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Simulated photoperiod influences testicular activity in quail via modulating local GnRHR-GnIHR, GH-R, Cnx-43 and 14-3-3

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ABSTRACT

The hypothalamo-hypophyseal-gonadal axis mediated differential photosexual responses in quail kept under different simulated photoperiodic conditions have been studied in details. Local testicular GnRH-GnIH and their receptor system has been hypothesized to be modulated in quail showing different photo-sexual responses and thus influence the testicular activity and steroidogenesis through local (paracrine and autocrine) action. To validate this hypothesis, we studied the expression of gonadotropin releasing hormone receptor (GnRH-R), gonadotropin inhibiting hormone receptor (GnIH-R) mRNA, growth hormone receptor (GH-R), proliferating cell nuclear antigen (PCNA), 14-3-3, Connexin-43 (Cnx-43), steroidogenic factor-1 (SF-1), Steroidogenic Acute Regulatory protein (StAR), steroidogenic enzyme (3β HSD) in testis as well as androgen receptor (AR) in testis and epididymis of photosensitive (PS), scotorefractory (SR), photorefractory (PR) and scotosensitive (SS) quail. Experimental findings clearly indicate the increased expression of GnIH-R mRNA and suppression of GnRH-R, GH-R, PCNA, 14-3-3, Connexin-43, SF-1, StAR, 3β HSD in testis as well as AR in testis and epididymis of PR and SS quail, while PS and SR quail exhibited the opposite results i.e., significantly decreased expression of GnIH-R mRNA and increased expression of GnRH-R, GH-R, PCNA, 14-3-3, Cnx-43, SF-1, StAR, 3β HSD in testis as well as AR in testis and epididymis. The significantly increased intra-testicular testosterone has been observed in the PS and SR quail while, PR and SS quail showed opposite results. Hence, we conclude that PS and SR quail showed significantly increased testicular activity and steroidogenesis while opposite pattern was observed in PR and SS quail.

1. Introduction

Previously, we reported that differential photosexual responses in quail kept under different photoperiodic conditions is shown through modulation of hypothalamic deep brain photoreceptors (DBPs: VA-opsin and Opsin-5) and reproductive neuropeptides (GnRH-I, II, and GnIH) and subsequent testicular stimulation/inhibition [1]. In a separate study, we have also shown that GnIH induce the P53 dependent Bax mediated testicular apoptosis in PR and SS quail while in PS and SS quail opposite effects have been observed [2]. Although, the long and short photoperiod induced differential photo-sexual responses and the involvement of hypothalamic-pituitary-gonadal (HPG) axis are extensively documented in various avian species. In general, long photoperiod is gonado-stimulatory and short day length is gonado-inhibitory. Increased day length of summer stimulates the onset of gonadal activities in photoperiodic avian species and these birds are said to be photosensitive (PS). Decreased day length initiates post-reproductive gonadal regression making them photorefractory (PR). During PR phase, gonads regress when days are still long than that required for gonadal recrudescence [3]. During 'absolute' PR condition, the gonads cannot be re-stimulated by any amount of photoperiod, even not with the continuous condition of light (24L: 0D). On the other hand, some birds, showing the 'relative refractoriness', remain continuously in breeding condition under constant long days but gonads regress if they are shifted to relatively short days. Various species including quail [4–6] are reported to show this type of refractoriness. Hypothalamus is considered as the regulatory site of photorefractoriness [7–8]. Marked decrease in LH and FSH is demonstrated as the reason behind the post reproductive gonadal regression [9–10]. Exposing these 'photo-refractory' birds to short day length, may restore the ability of photo-sensitivity in them by terminating the photorefractoriness.

On the other hand, seasonal gonadal growth in short day breeders is initiated with decreasing daylength and breeding occurs during short days of winter. Like long day breeders, these species also undergo the phases of scotosensitivity (sensitive to inhibitory effects of short days) and scotorefractoriness (refractory to the inhibitory effects of short days

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i.e. gonads develop even under short days). In such species, constant short days mediate the gonadal regression and birds with quiescent gonads are referred as scotosensitive (SS). But, after sometime, spontaneous gonadal growth occurs and birds are said to be scotorefractory (SR). This adaptive mechanism enables this species to breed even under short days [11–13]. Thus phenomenon of avian photoperiodism is complex. In fact, gonadal development and regression may occur in both long and short daylength exposed birds. The plausible reason behind these differential photo-sexual responses is may be because of the sensitivity and refractoriness to a particular daylength which develops after certain period of exposure to the same daylength and is termed as photo/scoto-sensitive and refractory phase [14–16].

GnRH-I and GnIH and their receptors [2,17–19] have been documented in quail testis. GnIH, through its receptor inhibits testicular activity, growth and testosterone production [20–22] in birds and mammals [17,23]. Presence of growth hormone (GH) is documented in human and chicken testis [24–25] as well as mediates the reproductive growth and differentiation [26]. Testicular expression of this hormone and its receptors indicate its autocrine, paracrine, and/or intracrine actions in avian reproduction [25]. Testicular GH influences steroidogenesis and gametogenesis [26]. GHR is unanimously present in the plasma membrane, cytoplasm as well as in the nucleus of various cell types [27–28]. PCNA is the auxiliary protein of DNA polymerase and serves in DNA replication [29–30]. Because PCNA is a potent marker of proliferating cells involved in DNA synthesis, its expression was monitored in the testis of different photoperiodic quail phenotypes to evaluate the testicular growth and activity.

The intercellular channels, made up of gap junctional proteins (predominantly Connexin-43: Cnx-43), play very important role in Sertoli cell proliferation and differentiation [31]. Sertoli cells support sperm development through nutrient supply and connexins are the part of nutrient channel [32]. Presence of connexins has also been demonstrated in frog as well as human testis, suggesting the critical role of these channels in the maintenance of spermatogenesis in humans but also in non-mammalian vertebrates [33–35].

The 14-3-3 protein family isoforms influence diverse cellular processes through binding and interacting with a wide range of proteins [36–37]. 14-3-3 binding and interactions has been demonstrated with several intra-cellular phosphatases, kinases and transmembrane receptors through homo- and heterodimerization [36–37]. These functions are crucial for the cell differentiation and maintenance of the physiological function in differentiated cells [38–39]. 14-3-3 theta isoform serves an essential role in regulating cell-cell interaction and cell adhesion and maintains the blood-testis barrier [40]. 14-3-3 also plays crucial role in reorganizing the Sertoli-cell cytoskeleton and Sertoli-cell signaling [41].

Steroidogenic factor 1 (SF-1) is a master regulator of gonadal and adrenal steroidogenesis [42–43]. However, it has been shown that SF1 also regulates other genes that are involved in various cellular processes [44]. StAR (Steroidogenic Acute Regulatory protein) mediates transportation of cholesterol which is a precursor of steroid synthesis [45]. In mammals, androgen is essential in maintaining the spermatogenesis. It also regulates the luminal microenvironment and guarantees the proper transport, maturation and storage of sperms [46–47]. Androgen receptor (AR) is known to mediate the physiological actions of testosterone and dihydrotestosterone but little is known about the differential AR expression in the testes and epididymis of different quail phenotypes (PS, SR, PR and SS).

Although, we have evaluated the differential simulated photoperiodic condition induced modulation of hypothalamic deep brain photoreceptors (DBPS: Opsin-5 and VA-opsin) and GnRH-GnIH system [1]. Further, we hypothesized that these different simulated photoperiodic conditions may also modulate testicular activity and steroidogenesis through influencing the local testicular GnRH-GnIH system.

In the present study, we have investigated the effect of different simulated photoperiodic conditions on the local testicular GnRH-GnIH receptor system (GnRH-R and GnIH-R), GH-R, PCNA, Cnx-43 and multifunctional 14-3-3, steroidogenic proteins (SF-1, StAR) and enzyme (3β HSD) in testis and AR in testis and epididymis of photosensitive (PS), scotorefractory (SR), photorefractory (PR) and scotosensitive (SS) quail.

Table 1
Details of the antibodies used in the present study.

Antibodies	Catalog. no. and/or sources	Dilution
GH-R (mouse monoclonal)	sc-137185; Santa Cruz Biotechnology, USA	1:100 (IF)
GnRH-R (mouse monoclonal)	sc-8682; Santa Cruz Biotechnology, USA	1:25 (IF)
14-3-3 (rabbit polyclonal)	Dr. David Pallas, Winship Cancer Institute, Emory University School of Medicine, Atlanta, USA	1:100 (IF) 1:1500 (WB)
Cnx-43 (mouse monoclonal)	sc-13558; Santa Cruz Biotechnology, USA	1:100 (IF) 1:1000 (WB)
PCNA (mouse monoclonal)	Prof. Catherine Green, Nuffield Department of Medicine, University of Oxford, UK	1:1000 (WB)
SF-1 (rabbit polyclonal)	Prof. Ken-ichirou Morohashi, Department of Molecular Biology, Graduate School of Medical Sciences, Kyushu University, Japan	1:1000 (IF) 1:2000 (WB)
StAR (rabbit polyclonal)	Prof. Douglas Stocco, Texas Tech University Health sciences centre, Lubbock, Texas, USA	1:1000 (IF) 1:2000 (WB)
3β-HSD (rabbit polyclonal)	Prof. J. Ian Mason, University of Edinburgh, MRC Centre for Reproductive Health, UK	1:1000 (WB)
AR (rabbit polyclonal)	Prof. Frank Claessens, Department of Cellular and Molecular Medicine, University of Leuven, Leuven, Belgium	1:200 (IF) 1:1000 (WB)
β-actin (mouse monoclonal)	A00702-40; Genscript, USA	1:2500 (WB)

2. Materials and Methods

To study photoperiod induced alterations in testicular activity and steroidogenesis in quail, sexually mature six week old male Japanese quail were acclimatized under laboratory conditions in a photoperiodically controlled room. For inducing different photoperiodic conditions (PS, PR, SS and SR), these adult male Japanese quail were randomly divided into two groups and maintained under long day condition (16L: 8D) (n = 48) or short day condition (6L: 18D) (n = 48) for 9 weeks. Long day birds were further divided into two sub-groups. Birds in Group I (n = 24) were continued to be exposed to long daylength conditions (16L:8D) for an additional four weeks; this condition maintained photosensitivity (PS) and quail continued to be reproductively active. Birds of Group II (n = 24) were transferred to intermediate daylength (13.5L:10.5D) for four weeks, condition that resulted in relative photorefractoriness (PR) and suppression of cloacal gland and testes. Short day birds, after 9 weeks of exposure to short daylength conditions were scotosensitive (SS)/sexually quiescent, with completely regressed cloacal gland and testes size. These quails were also randomly divided into two sub-groups. Birds in Group III (SS) (n = 24) were sacrificed and processed as described below. Rest of the birds i.e., Group IV (n = 24) were continued to be exposed to short daylength conditions (6L: 18D) for an additional four weeks; to attain the scotorefractory (SR) condition. All experimental procedures were approved by Institutional Animal Ethical Committee, Institute of Science, Banaras Hindu University (BHU), Varanasi (Ethical committee letter No.: F.Sc./88/IAEC/2016-17/1402) and the ARRIVE guidelines were followed to report this study.