Published in final edited form as:

Curr Opin Endocr Metab Res. 2018 October ; 2: 18–23. doi:10.1016/j.coemr.2018.01.010.

Thyroid Hormone Deiodination and Action in the Gonads

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Abstract

Alterations in thyroid hormone (TH) status during developmental or adult age are associated with abnormal gonadal development and function in human and animal models. In the developing testis, aberrant levels of TH action lead to disruptions in cell proliferation and differentiation, ultimately influencing testicular size, spermatogenesis, steroidogenesis and male fertility. Recent work is increasingly identifying the systems and signaling pathways in gonadal cells that are affected by TH, as well as delineating the major factors that determine TH signaling in gonadal tissue. The TH receptor alpha, the monocarboxylate transporter 8 and the type 3 deiodinase appear to be major contributors to the timely regulation of TH action in the developing testis.

Keywords

thyroid hormone; testis; ovary; thyroid hormone deiodinase; thyroid hormone receptor; thyroid hormone transporter; Sertoli cell; Leydig cell; *Dio1*; *Dio2*; *Dio3*; *Mct8*; *Thra*; *Thrb*

1. Introduction

Thyroid hormones (THs) are well known for their critical roles in the development of the central nervous system and in the regulation of metabolism and heart physiology. However, the presence of thyroid hormone receptors in most cell types and the effects of THs observed by investigators studying different physiological systems are evidence of the broad actions of these hormones across tissues. These include the gonads.

Work from recent years has substantially advanced our understanding of the mechanisms that regulate TH action at the tissue and cellular level. This research is increasingly revealing that TH action not only depends on the serum concentrations of the hormones, but also on tissue-specific pre-receptor processes. Circulating levels of thyroxine (T4) and 3,5,3'-

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triiodothyronine (T3), largely dictated by the hypothalamic-pituitary-thyroid axis, are the initial determinant of TH action in peripheral tissues. However, the local complement of TH transporters, deiodinases and receptors are critically positioned to adjust TH availability and action to levels suiting the specific developmental requirements of the target cells [1]. Furthermore, pre-receptor mechanisms may produce a local level of TH action that greatly diverges from what can be predicted from the serum concentrations of the hormones [2–4].

Despite these advances, there is still very limited information about the gonadal determinants of TH action and their specific importance for the pathophysiology of the reproductive axis. In this regard, further studies are warranted, since substantial evidence from the last two decades has shown that abnormalities in circulating levels of TH have a significant impact on gonadal development and may adversely affect reproductive function. In the context of previously established knowledge, here we review the most recent studies advancing our understanding of the functional importance and mechanisms of TH action in the gonads.

2. TH status and the gonads

TH status influences male reproductive function and testis development [5, 6]. Numerous studies using rodent models of hypo- or hyperthyroidism have shown that altered circulating levels of TH cause significant abnormalities in the proliferation and differentiation of testicular cells, changes in testis size and functional and hormonal alterations in the male reproductive axis [5]. Consistent with these previous observations, recent findings indicate that treatment of rats with the antithyroid compound propylthiouracyl from late gestation to weaning age lead in adult life to enlarged testis, increased number of Sertoli cells (SCs) and normal number of Leydig cells [7]. In mice, antithyroid treatment from birth influences the prepubertal testis by increasing estrogen content and aromatase expression and by down-regulating TH receptor alpha (*Thra*) and critical genes in the steroidogenic pathway including SF-1, StAR, CYP11A1 and 3-beta-HSD [8]. These abnormalities are also associated with decreased testicular expression of glucose transporters and testicular glucose content and increased apoptosis of germ cells [9]. About the latter observation, studies in *Akt1*^{−/−} mice made hypothyroid during lactation suggest that *Akt1* exerts a protective function and prevents germ cell apoptosis in hypothyroidism [10].

A very consistent observation in the literature is that T3 promotes the arrest of SC proliferation and that neonatal hypothyroidism extends their period of proliferation and delays their differentiation [5]. Observations in a rat model of fetal and neonatal hypothyroidism further supports the hypothesis that the larger testis size cause by developmental hypothyroidism is the result of extended SC proliferation [11]. This effect is not observed in a hypothyroidism mouse model with a genetic deficiency in nitric oxide synthase 2 [12], suggesting that nitric oxide synthesis may be affected by TH status and is partially mediating the mechanism by which TH influence SC proliferation and testis size. On the other hand, the arrested proliferation of SCs caused by early TH action may involve the inhibition of cell-cycle regulatory genes including cyclins A2 and D1, and the suppression of the PI3K/ Akt pathway, as determined by a study using SCs from piglets [13]. TH may also have a role in the establishment of the blood/testis barrier by promoting

maturational changes in the expression of connexin 43 [14, 15]. The consequences of an altered TH status on testicular cell proliferation is still noted when the onset of hypo- or hyperthyroidism occurs at pre-pubertal age. In this model, hyperthyroidism –but not hypothyroidism–was associated with a reduction in the diameter of seminiferous tubules and an elevation in the number of Leydig cells [16]. Taken together, these recent studies further demonstrate that alterations in the normal developmental course of TH state lead to adverse testicular outcomes.

Altered TH states are also associated with impaired ovarian physiology and reduced reproductive function, indicating an important role for TH action in the ovary. A study in granulosa and cervical cells from a small cohort of infertile women showed a significant reduction in markers of fertility, including gremlin-1 and prostaglandin synthase 2. Interestingly, these reductions were associated with an altered profile in the expression of TH receptors indicative of reduced TH action [17]*, suggesting a direct link between female infertility and reduced TH receptor signaling in reproductive tissues. In addition, chronic hypothyroidism in adult female rats reduces the ovarian reserve and follicle size [18]. These findings are consistent with observations from studies in adult women and adolescents girls showing an association between autoimmune thyroiditis and reduced ovarian reserve [19, 20], although it is unclear whether this is the direct result of decreased TH action in the ovary or a consequence of the autoimmune disease [21].

Ovarian alterations in particular molecular pathways are also associated with abnormal TH status. As observed in the testis, studies in immature hyper- and hypothyroid rats indicate that TH receptor alpha (*Thra*) and nitric oxide signaling contribute to ovarian follicular development [22]. These altered TH states also influence the physiology of glucose transport in the ovary, especially in response to chorionic gonatropin [23], suggesting that ovarian TH action levels may impact ovarian growth and maturation. In a rat model, hyperthyroidism achieved by excessive iodine intake leads to a stimulation of steroidogenic pathways and estradiol excess [24], consistent with studies cited above associating hypothyroidism with decreased testicular steroidogenesis and supporting the notion that TH signaling correlates with steroidogenesis in male and female gonads.

3. Molecular determinants of TH action in the gonads

The impact of altered TH status on the gonads suggests the presence of TH transporters and receptors in these tissues. Seminal work in rodents demonstrated that THRA is the most abundant receptor in the testis [25] and recent evidence shows expression of TH transporters in the chicken testis [26]. In addition, expression of all three deiodinases has been detected in the testis and ovary at different neonatal ages [27]. The mouse ENCODE Consortium [28, 29] has made available gene expression data for adult mouse tissues. These data reveals that, for both the testis and the ovary (Fig. 1A), *Thra* is the most abundant receptor and *Lat1* (*Slc7a5*) and *Lat2* (*Slc7a8*) are the most abundant transporters, with the *Mct8* (*Slc16a2*) transporter also expressed at notable levels in the adult ovary. Although ENCODE data indicates negligible expression of T4 transporter OATP1C1 (organ anion transporting polypeptide 1C1, also *Slco1c1*) in the adult mouse testis, its expression has been observed in human Leydig cells [5], but no information is available about its relevance for Leydig cell

function. In addition, other transporters from the same family including *Slco6b1* and *Slco6c1* exhibit expression that is highly specific to the testis [5], suggesting an important role in this tissue. However, given their ability to transport other molecules, it is uncertain whether their function in the testis is critical for the transport of THs.

According to these data, deiodinases *Dio1* and *Dio2* are expressed at very low levels with *Dio2* showing higher expression in the ovary and *Dio1* in the testis. (No ENCODE data on deiodinase *Dio3* expression was available at the time of writing this article). The gonadal expression of the different deiodinases may vary significantly across developmental stages [27]. Furthermore, rodent data from additional studies (Fig. 1B) have shown that the testicular expression of THRA, MCT8 and DIO3 declines dramatically from neonatal age into adulthood [25, 30]**. The major changes in the expression of these proteins suggest very different regulation and requirements for testicular TH action depending on the developmental stage.

3.1. TH transporters in the testis

Although *Lat1* and *Lat2* are the most abundant TH transporters in the adult mouse testis [29], little is known about their physiological significance or their cell-specific expression in the testis. Testicular *Mct8* expression is low in the adult, but high during neonatal life [30]** (Fig.1B). MCT8-deficient male mice show normal fertility and significantly enlarged testis [30]**. This phenotype is consistent with reduced TH transport and action in testicular cells during neonatal life, a time of active proliferation of SCs and spermatogonia that is known to be extended by hypothyroidism [5]. The larger testis of MCT8-deficient mice suggests that T3 action dependent on MCT8-mediated T3 transport influences processes that ultimately impact SC proliferation, as the number of these cells is a major determinant of testis size [31].

3.2. TH deiodinases and testis development and function

Although detectable, the testis reveals relatively low levels of expression of the DIO1 and DIO2 deiodinases, which are capable of converting T4 to T3, thereby enhancing T3 availability and action [32]. In addition, male mice deficient in DIO1 or DIO2 do not exhibit a fertility or testicular phenotype, suggesting no critical role for these deiodinases on testis development and function.

In contrast, the neonatal testis exhibits high levels of expression of the deiodinase DIO3 [30]**, whose function is to inactivate both T4 and T3 by converting them into compounds with negligible affinity for the TH receptors [32]. DIO3 expression is still detectable in the adult testis, but at much lower levels. This marked developmental pattern of expression suggests a role for DIO3 in preventing premature T3 action in the neonatal testis, allowing for an appropriate period of proliferation of testicular cells. Consistent with this notion are the observations in DIO3-deficient mouse neonates showing elevated testicular expression of T3-dependent genes, arrested proliferation of testicular cells and premature lumen formation in seminiferous tubules [30]**. This leads to a marked reduction in testis size that is noticeable during neonatal life and persists into adult age [30]**. This testicular phenotype is associated with impaired spermatogenesis, delayed puberty, reduced serum testosterone

and increased serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [30]**. Furthermore, adult *Dio3*^{-/-} male mice exhibit reduced fertility, increased hypothalamic expression of gonadotropin releasing hormone, pituitary expression of FSH and LH, testicular expression of follistatin and the inhibin family of genes [30]**. Some of these abnormalities, including reduced testis size, arrested cell proliferation and elevated FSH have been also observed in animals that received pharmacological doses of TH during neonatal life [33], suggesting that direct effects of TH in the neonatal testis are largely causing the phenotype of *Dio3*^{-/-} mice. However, adverse effects of T3 excess in other tissues of the reproductive axis may also contribute to the observed abnormalities. Overall, these findings demonstrate a critical role for DIO3 in testis development and function.

Of note, *Dio3* is an imprinted gene that is preferentially expressed from the paternal allele in most tissues [34], an observation that raises the possibility of a parent-of-origin testicular phenotype in mice heterozygous for the inactivating *Dio3* mutation. Consistent with *Dio3* imprinting, mice that inherit the mutated allele from the father show slightly reduced testis size [30]**. This parent-of-origin phenotype occurs despite the fact that *Dio3* expression in the neonatal testis is essentially biallelic, as mice with inactivation of the paternally- or maternally-inherited *Dio3* allele exhibit similar levels of testicular *Dio3* expression [35]*. This suggests that the lack of systemic T3 clearance rather than testicular T3 clearance is largely responsible for the testicular phenotype of these animals. This idea is further supported by the increased serum T3 in mouse neonates that inherited the *Dio3* mutation from their fathers, while this parameter is normal in those that inherited the *Dio3* mutation from their mothers.

The marked testis size reduction of DIO3-deficient mice is less severe with a genetic loss of function in DIO2 [30]**. The increased neonatal expression of T3-dependent genes and the adult elevation in FSH and follistatin of *Dio3*^{-/-} mice are also largely corrected with DIO2 deficiency [30]**. The less severe phenotype of mice with double DIO3/DIO2 deficiency suggests that T3 generation by DIO2 contributes significantly to the systemic and gonadal T3 excess that occurs in *Dio3*^{-/-} mice during neonatal life.

Similarly, genetic ablation of the transporter MCT8 in *Dio3*^{-/-} mice results in a partial normalization of testicular size and neonatal expression of T3-dependent genes in the testis [30]**. Adult testicular expression of follistatin is fully normalized, but the elevated testicular expression of inhibin B and pituitary expression of FSH are exacerbated [30]**. These observations suggest that cellular T3 transport by MCT8 contributes significantly to the effects of T3 excess in the testes, but probably less to the effects of DIO3-deficiency in other tissues of the male reproductive axis.

3.3. THRA in the testis

Previous expression data on TH receptors had suggested a prominent role for *Thra* in testicular TH action. *Thra* mRNA and protein are present in the testis at much higher levels than those of TH receptor beta (*Thrb*) [36], and these observations are consistent with recent gene expression data from the ENCODE project in the mouse adult testis (Fig. 1A) [29]. The predominant role of THRA in the developing testis is demonstrated by the effect of *Thra* ablation in models of neonatal thyrotoxicosis. THRA deficiency fully normalizes testis size

in mice treated with T3 during neonatal age [33, 37], or in mice deficient in DIO3 [30]**. In the latter, TRHA-deficiency also completely reverse the hormonal and gene expression abnormalities.

In addition, mice carrying a transgene expressing a dominant negative form of the THRA globally or specifically in SCs exhibit enlarged testes [38, 39]** and a lengthening in the period of SC proliferation that is partially mediated by signaling through the Cdk4/c-myc pathway. Increases in SC proliferation, testis weight and sperm reserved are also observed in mice depleted of a truncated form of THRA present in the mitochondrial matrix [40]**, suggesting that mitochondrial abnormalities partly contribute to the effects of TH signaling on SCs.

The testis size phenotype of mice expressing a dominant negative THRA in SCs is not exacerbated by additional transgene expression in Leydig cells [39]**, consistent with previous literature suggesting a predominant role of SC number in the determination of testis size [31]. This finding does not negate a role for THRA in Leydig cells. In this regard, and consistent with the observed effects of TH status on steroidogenesis reviewed above, an *in vitro* study in primary Leydig cells shows that THRA-dependent TH signaling activates directly, or indirectly through the up-regulation of the transcription factor *Nur77* (*Nr4a1*), the promoters of steroidogenic enzymes [41]*. Overall, these observations demonstrate a central role for THRA in mediating TH signaling in the testis and in both Sertoli and Leydig cells, with important consequences for testis development and steroidogenesis.

4. Summary

Published evidence, especially on the testis, indicate that TH status exert a significant influence on the development and function of the gonads. TH impacts the balance of proliferation and differentiation in testicular cells, affecting testis size, spermatogenesis and steroidogenesis. A number of TH transporters, deiodinases and receptors are present in gonadal tissue, with their expression varying significantly with development and possibly with cell type. DIO3, MCT8 and THRA appear to play a critical role in testis development and function, but much work is still needed to understand the cellular and molecular mechanisms regulating TH action in the gonads and to determine their relevance to male and female reproductive function.

Acknowledgments

The author is grateful to M. Martinez for reviewing this manuscript. This work was supported the National Institutes of Health (grants DK095908 and MH096050).

Abbreviations

TH(s)	thyroid hormone(s)
SC(s)	Sertoli cell(s)

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Figure 1.

A, Mouse gonadal expression data generated by the ENCODE project [29] of genes implicated in TH action (Note that data on *Dio3* expression was not available at this time). RPKM, reads per kilobase of transcript per million mapped reads; B, Relative neonatal and adult expression levels of *Mct8*, *Dio3* and *Thra* in the rodent testis (adapted from Jannini *et al.* and Martinez *et al.* [30, 42]).

Figure 2.

Testis size and levels of TH signaling. Testis size relative to body weight and illustrative composite photograph in mouse models with genetic deficiencies in different molecular components involved in TH action (adapted from Martinez *et al.* [30]).