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Decreased anxiety- and depression-like behaviors and hyperactivity in a type 3 deiodinase-deficient mouse showing brain thyrotoxicosis and peripheral hypothyroidism

J. Patrizia Stohn, M. Elena Martinez, Arturo Hernandez (Ph.D.) (Faculty Scientist II)*

Department of Molecular Medicine, Maine Medical Center Research Institute, Maine Medical Center, 81 Research Drive, Scarborough, ME, 04074, United States

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ABSTRACT

Hypo- and hyperthyroid states, as well as functional abnormalities in the hypothalamic-pituitary-thyroid axis have been associated with psychiatric conditions like anxiety and depression. However, the nature of this relationship is poorly understood since it is difficult to ascertain the thyroid status of the brain in humans. Data from animal models indicate that the brain exhibits efficient homeostatic mechanisms that maintain local levels of the active thyroid hormone, triiodothyronine (T3) within a narrow range. To better understand the consequences of peripheral and central thyroid status for mood-related behaviors, we used a mouse model of type 3 deiodinase (DIO3) deficiency (*Dio3* -/- mouse). This enzyme inactivates thyroid hormone and is highly expressed in the adult central nervous system. Adult *Dio3* -/- mice exhibit elevated levels of T3-dependent gene expression in the brain, despite peripheral hypothyroidism as indicated by low circulating levels of thyroxine and T3. *Dio3* -/- mice of both sexes exhibit hyperactivity and significantly decreased anxiety-like behavior, as measured by longer time spent in the open arms of the elevated plus maze and in the light area of the light/dark box. During the tail suspension, they stayed immobile for a significantly shorter time than their wild-type littermates, suggesting decreased depression-like behavior. These results indicate that increased thyroid hormone in the brain, not necessarily in peripheral tissues, correlates with hyperactivity and with decreases in anxiety and depression-like behaviors. Our results also underscore the importance of DIO3 as a determinant of behavior by locally regulating the brain levels of thyroid hormone.

1. Introduction

The relationship between thyroid hormone status and the susceptibility and outcome of psychiatric conditions, especially mood disorders, is not well understood. Although abnormal thyroid parameters in the serum have been associated with several of these conditions, findings may vary between different human studies and the nature of that relationship remains unclear (Dayan and Panicker, 2013; Fava et al., 1995; Haggerty and Prange, 1995).

Some human studies indicate that hypothyroidism is associated with an increased risk of anxiety, depression and suicide (Chueire et al., 2007; Cleare et al., 1995; Constant et al., 2005; Custro et al., 1994; Sinai et al., 2009). Hypothyroid patients with certain single nucleotide polymorphisms in the thyroid hormone transporter OATP1C1 are more susceptible to develop depression (van der Deure et al., 2008). In addition, abnormal parameters in thyroid function have been associated with postpartum depression risk (Albacar et al., 2010; Lucas et al., 2001; Pedersen et al., 2007; Plaza et al., 2010), and serum thyroid hormone concentrations have been reported to influence depression severity (Berent et al., 2014; Joffe and Marriott, 2000). Furthermore, altered levels of TSH in response to the administration of thyrotropin-releasing hormone (TRH) are also associated with depression severity or susceptibility (Duval et al., 1994; Kim et al., 2015; Kirkegaard and Faber, 1986; Targum et al., 1984), suggesting a relationship between the disorder and the setup of the hypothalamic-pituitary-thyroid (HPT) axis. Even the use of thyroid hormones, together with antidepressants, appears to improve outcomes (Bauer et al., 2002; Bauer et al., 2005; Joffe and Sokolov, 2000; Joffe et al., 1995; Prange, 1996), in some cases probably due to the influences of certain antidepressants on the HPT axis (Shelton et al., 1993) or the local availability of thyroid hormone in

* Corresponding author at: Maine Medical Center Research Institute, Scarborough, ME 04074, USA.
E-mail address: hernaa@mmc.org (A. Hernandez).

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the brain regions (Baumgartner et al., 1994; Campos-Barros et al., 1994).

Although a majority of research supports an association between hypothyroidism and depression, the results from other studies do not align with this idea. Park et al. reported no association between thyroid disease and depression in older men (Park et al., 2010), Frye et al. found no correlation between depression and thyroid parameters in cerebrospinal fluid (Frye et al., 1999), and Medici et al. observed that an increased risk of depression is associated with low serum TSH (Medici et al., 2014). There might be significant differences in the experimental design and populations between these and other studies, but the significant inconsistency of findings has lead to the suggestion that thyroid tests offer misleading information for psychiatric patients (Lasser and Baldessarini, 1997).

Studies in animals largely confirm the occurrence of increased anxiety- and depression-like behaviors in rodent models of hypothyroidism (Darbra et al., 2003; Ge et al., 2014; Yu et al., 2015). These abnormal behaviors are observed in mice with a hypofunctional thyroid hormone receptor alpha1 (Buras et al., 2014), and are normalized with T3 treatment (Venero et al., 2005). The depression-related phenotypes observed in mice with an altered HPT axis (Shukla et al., 2010; Zeng et al., 2007), also supports its association with mood disorders. There are less published work on the psychiatric effects of excessive exposure to thyroid hormone, but hyperthyroidism has also been associated with mood alterations (Demet et al., 2002; Simon et al., 2002), suggesting that thyroid hormone action in the brain needs to be maintained at appropriate levels for normal behavioral outcomes.

One potential explanation for the disparity of some findings is the relatively loose relationship between thyroid hormone parameters in the serum and actual thyroid hormone signaling in the brain. The latter is greatly influenced, not as much by circulating levels of thyroid hormones, but by local determinants of thyroid hormone action, including thyroid hormone transporters and deiodinase enzymes (Bianco, 2011). When these factors are genetically altered in mouse models, a marked divergence may exist between the thyroid status of the serum and that of the brain (Bianco, 2011). We hypothesized that the thyroid hormone status of the brain is more consequential than that of the serum for mood-related behaviors that depend on thyroid hormones.

One critical determinant of thyroid hormone action in the central nervous system is the type 3 deiodinase (DIO3), an enzyme encoded by the imprinted gene *Dio3* (Hernandez et al., 2002) that inactivates thyroid hormone (Hernandez, 2005). During adult life, *Dio3* -/- mice exhibit systemic hypothyroidism due to functional deficits in the HPT axis, but marked brain thyrotoxicosis due to impaired T3 clearance in the central nervous system (Hernandez et al., 2010).

Here we use this mouse model of divergence between the peripheral and central thyroid state to better understand the relationship between mood disorders and thyroid hormone status in the brain versus that in the circulation. We find that Dio3 −/- mice exhibit hyperactivity and a reduction in anxiety- and depression-like behaviors despite their peripheral hypothyroidism. The new data shed additional light on the relationship between thyroid hormone status and mood disorders and underscores the potential relevance of DIO3 for these conditions.

2. Methods

2.1. Animals

Male (C57Bl/6J genetic background) and female (129/SvJ genetic background) mice were mated to generate the experimental animals used for behavioral analysis and gene expression. Both breeders were heterozygous for an inactivating mutation in the gene that codes for the D3 enzyme, Dio3 (Hernandez et al., 2006). Thus, the *Dio3* +/+ and *Dio3* -/- littermates so generated are on a defined 50:50 129/SvJ/C57Bl/6J mixed genetic background. The genomic heterozygosity of experimental animals reflects better the genetic variation in the human population and avoids extreme baseline behavior that may exist in inbred strains preventing the detection of a behavioral phenotype. All mice were kept in a 12 h light/dark cycle and fed regular chow *ad libitum*. Adult animals (4–5 month old) were sacrificed by CO_2 asphyxiation. Mice carrying the FINDT3 transgene used for β-galactosidase staining of brain sections were also littermates but in a heterogeneous 129/SvJ/C57Bl/6J mixed genetic background. Brains from these mice were collected and processed for β-galactosidase staining of coronal section as previously described (Hernandez et al., 2010).

For the behavioral tests, two different cohorts of mice (Dartmouth cohort and the MMCRI cohort) were generated at different Institutions and tested at different facilities. One was generated at the Geisel School of Medicine at Dartmouth (Dartmouth cohort) and the other at MMCRI (MMCRI cohort). For the Dartmouth cohort, the testing schedule was as follows: open filed test at day one, elevated plus maze at day four, marble burying test at day 8, tail suspension test at day 15, and forced swimming test at day 22. For the MMCRI cohort, the testing regime was: light/dark box test at day one, elevated plus maze test at day four, elevated zero maze at day 8, and marble burying test at day 11. The shorter time intervals between tests is based on battery testing done at The Jackson Laboratory (O'Leary et al., 2013). A total of 103 mice were used in the behavioral studies. The Dartmouth cohort comprised 56 mice (15 *Dio3* +/+ males, 14 *Dio3* -/- males, 16 *Dio3* +/+ females and 11 *Dio3* -/- females), while the MMCRI cohort comprised 47 mice (17 *Dio3* +/+ males, 9 *Dio3* -/- males, 9 *Dio3* +/+ females and 12 *Dio3* -/- females). Not all mice were used in all tests. The number of mice from which data was obtained for a given test is indicated in the figure legends. The Institutional Animal Care and Use Committees of the Geisel School of Medicine at Dartmouth and the Maine Medical Center Research Institute approved all procedures and behavioral tests. The animal work was performed following the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Quantitative real time RT-PCR (qRT-PCR)

The neocortex was harvested and frozen on dry ice, and total RNA was extracted using the RNeasy kit from Qiagen (Valencia, CA). Total RNA (1 μg) was reverse transcribed with M-MLV reverse transcriptase in the presence of random hexamers at 42 °C for 1 h. Reverse transcription reactions were diluted appropriately and aliquots were used as templates in duplicate real-time PCR reactions for each of the selected genes. Reactions were run in a 7300 RT PCR System (Applied Biosystems) using the SYBR® Select Master Mix from Applied Biosystems. Real time PCR reactions underwent an initial 10 min denaturing step, followed by 36 cycles of a denaturing step (94 °C for 30 s) and an annealing/extension step (60 °C for one minute). The sequence of the primers used was (5′ to 3′): *Itih3*, GCACGTTCAGTTGGCTAGAC and CCATCTCCAAAGGACACCAC; *Hr*, AGCACTGTGTGGCATGTGTT and AACCCTGCATCCAAGTAGCA; *Rn18s*, GGAGTATGGTTGCAAAGCTG and TCGCTCCACCAACTAAGAAC; *Sema7a*, AAGTGGTCGTTCACCGCATG and CCACCACCTTGTGAATGGTG; *Aldh1a1*, CCTTGCATTGTGTTTGCAGATG and GCTCGCTCAACACTCCTTTTC; *Shh*, GGACGTAAGTCCTTCACCAG and TTCTGTGAAAGCAGAGAACTCC; *Htr2c*, ACTTGTCATGCCCCTGTCTC and CCGCGAATTGAACCGGCTAT; *Bdnf*, TGCAGGGGCATAGACAAAAGG and CTTATGAATCGCCAGCCAATTCTC; *Thra*, CTTTGA-