General and Comparative Endocrinology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Effect of neonatal hypothyroidism on prepubertal mouse testis in relation to thyroid hormone receptor alpha 1 (THRα1)

Debarshi Sarkar, Shio Kumar Singh *

Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221005, India

ARTICLE INFO

Article history:
Received 29 March 2016
Revised 30 June 2016
Accepted 1 August 2016
Available online xxxx

Keywords:
Hypothyroidism
SF-1
Testicular steroidogenesis
Spermatogenesis
THRα1
Mouse

ABSTRACT

Thyroid hormones (THs) are important for growth and development of many tissues, and altered thyroid status affects various organs and systems. Testis also is considered as a thyroid hormone responsive organ. Though THs play an important role in regulation of testicular steroidogenesis and spermatogenesis, the exact mechanism of this regulation remains poorly understood. The present study, therefore, is designed to examine the effect of neonatal hypothyroidism on prepubertal Parkes (P) strain mice testis in relation to thyroid hormone receptor alpha 1 (THRα1). Hypothyroidism was induced by administration of 6-propyl-2-thiouracil (PTU) in mother's drinking water from birth to day 28; on postnatal day (PND) 21 only pups, and on PND 28, both pups and lactating dams were euthanized. Serum T_3 and T_4 were markedly reduced in pups at PND 28 and in lactating mothers, while serum and intra-testicular testosterone levels were considerably decreased in pups of both age groups. Further, serum and intra-testicular levels of estrogen were significantly increased in hypothyroid mice at PND 28 with concomitant increase in CYP19 expression. Histologically, marked changes were noticed in testes of PTU-treated mice; immunohistochemical and western blot analyses of testes in treated mice also revealed marked decrease in the expression of THRα1 at both age groups. Semiquantitative RT-PCR and western blot analyses also showed reductions in both testicular mRNA and protein levels of SF-1, StAR, CYP11A1 and 3β-HSD in these mice. In conclusion, our results suggest that neonatal hypothyroidism alters localization and expression of THRα1 and impairs testicular steroidogenesis by down-regulating the expression SF-1, thereby affecting spermatogenesis in prepubertal mice.

1. Introduction

Transient neonatal hypothyroidism in rats results in increased testicular size and daily sperm production in adult life due to an increase in the number of Sertoli cells, Leydig cells and germ cells (Cooke and Meisami, 1991; Hess et al., 1993). Simorangkir et al. (1997) have reported that despite greater testicular mass in adult life, testis mass and germ cell numbers remain significantly reduced in hypothyroid juvenile rats. The reason for this unlikeness has not been critically evaluated so far. The effect of transient neonatal hypothyroidism on the testes of immature rats is reflected mainly by the absence of round spermatids due to incomplete meiosis (Simorangkir et al., 1997), thus depicting the possible role of thyroid hormones (THs) on germ cell survival and proliferation. Earlier reports show that the effect of thyroid hormones on germ cells is mediated mainly via Sertoli cells (Van Haaster et al., 1992, 1993). The question now arises as to how thyroid hormones could act as regulator for germ cell survival if the latter cells do not express thyroid hormone receptors (TRs)? Till date, the presence of thyroid hormone receptor alpha 1 (THRα1) in the testis remains a matter of controversy. Jannini et al. (1994) and Holsberger et al. (2005) have shown that the THRα1 mRNA and protein are abundant in developing rat testis, especially in Sertoli cells. THRα1 is the predominant isoform expressed in germ cells from intermediate spermatogonia to pachytene spermatocyte (Buzzard et al., 2000) and in Sertoli cells during proliferative phase of their development (Buzzard et al., 2000; Canale et al., 2001). In mice testes, THRα1 is crucial for mediating the effects of T_3 on Sertoli cell development other than thyroid receptor beta 1 (TRβ1) and thyroid receptor beta 2 (TRβ2) (Buzzard et al., 2000). Further, several studies also indicate that THRα1 is present in the adult testis, thus suggesting that the T_3-binding capacity is not completely absent in adult testis (Canale et al., 2001).

Studies have shown that the altered thyroid status in prepubertal rat has profound effect on Leydig cell differentiation (Mendis-Handagama and Siril Ariyaratne, 2005) and the transient neonatal hypothyroidism increases the number of Leydig cells in adult rat

* Corresponding author.
E-mail address: shioks@rediffmail.com (S.K. Singh).

http://dx.doi.org/10.1016/j.ygcen.2016.08.001
0016-6480/

testis (Hardy et al., 1993; Mendis-Handagama and Sharma, 1994) by arresting differentiation of these cells and allowing their continuous proliferation (Teerds et al., 1998). Treatment of Leydig cells with T_3 and hCG resulted in additive augmentation to the level of steroidogenic acute regulatory (StAR) protein and steroid production via increased expression of SF-1 (Ikeda et al., 1994; Manna et al., 1999). The nuclear receptor family of transcription factors such as steroidogenic factor-1 (SF-1) regulates the expression of StAR protein, cytochrome P450scc (CYP11A1) and 3β-hydroxysteroid dehydrogenase (3β-HSD) genes (Morohashi and Omura, 1996). Earlier reports have also shown that during hypothyroid conditions the activities of Leydig cell 3β- and 17β-HSDs and aromatase are also decreased (Antony et al., 1995; Ando et al., 2001). Down-regulation of aromatase activity is further associated with decreased estrogen production during altered thyroid condition. On the other hand, chronic T_3 treatment of Leydig cells increases the mRNA level of CYP11A1 with down-regulation in mRNA levels of 3β-HSD (Manna et al., 2001). Fluctuations in circulating THs also have the ability to disrupt androgen biosynthesis (Flood et al., 2013). Testosterone, on the other hand, is the prime hormone responsible for the sustenance of spermatogenesis and germ cell kinetics (Dohle et al., 2003; Smith and Walker, 2014).

Most of the studies so far have mainly described the inhibitory role of transient neonatal hypothyroidism on adult testis in terms of testosterone production despite increased number of Leydig cells (Cooke and Meisami, 1991). Further, role of THs deficiency in early developmental period and its function on testis in relation to THRα1 and steroidogenesis in immature mice remain unclear. The present study, therefore, is conducted in a comprehensive way to determine the effect of neonatal hypothyroidism on testicular steroidogenesis, taking SF-1 as a key mediator which might be a link between hypothyroidism and decreased testicular steroidogenesis. Thus, the objectives of the present study are (i) to evaluate the effect of neonatal hypothyroidism on testicular steroidogenesis and its possible association with spermatogenesis in prepubertal mice; and (ii) to study the effect of neonatal hypothyroidism on localization and expression of THRα1 in prepubertal mice testes.

Parkes strain male mice were used in the present investigation, which we have been using for an animal model (Joshi and Singh, 2015; Sarkar et al., 2015).

2. Materials and methods

2.1. Chemicals

Chemicals used in the present study were of analytical grade and purchased from HiMedia Laboratories and Merck India Ltd., Mumbai, unless stated otherwise. All the primers were procured from Eurofins genomics India Ltd., Bangalore. Primary antibodies used in the present study, except for SF-1 (kind gift from Prof K.I. Morohashi, Kyushu University, Japan), were obtained either from Santa Cruz Biotechnology Inc, CA, USA or Thermo Pierce, Rockford, IL, USA. anti-mouse and anti-rabbit horseradish peroxidase-conjugated IgG were purchased from GeNei, Bangalore, India, while anti-goat horseradish peroxidase-conjugated IgG was procured from Santa Cruz Biotechnology Inc, CA, USA.

2.2. Animal maintenance

Male P mice of postnatal day 21 and 28 (PND 21 and 28) were used in this study and they were obtained from litters of our mice colony in the Department of Zoology, Banaras Hindu University. PND 21 was selected because, in mouse haploid spermatids are generated by day 20 (Print and Loveland, 2000). PND 28, on the other hand, was selected to encompass the period of the activation of blood testis barrier (Franca et al., 2012; Murphy et al., 2014). The animals were maintained under hygienic conditions in well-ventilated rooms at 23 ± 2 °C with 12 h photoperiod and relative humidity of 50 ± 20%. On the day of birth, the litter size was culled to 6–8 pups per mother. Mice were maintained on pellet food (Amrut Laboratory Animal Feeds, Pune, India) and drinking water *ad libitum*. Each group of experimental animals was housed separately in polypropylene cages (450 mm × 270 mm × 150 mm) with dry rice husk as bedding material. General health and body weight of the animals were monitored regularly throughout the treatment schedule. Animals were maintained in accordance with the guidelines of the Banaras Hindu University Animal Ethics Committee as per approval of committee for the purpose for control and supervision of experimental animals (CPCSEA), Government of India (No. 1802/G0/Re/S/15/CPCSEA).

2.3. Experimental design

The experimental protocol of this study was based on a report in which 6-propyl-2-thiouracil (PTU) in drinking water of lactating dams induced neonatal hypothyroidism in rat pups (Gilleron et al., 2006). Lactating female P mice were divided into two groups, each comprising nine (n = 9) individuals. In the treated group (HT), pups were made hypothyroid by adding 0.1% (w/v) PTU (Sigma-Aldrich, St. Louis, USA) to the drinking water of lactating dams from birth to day 28. Fresh PTU was provided to mothers every 3–4 days up to day 28. Lactating dams in control group (CN) received normal drinking water *ad libitum*. Male mice from both groups were killed on PND 21 and 28.

2.4. Autopsy, blood and tissue collection

After recording final body weights at PND 21, only male pups were sacrificed, whereas on PND 28, both male pups and lactating dams were euthanized by decapitation under mild ether anaesthesia. Trunk blood was collected; serum was separated and stored at −80 °C until further use. For histological and immunohistochemical studies, testes from either left or the right side were randomly excised from five mice in each group, while testes from the other side of the above five and those from the remaining four mice were excised, blotted free of blood, weighed and kept frozen at −80 °C until further use.

2.5. Estimation of hormones

Testosterone (T), 17β-estradiol (E_2) and thyroid hormones (T_3 and T_4) were measured in the present investigation. The serum and intra-testicular levels of T and E_2 were measured by ELISA as per manufacturer's instruction, using a highly sensitive and specific commercial kit (DiaMetra, Segrate, Italy). Serum levels of T and E_2 were measured from individual mouse of PND 21 and 28 in each group (n = 9). Estimations of intra-testicular T and E_2 were performed following the protocol described by Jeyaraj et al. (2005), with minor modifications. Briefly, whole testis was homogenized in 1 ml of phosphate-buffer saline (PBS) via sonication. The homogenate (1 ml) was then extracted with diethyl ether, evaporated and resuspended in PBS for hormone estimations. The sensitivity of the assays for T and E_2 was 0.07 ng/ml and 8.68 pg/ml, respectively. All samples were quantified in single assay with intra- and inter-assay coefficient of variations being 5.8% and 10.5%, respectively for T, while the intra- and inter-assay coefficient of variations for E_2 were 9% and 10%, respectively. The ELISA kit for E_2 was shown to possess 100% cross-reactivity for 17β-estradiol.

Serum levels of T_3 and T_4 were measured in 400 μl of serum by a solid-phase competitive chemiluminescent enzyme immunoassay on the Siemens ADVIA Centaur XP assay system (Siemens AG,

Please cite this article in press as: Sarkar, D., Singh, S.K. Effect of neonatal hypothyroidism on prepubertal mouse testis in relation to thyroid hormone receptor alpha 1 (THRα1). Gen. Comp. Endocrinol. (2016), http://dx.doi.org/10.1016/j.ygcen.2016.08.001