant difference in total rear time ($F(3, 34) = 11.374$, $p < .01$) and total number of rears between treatment groups ($F(3, 34) = 13.370$, $p < .01$). The 1D-TST group reared significantly less times than the euthyroid ($t_{q.05}(4,34)=2.747$, $p< .01$), hypothyroid ($t_{q.05}(4,34)=1.865$, $p< .01$), and 6D-TST groups ($t_{q.05}(4,34)=2.551$, $p< .01$), and spent significantly fewer seconds rearing than both euthyroid ($t_{q.05}(4,34)=1.716$, $p< .01$) and 6D-TST groups ($t_{q.05}(4,34)=1.558$, $p< .01$). Also, the hypothyroid group spent significantly less time rearing than the euthyroid group ($t_{q.05}(4,34)=1.082$, $p< .05$). There was a significant difference in distance traveled between treatment groups, $F(3, 34) = 3.826$, $p < .05$. The single trail tail suspension treatment group traveled a significantly shorter distance than both the euthyroid ($t_{q.05}(4,34)=0.035$, $p< .05$) and 6D-TST groups ($t_{q.05}(4,34)=0.032$, $p< .05$). There was no significant difference in number of seconds traveled between treatment groups ($F(3, 34) = .167$, $p > .05$). There was no significant difference in number of quadrant crossings between treatment groups ($F(3, 34) = 1.962$, $p > .05$).

There was no significant difference in immobility between 1D-TST and 6D-TST groups ($t(18) = -1.605$, $p > .05$). However, the 1D-TST group had significantly greater total activity ($t(18) = 2.721$, $p < .05$) and activity >2 vlts ($t(18) = 2.886$, $p < .05$) than the 6D-TST group. In addition, the 1D-TST group had significantly fewer boli than the 6D-TST group, ($t(18) = 2.886$, $p < .05$).

Discussion

The thiouracil effectively induced hypothyroidism in the experimental drug (hypothyroid) group. Specifically, the T4 assay showed a significant reduction (approximately 50%) in the amount of thyroxine in the thiouracil treated group compared to the control group. Due to the

depressive symptoms that are typical of hypothyroidism and the proposed relationship between the release of ACTH and hypothyroidism, the six day tail suspension testing group had significantly fewer movements for total activity and activity >2 volts than both the single-trail. This lack of movement also translates into the open field test for anxiety, with the thiouracil and single trial tail suspension groups having significantly fewer rears than the six-day and no-tail suspension groups. Also, the single trial tail suspension group traveled a shorter total distance within the chamber than both control and six-day tail suspension groups. This promotes the notion that the symptomology of hypothyroidism and depression are near equivalent. Lastly, the plasma analyses of total thyroxine levels confirmed that thiouracil produced hypothyroidism in our subjects. The implications of these results include a better understanding of how stress can alter the neuroendocrine system to produce hypothyroidism and how this can impact patients that are diagnosed with major depression. These studies also help clarify the relationship between thyroid status and major depression and consequently help to clarify the current confusion of the appropriate treatments for the depression associated with hypothyroidism and whether thyroid status needs to be considered when treating major depression.

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