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Review

Thyroid hormones in male reproductive development: Evidence for direct crosstalk between the androgen and thyroid hormone axes

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ABSTRACT

Thyroid hormones (THs) exert a broad range of effects on development in vertebrate species, demonstrating connections in nearly every biological endocrine system. In particular, studies have shown that THs play a role in sexual differentiation and gonadal development in mammalian and non-mammalian species. There is considerable evidence that the effects of THs on reproductive development are mediated through the female hormonal axis; however, recent findings suggest a more direct crosstalk between THs and the androgen axis. These findings demonstrate that THs have considerable influence in the sexual ontogeny of male vertebrates, through direct interactions with select sex-determining-genes and regulation of gonadotropin production in the hypothalamus–pituitary–gonad axis. THs also regulate androgen biosynthesis and signaling through direct and indirect regulation of steroidogenic enzyme expression and activity. Novel promoter analysis presented in this work demonstrates the potential for direct and vertebrate wide crosstalk at the transcriptional level in mice (*Mus musculus*), Western clawed frogs (*Silurana tropicalis*) and medaka (*Oryzias latipes*). Cumulative evidence from previous studies; coupled with novel promoter analysis suggests mechanisms for a more direct crosstalk between the TH and male reproductive axes across vertebrate species.

1. Introduction

Thyroid hormones (THs) influence many developmental processes, including reproduction. However, the specific mechanisms underlying this hormonal interaction are largely debated. Initially, it was believed that the female axis solely mediated the effects of THs on the reproductive development of either sex. Consequently, the interrelationships between the estrogen and THs axes are well established and have been previously reviewed (teleost fish (Habibi et al., 2012; Liu et al., 2011); mammals (Doufas and Mastorakos, 2000; Vasudevan et al., 2002)). Over the last two decades, a more direct crosstalk between the androgen and TH axes has also been suggested (Wagner et al., 2008, 2009). However, the molecular basis for cross-regulation between these two hormonal pathways is still largely unexplored and has not been extensively reviewed. In this comparative review, we provide further evidence for direct crosstalk between the androgen and TH axes throughout male reproductive development, weakening the proposal that the female reproductive axis solely mediates TH effects.

TH regulation has been implicated in the reproductive development of many different vertebrate species (rainbow trout, *Oncorhynchus mykiss* (Holloway et al., 1999); zebrafish, *Danio rerio* (Filby et al., 2007); African clawed frog, *Xenopus laevis* (Goleman et al., 2002); Western clawed frog, *Silurana tropicalis* (Duarte-Guterman et al., 2010; Duarte-Guterman and Trudeau, 2011; Langlois et al., 2010a, 2011); Bocage's Wall Lizard, *Podarcis bocagei* (Bicho et al., 2013); Indian garden lizard, *Calotes versicolor* (Haldarmisra and Thapliyal, 1981); American tree sparrows, *Spizella arborea* (Reinert and Wilson, 1996); Indian finch, Lal munia, *Estrilda amandava* (Thapliyal and Pandha, 1967); red munia, *E. amandava* (Saxena et al., 2011); sheep, *Ovis aries* (Karch et al., 1995); rat, *Rattus norvegicus* (Tamura et al., 1998)). This cross regulation has been studied extensively on the physiological level, with many studies examining the role of the TH axis in testes function and development (*X. laevis* (Goleman et al., 2002); Hokkaido salamander, *Hynobius retardatus* (Kanki and Wakahara, 1999); *P. bocagei* (Bicho et al., 2013); *C. versicolor* (Haldarmisra and Thapliyal, 1981); *Lonchura punctulata* (Gupta and Thapliyal, 1984), *E. amandava* (Saxena et al., 2011; Thapliyal and Pandha, 1967); Chicken, *Gallus gallus* (Akhlaghi and Zamiri, 2007); *O. aries* (Parkinson et al., 1995); *R. norvegicus* (Anbalagan et al., 2010; Cristovao et al., 2002;

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Holsberger and Cooke, 2005; Jannini et al., 1995; Lagu et al., 2005; Marchlewska et al., 2011; Wagner et al., 2008, 2009; Wajner et al., 2009); *Homo sapiens* (Maran, 2003)). Therefore, we focus primarily on transcriptional, hormonal and cellular responses within the male reproductive-axis to elucidate the molecular controls behind this hormonal interaction. We reviewed information concerning both mammalian and non-mammalian species to stress the conserved nature of TH regulation in male reproductive development. We acknowledge that species-specific variation in the interaction between THs and male reproduction does exist; however, crosstalk between the androgen and TH axes appear to be maintained in some degree across vertebrates. We believe our findings effectively demonstrate that THs have considerable influence in the sexual ontogeny of male vertebrates and strengthen the proposal of cross-regulation between the androgen- and TH-axes.

2. Androgenic involvement in TH biosynthesis

An understanding of the TH hierarchy enables us to better identify and elucidate points where crosstalk is possible. The hypothalamus–pituitary–thyroid axis (HPT) has been extensively reviewed in vertebrate species (teleosts and amphibians (Brown and Cai, 2007; Carr and Patino, 2011); mammals, (Wagner et al., 2008, 2009)). Previous reviews solely examined the regulatory role of THs in reproductive development. Here we focus on the potential for cross-regulation between androgenic factors and THs throughout TH biosynthesis in a brief outline of the HPT structure.

Considering the extent of the proposed regulatory role of THs in the androgen axis, an understanding of TH biosynthesis could assist in elucidating where potential crosstalk between androgens and THs can occur. Throughout normal thyroid gland functioning, the thyroid-releasing hormone (TRH) is released from the hypothalamus. TRH then triggers the release of thyroid-stimulating hormone (TSH) or thyrotropin, from thyrotrope cells in the *pars distalis* of the adenohypophysis (Fig. 1). Studies show that TRHs and TSHs are susceptible to regulation by other endocrine systems at the hypothalamic and pituitary levels. Gonadotropin-releasing hormones (GnRHs), from the hypothalamus–pituitary–gonad axis (HPG) interfere with the TH axis, increasing TSH secretion (Northern leopard frog, *Rana pipiens* (Denver, 1988); American bullfrog, *Rana catesbeina* (Okada, 2004)). An increase in TSH production would lead to a subsequent increase in TH synthesis. TSH binds to receptors on the thyroid follicle cell membrane, stimulating the biosynthesis of the iodine-containing THs, tetraiodothyronine or thyroxine (T4), and triiodothyronine (T3). T4 is the principle form of TH secreted from the thyroid gland; however, it is quickly metabolized into T3, the more potent form of TH. In addition to increased TSH concentrations, a number of studies have demonstrated that exposure to GnRH induces T4 secretion, increasing T4 production and serum concentrations in fish (Barfin flounder, *Verasper moseri* (Chiba et al., 2004); masu salmon, *Oncorhynchus masou* (Chiba et al., 2004); goldfish, *Carassius auratus* (Chiba et al., 2004) and amphibians (*R. pipiens* Denver, 1988; *A. mexicanum* (Jacobs and Kuhn, 1987);; Marsh frog, *Rana ridibunda* (Jacobs et al., 1988); Common frog, *Rana temporaria* (Jacobs et al., 1988); European frog, *Rana esculenta* (Jacobs et al., 1988)). However, no changes in circulating T3 concentrations were observed in fish in response to GnRH increases (*C. auratus* (Mackenzie et al., 1987)). Discrepancies between T4 and T3 level fluctuations suggest that gonadotropins can increase the baseline circulating TH concentration, but the appropriate deiodinase activity would have to be stimulated in order to increase the concentration of the active TH.

Deiodinases activate and deactivate THs via iodination and deiodination of their phenolic rings (Fig. 1; reviewed in Kohrle (1996) and Visser and Schoenmakers, 1992). Thus, the coordination of the expression and activity of the deiodinase enzymes in individual tissues regulates the concentration of active THs, according to the specific needs of the tissue. The spatiotemporal distribution and expression of deiodinases have been shown to respond to androgen signaling. For example, exposure to finasteride, a known disruptor of androgen biosynthesis, increases type II deiodinase (*dio2*) and decreases *dio3* mRNA levels in brain and liver tissues of pre-metamorphic tadpoles (Fig. 1; *S. tropicalis* (Langlois et al., 2011)), suggesting that TH axis is responsive to circulating androgen concentrations, and increases T3 concentrations accordingly. In support of observed deiodinases activity, T4 and T3 ratios and concentrations fluctuate with androgen levels. Testosterone (T) treatment had no effect on the plasma concentrations of T4, but reduced the T3 concentrations in rainbow trout (*Salmo-gairdneri Richardson* (Leatherland, 1985). Moreover, studies have identified androgen receptors (ar) in the thyroid gland of different vertebrate species (American alligator, *Alligator mississippiensis* (Bermudez et al., 2011); mammals, reviewed in Pelletier (2000)), suggesting that the androgen axis directly regulates TH synthesis. Androgenic regulation of both the synthesis and the peripheral metabolism of THs alone, demonstrates considerable crosstalk between the androgen and TH axes. Further research on these potential regulatory and feedback mechanisms will further strengthen the proposed mechanism.

3. TH-related machinery within gonadal tissues

Deiodinases are responsible for TH peripheral metabolism and thyroid receptors (trs) mediate TH activity at sites of action, both are present within gonadal tissues. Moreover, it has become clear that the distribution of TH-related machinery in gonadal tissues is highly sex-specific. Studies have identified deiodinases in the testes of vertebrate species, and the role of deiodinases within testicular functioning in mammalian species has been reviewed in detail (see reference Wagner et al. (2009)). In developing rats (*R. norvegicus*), dio1 and dio2 activity are higher in the testes compared to ovaries, whereas dio3 activities is higher in ovary tissue (Bates et al., 1999). Recently, similar observations have been confirmed in non-mammalian species. Testes of striped parrotfish (*Scarus iseri*) are characterized by higher *dio2* and *dio3* mRNA levels than ovaries (Johnson and Lema, 2011). Also, testes of *O. mykiss* are characterized by higher transcripts of *dio2*, and *dio2* expression is dependent on spermatogenic stages, increasing at the beginning of spermatogenesis (Sambroni et al., 2001). Moreover, Duarte-Guterman and Trudeau (2011) demonstrate that *dio1*, *dio2* and *dio3* mRNAs are significantly higher in testes compared to ovaries in the frog *S. tropicalis*. Altogether, this demonstrates that maintenance of a baseline level of active THs by deiodinases could be necessary to vertebrate testes development.

TRs mediate TH signaling and are crucial for testes development and function. The expression of *trs* in testicular tissues and their physiological implications in mammalian species have been reviewed thoroughly (Bagamasbad and Denver, 2011; Tsai-Morris et al., 1993; Valadares et al., 2008). *trα* and *trβ* genes code for a number of tr-isoforms including: *trα1*, *trα2*, *trα3*, *trβ1*, *trβ2*, and *trβ3*. These various tr-isoforms are expressed in a range of tissue types including testes (fish (Johnson and Lema, 2011; Sambroni et al., 2001); reptiles (Cardone et al., 2000) mammals (Buzzard et al., 2000; Holsberger and Cooke, 2005; Jannini et al., 1990, 1999; Williams, 2000, 2011). Apriletti et al. (1998) reviewed in detail the various modes of action of tr isoforms in mammals. The expression of *trs* in testes is dependent on circulating TH concentrations. Recent studies demonstrate that *tr* mRNA within gonadal tissues fluctuate with TH production, reinforcing auto-regulation by THs (De Paul et al., 2008; Wagner et al., 2008, 2009). Similar