Journal of Photochemistry & Photobiology, B: Biology 165 (2016) 351–358

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Photoperiodic modulation of thyroid hormone receptor (TR-α), deiodinase-2 (Dio-2) and glucose transporters (GLUT 1 and GLUT 4) expression in testis of adult golden hamster, *Mesocricetus auratus*

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ARTICLE INFO

Article history:
Received 4 August 2016
Accepted 29 October 2016
Available online 1 November 2016

Keywords:
Photoperiod
Golden hamster
Testis
Deiodinase-2
Thyroid hormone
GLUT 1–4

ABSTRACT

Phenomenon of seasonal reproduction is being regulated by changes in day length or photoperiod. The molecular mechanism underlying the event of photoperiodic regulation of testis and thyroid functions along with glucose uptake transporters has never been reported for golden hamster, *M. auratus*. The present study was performed to investigate the effect of photoperiod on the expression of key thyroid hormone receptor (TR-α), deiodinase-2 (Dio-2) and glucose uptake transporters (GLUT-1 & GLUT-4) in testicular germ cell and Leydig cells, and its correlation with the testicular androgen receptor (AR), germ cell proliferation factor (PCNA) and cell survival factor (Bcl-2) in testis of adult golden hamster, *Mesocricetus auratus*. Hamsters were exposed to different photoperiodic regimes i.e. critical photoperiod (CP), short day (SD) and long day (LD) for 10 weeks. LD induces upregulation of thyroidal and gonadal activity as evident by active thyroid gland and testicular histoarchitecture, peripheral total thyroid (tT3, tT4) and testosterone hormone profiles when compared with SD exposed hamsters. Further, LD increased the expression of testicular TR-α, Dio-2, GLUT-1, GLUT-4 along with testicular AR and glucose content thereby enhancing germ cell proliferation and survival as reflected by increased PCNA and Bcl-2 expression when compared to SD exposed hamsters. Thus, it can be suggested that testicular thyroid hormone status is being regulated by photoperiod and is possibly involved in seasonal adaptation to reproductive phenomenon of golden hamster.

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1. Introduction

Photoperiod regulates various season bound physiological changes (such as molting, hibernation, migration and reproduction) in various species where they utilize their intrinsic timing mechanism to anticipate changes in order to adopt their physiology for the coming seasons [1] and breed at a particular time of year to maximise the survival of their offspring. Photoperiodic alternation in the morphometric and steroidogenic factors have been studied under photo-inhibitory condition leading to full regression of testis in Syrian hamsters [2]. We previously reported for the first time about the effect of changing photoperiod on peripheral hormonal level along with expressional pattern for steroidogenic factors in regulation of testicular seasonality of golden hamster [3] but studies related to photoperiodic moderation of other endocrine gland function like thyroid hormone status that also regulates molecular mechanism of testicular seasonality has never been discussed in detailed.

Thyroid hormones have emerged as key elements in the synchronization of seasonal reproduction. They not only regulate the proliferation and differentiation of testicular Sertoli and Leydig cells but also regulate the expression of steroidogenic enzymes, biosynthesis of testosterone and expression of androgen receptor [4]. The action of thyroid hormone in target tissue is mediated by thyroid hormone receptors having two isoforms, TR-α and TR-β. The presence of thyroid hormone receptor alpha (TR-α) has been reported in mammalian testis [5–9]. Thyroid gland secrets pro-hormone thyroxine (T4) which needs to be converted into tri-iodothyronine (T3) in target cells. Deiodinase enzymes are essential control points for local thyroid hormone status as deiodinase-2 (Dio-2) catalyzes the process for conversion of thyroxine (T4) to bioactive form tri-iodothyronine (T3) in tissue specific manner and thus regulates intra-hypothalamic bio-availability of T3 [1,10,11] and is also highly expressed in germ cells of adult rat testis [12].

Studies related to seasonal changes in mammalian physiology have generally focused on reproductive function due to its high energetic costs and implication for organism fitness [13]. Seasonal fluctuations in reproductive status have evolved as adaptive mechanism to cope with seasonal fluctuations such as energy availability [14]. Glucose as energy substrate is of high priority to the organism to ensure and perform the multitude of cellular processes inherent to life and it also regulates steroidogenesis in testis [15] for the maintenance of normal

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http://dx.doi.org/10.1016/j.jphotobiol.2016.10.036
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reproductive function and reduction in glucose availability inhibits testicular functions [16] as in Sertoli cell it gets converted into lactate which acts as substrate for ATP production in germ cells [17] and is known to exhibit antiapoptotic effect on testicular germ cells [18,19]. Glucose uptake into cells involves its transfer across cell membranes through a family of related glucose uptake transporter proteins (GLUTs) [20]. Sertoli cells have specific glucose uptake transporters (GLUT 1 and GLUT 3) belonging to class 1 subfamily [21–23]. GLUT-4 controls the rate of glucose uptake in most of the mammalian cells [20,24]. Thyroid hormone has important endocrine effects on the maintenance of glucose homeostasis. Thyroid hormone is known to stimulate glucose uptake by increasing the GLUT 1 and GLUT 4 glucose transporter proteins [25] and thereby plays a pivotal role in controlling the local energy metabolism. We gathered from literature that the impact of energy availability in regulation of testicular function has never been investigated for any seasonal breeder in general and especially for golden hamster.

Previous studies reveals about the involvement of mediobasal hypothalamus (MBH) as centre for photoperiodism where intra hypothalamic bioavailability of T3 which is biologically active form of thyroid hormone, is governed by photoperiod dependent changes in deiodinase expression where long day induces the upregulation of deiodinase-2 (Dio-2) which is thyroid hormone activating enzyme, while short day inhibits Dio-2 expression [26]. Reciprocal switching of Dio-2 and Dio-3 gene activation in tanycytes surrounding the third ventricle affects gonadotropin releasing hormone (GnRH) release from median eminence (ME) and in turn modulates the release of pituitary gonadotropins [27]. Such photoperiodic regulation of thyroid hormone in hypothalamus appears to be an adaptive feature in birds and fishes reproduction [28,29].

To the best of our knowledge, molecular pathways involving photoperiodic moderation of TR-α, Dio-2, GLUT-1 and GLUT-4 expression in testis with reference to germ cell proliferation and survival of golden hamster has never been studied. Hence, we proposed that changing photoperiod would certainly have its role on local thyroid hormone status of testis that may regulate reproduction of seasonally breeding mammal. Thus, we investigated the photoperiodic modulation of thyroid function, testicular thyroid hormone receptor and its activating enzyme expression along with the testicular glucose uptake transporters to record their possible role in regulation of testicular germ cell proliferation and their survival in seasonally breeding mammal, *Mesocricetus auratus*.

2. Materials and Methods

All the experiments were conducted in accordance with institutional practice and within the framework of experimental animals (Scientific Procedure) Act 2007, of the Committee for the Purpose of Supervision and Control on Experiments on Animals (CPSCEA), Government of India, on animal welfare.

2.1. Animal Procurement and Maintenance

Golden hamsters were procured from Central Drug Research Institute (CDRI), Lucknow, India and colony was developed and maintained in the departmental animal house facility. Hamsters were kept under constant temperature (25 ± 2 °C) and light/dark cycle (critical photoperiod; 12.5 h light, 11.5 h dark; i.e. lights on at 07:00 a.m. and lights off at 07:30 p.m.). Animals were maintained in polypropylene cages (475 × 350 × 200 mm) and provided with commercial rodent pellet and water ad libitum.

2.2. Photoperiodic Treatments

Adult male golden hamsters (average weight 125 g, 90–100 days old) were randomly selected and divided into three experimental groups (N = 5/group) and exposed to different photoperiodic regimes as:

Group I: critical photoperiod (CP: 12.5 h light and 11.5 h dark)

Group II: short day photoperiod (SD: 8 h light and 16 h dark)

Group III: long day photoperiod (LD: 16 h light and 8 h dark).

Hamsters were exposed to photostimulatory long day condition and photoinhibitory short day condition for 10 weeks to achieve maximum testicular growth and regression respectively. The critical photoperiod exposed hamster group act as control.

2.3. Sample Collection and Processing

The animals were weighed and sacrificed at 11:00 h at the end of photoperiodic treatment under deep ether anaesthesia [3]. Testes and thyroid gland were removed aseptically, blotted dry, weighed and processed for histological and western blot analyses.

2.4. Histology

Testes and thyroid gland were immersed in 10% neutral formalin for 24 h and then further cut into small slices of 1–2 mm thickness and kept in fresh 10% neutral formalin for 24 h. These slices were then washed under running tap water to remove excess of fixative and then sequentially dehydrated. After clearing in xylene, the tissues were embedded in paraffin and cut into 5 μm sections. The sections were mounted on clean glass slides pre-coated with 1% gelatine. Deparaffinised sections were stained using hematoxylin and eosin stain. Morphology was observed under research microscope (Nikon, E 200, Japan) in randomly selected testis sections. The germinal epithelium height and thyroid epithelium cell height was measured with Motic software using Nikon-E200 as published in Verma and Krishna [30].

2.5. Immunohistochemical Analyses

The immunohistochemical analyses were performed according to method published in Mukherjee and Haldar [3]. Briefly, 5 μm thick testis sections were mounted on glass slides and were sequentially rehydrated. Endogenous peroxide activity was blocked by 0.1% H_2O_2 in methanol for 30 min at room temperature [RT] followed by washing with phosphate buffer saline (PBS) and incubated with horse blocking serum [1:100 in PBS; PK-6200, Vector laboratories, Burlingame, CA] for 2 h. Then sections were incubated with primary antibody against TR-α (SAB4502968, Lot#310345, rabbit polyclonal, Sigma, dilution 1:200) and Dio-2 (Ab77779, rabbit polyclonal, Abcam, dilution 1:200) overnight at 4 °C followed by washing thrice with PBS and sections were then incubated with biotinylated secondary antibody [rabbit IgG, PK-6200, Vector laboratories, Burlingame, CA, dilution 1:50]. After washing thrice with PBS, a pre-formed ABC reagent conjugated to the free biotin of the secondary antibody was administered. The immunoreactivity for TR-α was visualized using the 0.03% peroxidase substrate 3, 3-diaminobenzidine [DAB, Sigma chemicals St. Louis, USA] in 0.01 M Tris-Cl [pH 7.6] and 0.1% H_2O_2. For Dio-2 the sections were counter stained with hematoxylin, dehydrated, mounted with DPX and observed under research microscope (Nikon, E 200, Japan). Immunodetection was performed on at least 10 tubular cross sections per testis from each group.

2.6. Western Blot Analyses

The western blot analyses were performed following the method of Mukherjee and Haldar [3] and Mukherjee et al. [31]. Briefly, testes were homogenized and lysed in RIPA buffer containing aprotinin, sodium orthovanadate and phenyl methyl sulphonyl fluoride (PMSF) protease inhibitor cocktail and was centrifuged at 12,000*g* for 30 min at 4 °C.

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