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Testicular atrophy and reproductive quiescence in photorefractory and scotosensitive quail: Involvement of hypothalamic deep brain photoreceptors and GnRH-GnIH system

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ABSTRACT

Birds time their daily and seasonal activities in synchronization with circadian and annual periodicities in the environment, which is mainly provided by changes in photoperiod/day length conditions. Photoperiod appears to act at the level of eye, pineal and encephalic/deep brain photoperception and thus entrain the hypothalamic clock as well as reproductive circuitry in different avian species. In this article our focus of study is to elucidate out the underlying molecular mechanism of modulation of the hypothalamic reproductive circuitry following the photoperception through the hypothalamic photoreceptor cells and the subsequent alteration in the reproductive responses in quail, kept under different simulated photoperiodic conditions. Present study investigated the different simulated photoperiodic conditions induced hypothalamic DBP-GnRH-GnIH system mediated translation of photoperiodic information and subsequent exhibition of differential photosexual responses (scoto-/photo-sensitivity and refractoriness) in Japanese quail, *Coturnix coturnix japonica*. Paired testes weight and paired testicular volume increased 15.9 and 22.6-fold respectively in scotorefractory quail compare to that of scotosensitive phase and 12.8 and 24.3-fold in photosensitive quail compare to that of photorefractory phase. The pineal/eye melatonin (through melatonin receptor subtype $Mel_{1c}R$) and hypothalamic deep brain photoreceptor (DBPs) cells directly modulate the hypothalamic GnRH-I/II and GnIH system and thus exhibit testicular stimulation or regression in response to different photoperiodic conditions (PS, PR, SS and SR). The hypothalamic alteration of DBP(s) and GnRH-GnIH system thus may induce the testicular stimulation in PS and SR quail and testicular regression in SS and PR quail.

1. Introduction

Photoperiod is considered a main temporal factor for seasonal reproduction in many temperate and some tropical and subtropical avian species [1,2,3]. Some of the non-temperate species although do not use annual day length to time their reproduction but respond to artificial photoperiod and their gonad develop or regress accordingly [3]. Changes in gonadal functions mediated in response to photoperiod through the hypothalamic-pituitary-gonadal (HPG) axis are extensively documented in birds. In general, long day length or photoperiod is gonado-stimulatory and short day length is gonado-inhibitory and hence increasing or increased day length of spring and summer i.e., modulation from short to long day length stimulates the onset of seasonal reproductive activities in photoperiodic avian species and these birds are said to be photosensitive (PS) [1,3,4–6]. Similarly, decreasing or decreased day length of post summer or winter markedly initiate post-reproductive testicular regression and these photoperiodic species also become photorefractory (PR) when their reproductive system is no more sensitive to long days and gonads regress when days are still long i.e., longer than that which initiated gonadal recrudescence [7].

On the other hand, seasonal gonadal growth in short day breeders is initiated with decreasing day length 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 i.e. gonads develop even under short days). In such species, when maintained under constant short days, initially gonad regress/remain in quiescent condition and birds are referred as scotosensitive (SS) but after sometime spontaneous gonadal growth occurs because short days are no more inhibitory to the reproductive system and birds are said to be scotorefractory (SR). This adaptive mechanism enables the species to breed even under short days [4,8,9]. Thus phenomenon of avian photoperiodism (i.e., long days are gonado-stimulatory and short days are gonado-inhibitory) is not so simple. In fact, gonadal development and

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regression as well, may occur in both long and short day length exposed birds, underlying mechanism of which is the sensitivity and refractoriness to a particular day length which develops after certain period of exposure to the same day length and is termed as photo/scotosensitive and refractory phase [4,5,9–11].

Photoperiodic responses are dependent on an interaction between endogenous circadian clocks [12–15] and encephalic photoreceptors. Unlike mammals, birds do not use melatonin to relay photoperiodic information; they use photoperiodic information directly through photoreceptors located within the mediobasal hypothalamus [16–19]. The mechanisms by which photoperiodic responses influence seasonal reproductive physiology in birds are complex [20–23]. The most interesting fact in avian photoperception which differs entirely from that of mammals is the transmission of light information occurs via three distinct/different pathways: the eyes, the pineal, and the deep brain [24]. In spite of the established fact that reproduction in birds do not require the seasonal changes in melatonin secretion [25,26], there are some evidences confirming the involvement of melatonin in the regulation of gonadotropin secretion and gonadal activity [27–29]. Thus, Ubuka et al. [30] investigated the action of melatonin on the expression of GnIH in the excellent photoperiodic poultry species, quail. As the pineal gland and eyes are the major sources of melatonin in quail [31], Ubuka and his group [30] demonstrated that pinealectomized (Px) quail combined with orbital enucleation (Ex) (Px plus Ex) showed significantly decreased expression of GnIH precursor mRNA and GnIH peptide while melatonin administration in the same quail reversed the GnIH precursor mRNA and GnIH peptide expression pattern in dose-dependent manner. Additionally, a melatonin receptor subtype (*Mel1c* mRNA) was expressed in GnIH-*ir* neurons in the paraventricular nucleus (PVN) and the binding of melatonin was further confirmed by receptor autoradiography in the PVN. Thus melatonin acts directly on GnIH neurons through its receptor to induce expression of GnIH [30]. A later study by Chowdhury et al., again confirmed that melatonin can stimulate GnIH release from the quail hypothalamus [32].

A variety of opsins have been suggested as the photopigment involved [33–35]. Photostimulation is essentially controlled by photoperiod through altering the rate of secretion of gonadotropin releasing hormone I/II (GnRH I/II) from the median eminence. Along the 3rd ventricle, the bilaterally distributed GnRH I cells send long projections to the median eminence where GnRH I is secreted [36].

These conditions under constant long (photosensitive/photorefractory) and short day length (scotosensitive/scotorefractory) develop at the hypothalamic level and the effects were observed at the gonadal level but its underlying mechanism still remains unclear. In photosensitive avian species, long day length initially induces testicular development. Long-term exposure to long-day photoperiod, however, inhibits the hypothalamo-pituitary-gonadal response to stimulatory long days and results in the onset of photorefractoriness. Inhibition of the HPG axis gives rise to a marked increase in GnIH and prolactin concentrations and a marked decrease in GnRH. Subsequently, gonadotropins- LH and FSH concentrations drop to undetectable values [3,10]. These hormonal changes, along with elevated prolactin concentrations, appeared to be responsible for rapid atrophy in testicular volume, activity and function [11].

In young Japanese quail, daily photoperiod exerts a definite effect on gonadal development and precocious sexual development can be attained by stimulatory photoperiods [37]. Reports indicate that quail not only respond to long days, which are gonado-stimulatory but may also reproduce under short days. Although, longer time is required for the bird to prepare for breeding under short day conditions (scotorefractoriness). Thus, both the phenomenon of photo/scotosensitivity and photo/scotorefractoriness is displayed by this photoperiodic poultry species [4–5,11,38–39]. The hypothalamic reproduction regulatory peptides (GnRH-GnIH system) alter the hypophyseal hormones (LH, FSH and prolactin) and subsequently control/regulate the steroidogenesis as well as spermatogenesis [40–41]. It is also the fact that hypothalamic GnIH has its receptor on GnRH neurons and pituitary gonadotropes and hence alters the gonadotropin synthesis and release [42–45].

There is strong evidence that photoreceptors may exist in several regions of the avian brain, particularly in the medio-basal hypothalamus [35,46–47]. However, there is compelling evidence that all of the components required to generate a photoperiodic response in birds reside within the hypothalamus. Till date, the differential photosexual responses observed in these four different quail phenotypes the hypothalamic photopigment(s) and the hypothalamic GnRH-GnIH system of all these quail phenotypes and the underlying mechanisms/pathways that required mediating these responses, remain to be determined.

To explore the underlying molecular mechanism we hypothesize that decreased expression/down regulation in hypothalamic DBP(s) may stimulate the hypothalamic GnIH system but inhibit the hypothalamic GnRH-I/II and thus induce the testicular atrophy/regression in the photorefractory and scotosensitive quail. While, on the other hand, in photosensitive quail, it is hypothesized that the appropriate/sufficient photoperiodic cues support the DBP(s) mediated potentiation of GnRH synthesis and release and thus inhibit the melatonin mediated GnIH synthesis and release and thus lead to testicular stimulation/hypertrophy. In scotosensitive quail, the deficit in the optimal light cues may elicit the testicular regression through the melatonin mediated hypothalamic GnIH activation and/or may be through the down regulation of hypothalamic GnRH-I/overexpression of GnIH. As the scotorefractory quail pass through the transient phase of testicular quiescence and exhibit the scotosensitivity but when maintained for prolonged period under the same conditions, showed restoration in the testicular activity and become scotorefractory. In scotorefractory quail testis, it is anticipated that there may be an increase in hypothalamic GnRH-I/II and down regulation in GnIH expression, due to the transition/shift from the sexual quiescence to full breeding condition.

In this study we have focused on the involvement of hypothalamic deep brain photoreceptors (VA-opsin and Opsin-5), hypothalamic GnRH-GnIH system and their impact on the testicular stimulation in photosensitive/scotorefractory quail and regression in the photorefractory/scotosensitive quail. To test the above hypothesis we have studied the contribution of the hypothalamic deep brain photoreceptors (VA-opsin and Opsin-5), hypothalamic GnRH-GnIH system in the testicular stimulation and regression in all these quail phenotypes.

This article highlights the role of simulated photoperiodic conditions in mediating testicular hypertrophy in PS/SR quail and quiescence in SS/PR quail.

2. Materials and Methods

2.1. Quail and Different Simulated Photoperiodic Conditions

Sexually mature six week old male Japanese quail (*Coturnix coturnix japonica*), raised under long photoperiod (16L: 8D) and having developed gonads, were purchased from Central Avian Research Institute (CARI), Izzatnagar, India and acclimatized under laboratory conditions in a photoperiodically controlled room. These adult male Japanese quail (n = 64) were randomly divided into two groups and were maintained under long day condition (16L: 8D) (n = 32) or short day condition (6L: 18D) (n = 32) for 9 weeks and provided commercial poultry feed and water ad libitum. Thirty-two long day birds were randomly divided into two groups. Birds in Group I (n = 16) were continued to be exposed to long day length 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 = 16) were transferred to intermediate day length (13.5L: 10.5D) for four weeks, condition that resulted in relative photorefractoriness (PR) and suppression of cloacal gland and testes. Thirty-two short day birds, after 9 weeks of exposure to short day length conditions were scotosensitive (SS)/sexually quiescent, with completely regressed cloacal