Bioscience Discovery, 8(2):177-183, April - 2017

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Print & Online, Open Access, Research Journal Available on http://jbsd.in

ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)

Research Article

Administration of 5-HTP and L-DOPA at specific time interval influences testicular lipid profile and plasma testosterone level in Indian Garden Lizard, *Calotes versicolor*

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Article Info

Received: 01-03-2017,
Revised: 26-03-2017,
Accepted: 30-03-2017

Keywords:
Garden lizard, 5-HTP, L-DOPA, testicular lipid profile, plasma testosterone

Abstract

Present investigation was undertaken to test whether the administration of serotonin and dopamine precursor drugs (5-HTP and L-DOPA) at the same time (0-hr) and at the interval of 12 hr (12- hr) can influence testicular lipid profile and plasma testosterone level in Indian Garden lizard, *Calotes versicolor*. Experiments were performed during late progressive phase of reproductive cycle. Male lizards were divided into three groups. Group I received two daily injection of normal saline and served as control. Group II administered 5-HTP and L-DOPA at the same time (0-hr) and group III injected 5-HTP and L-POPA at 12-hr interval. Results indicate that, following 0-hr treatment, concentration of total cholesterol, lipids and triglycerides in testis were found high and that of 12-hr treated groups were low in comparison to control. Plasma testosterone concentration was significantly higher in 12-hr and low in 0-hr group when compared with control. The lower concentration of lipid profile in the gonad may represent greater conversion of it's into testosterone. On the basis of the results of the present study it may be concluded that daily injections of 5-HTPand L-DOPA given at the same time (0-hr) and 12-hr apart actually affected the neuroendocrine-gonadal axis and their hormonal expression (i.e. LHRH, LH and Testosterone).

INTRODUCTION

Among seasonal breeders, the lipid content of gonad undergoes variations along with changes in gonadal activity. Histochemical studies show the presence of lipid in the interstitium during the active period and great accumulation within the seminiferous tubules during the inactive phase (Lofts, 1969; Reddy & Prasad, 1971; Kanwar *et al.*, 1977). It is also known that cholesterol concentration is low in the active (Reddy & Prasad, 1971; Arslan & Jalali, 1973) and high in the inactive testis (Simmons, 1969; Hafiez & Bartke, 1972). The lower concentration of cholesterol in the active gonad may represent greater conversion of cholesterol into steroidal hormones (Hall *et al.*, 1969; Kanwar *et al.*, 1977).

The steroid hormone testosterone (T) is ubiquitious in vertebrates and serves important organizational and activational roles in reproduction and behavior (Hadley, 1992; Wingfield *et al.*, 2001; Alonso-Alvarez *et al.*, 2002). One prominent role of T is its involvement in aggression, especially in the context of territoriality (for overviews, see Wingfield *et al.*, 1990; Moore & Marler, 1988). Temperate-zone birds have high T-levels during the breeding season and especially during the courtship phase (Wingfield *et al*., 1987). Paradoxically, many tropical birds have only very low plasma T-levels, but are neverthless highly aggressive and territorial (Dittami & Gwinner, 1990; Levin & Wingfield, 1992; Wingfield *et al*., 1992). Furthermore, many tropical birds are territorial year-round despite the fact that they reproduce seasonally (Dittami & Gwinner, 1990; Wikelski *et al*., 1999). It is well known that the vernal increase in day length stimulates gonadal development and increases plasma levels of T (Farner & Follett, 1979; Farner & Gwinner, 1980; Wingfield & Farner, 1980). Although photoperiodically induced gonadal development in the laboratory results in complete spermatogenesis accompanied by development of secondary sex characteristics, field investigations have shown that the temporal pattern of circulating levels of T under natural conditions are quite different from those of an artificially induced cycle in the laboratory, and the absolute levels of T are up to 10 fold higher in males sampled in the field (Wingfield & Farner, 1980). These data suggest that environmental factors, in addition to day length, can influence the secretion of these hormones. Regulation of gonadotrophin secretion by change in its rate of synthesis and pituitary cell sensitivity to gonadotrophin releasing hormone GnRH/LHRH, is modulated by neurotransmitters (Kumari & Saxena, 1997), primary those of hypothalamic origin. In mammals, however, the role of central dopaminergic neuronal system for LH release continues to be controversial. It exerts both stimulatory and inhibitory influence on the LH release (Korden & Glowinski, 1972). In birds also, there are some pharmacological studies (using agonists and antagonists for catecholamines) investigating the role of dopaminergic neural system. The dopaminergic system may regulate gonadotropin levels by acting on LHRH neuronal terminal either at the level of anterior/mediobasal hypothalamus and/or at the level of median eminence. However, very little information is available on subtorpical wild finch.

There is complete lack of information regarding the role of circadian neurotransmitter activity on testicular lipids variation. As far as concerned with the circulating testosterone level, it is only reported in a single avian species, weaver bird, *Ploceus philippinus* which is photoperiodic passerine (Das, 1999). The present study has therefore, been undertaken to investigate the role of circadian neurotransmitter activity on testicular lipid profiles and plasma testosterone level in a seasonally breeding reptilian species. It is envisaged that the results from the study will help to understand the role of circadian phase relationship of serotonergic and dopaminergic neural oscillations and its impact on reproductive axis and seasonal reproductive cycles (Qureshi *et al*, 2010; Chaturvedi & Yadav, 2013; Prasad & Thakur, 2015; Yadav & Chaturvedi, 2014, 2015, 2016)

MATERIALS AND METHODS

During late progressive phase (April-May) adult male Indian Garden Lizard, *Calotes versicolor* weighing 38 gm were divided into three groups of six Lizards each. Group I received two daily injections of normal saline and served as control. Group II administered 5-HTP and L-DOPA at the same time (i.e.0-hr) and Group III injected 5-HTP and L-DOPA at 12-hr interval. At the termination of the study, when Lizards were sacrificed by decapitation, (a) blood was collected in heparinized tubes, plasma separated and stored at -20^{0}c until assayed for plasma testosterone concentration by Radioimmunoassay using commercial RIA kit. (b) testes were removed and processed for the estimation of lipid profiles. Methods used are: 1) Phosphovanillin method for Total lipids 2) CHOD-PAP method for Total cholesterol 3) Enzyme calorimetric test for Triglycerides. Statistical analysis was done by student's ‘t’ test and analysis of variance, (ANOVA) (Bruning & Kintz, 1977).

RESULTS AND DISCUSSION

Testicular lipids: Following 0-hr treatment, concentration of total cholesterol, total lipids and triglycerides in testes were found significantly high and that of 12-hr treated groups were low in comparison to control (Fig-1).

Plasma testosterone: In contrast to testicular lipids, plasma testosterone concentration was significantly higher in 12-hr Lizards and low in those Lizards which were treated with 5-HTP and L-DOPA at 0-hr interval (Fig-1).

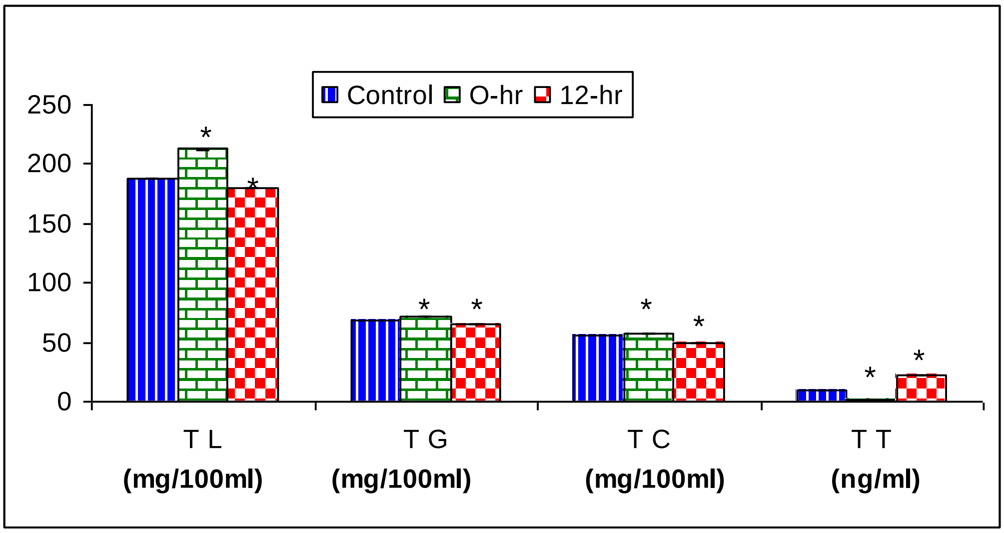
In general, with the changes in the gametogenetic activity, the lipid contents of interstitial Leydig cells and seminiferous tubules show cyclical variations. As the spermatogenetic process advances inside the tubules, lipid from the interstitial Leydig cells decreases and reaches a minimum when the process is at its peak, probably due to the conversion of lipids into gonadal steroids (Tam *et al.,* 1969).

With the decrease or cessation in the spermatogenetic activity, the seminiferous tubules regress, and cholesterol-positive lipid accumulates inside them. The rapid depletion of the interstitial lipids at the height of androgen production is a common feature of the avian testicular cycle (Marshall, 1955) and also occurs in some poikilothermic vertebrates (Lofts, 1968). After completion of spermatogenetic activity, the tubules regress and accumulate large quantities of lipids inside them. In another local species of non-migratory bird, *Ploceus philippinus* (Thapliyal & Saxena, 1964), lipid was present inside the tubules from August to February when the testes were regressing or had regressed. Concomitantly, the interstitium at that time contained no lipid. But during the gonadal active phase, the picture was reversed, *viz*, there was no lipid in the seminiferous tubules but plenty was present within the tubular areas.

The seasonal accumulation and depletion of lipid from seminiferous tubules and interstitial Leydig cells similar to spotted munia and the weaver bird (Thapliyal & Saxena, 1964), have been reported in a number of other seasonally breeding vertebrates (Fishes - Lofts & Marshall, 1957; Upadhya & Guraya, 1971; Amphibians - Guraya, 1972; Lofts, 1974; Reptiles - Lofts, 1969; Upadhya & Guraya, 1972; Guraya, 1973; Birds - John & George, 1966; Lofts & Murton, 1973). Among mammals, testicular lipid cycle has been studied in laboratory rat (Lacy & Lofts, 1965; Lacy, 1967), vole (Marshall & Wilkinson, 1956), deer (Wislocki, 1949), and mole (Lofts, 1960). But in seasonally breeding mole *Talpa europea* (Lofts, 1960) and vole *Microtus orcadensis* (Marshall & Wilkinson, 1956), there is no cyclical variations in the lipid contents within the seminiferous tubules. Further, hypophysectomy also induces dense accumulation of cholesterol-positive lipids inside the tubules (Clermont & Morgentaler, 1955; Steinberger & Nelson, 1955; Coombs & Marshall, 1956) similar to those observed after cessation of spermatogenetic cycle in seasonal breeders (Lofts and Boswell, 1961; Johnson, 1970; Lofts & Murton, 1973; Lofts, 1974). Presence of specific DA binding sites in anterior and posterior hypothalamus provide an evidence for hypothalamus to be the site of action of dopamine in birds. Further, neuro-anatomical studies on some bird species indicate presence of catecholaminergic nerve axons in the preoptic recess, paraventricular nucleus and in the region of ventromedialis where immunoreactive LHRH perikarya have also been observed in hypothalamus and/or at the level of median eminence where LHRH neuronal terminal converge near primary portal vessels (Farner *et al*., 1967). In weaver bird, *Ploceus Philippinus* dopamine exerts inhibitory influence on LH secretion by suppressing hypothalamic LHRH release (Kumari & Saxena, 1997).

It is evidenced that 12-hr relation of 5-HTP and L-DOPA induced activity of gonadal growth and that of 0-hr relation showed inhibitory effect in this species out of the season (Thakur, 2008). Present study also brings out that in *Calotes versicolor* when the testes are spermatogenetically activated by treatment of neurotransmitter precursor drugs (12-hr relation of 5-HTP and L-DOPA), the concentration of total cholesterol, lipids and triglycerides are significantly low and that of 0-hr treated Lizards, which inhibited the gonad spermatogenetically, have high values in comparison to normal Lizards. Since phospholipids and their fractions are also an important constituents of the testis and are very essential for the production of steroid hormones, it is proposed to extend it to include the study of phospholipids following temporal synergism of serotonergic and dopaminergic drugs. This would give us more information on the specific constituents affected and also on the dynamics within the gonads of reptilian species. Daily injections of L-DOPA given 12-hr after daily injections of 5-HTP, induced uterine growth and increased serum thyroxine and lutenizing hormone (LH) concentrations in scotosensitive female hamster maintained on short day lengths. On the other hand, daily injections of L-DOPA given at the same time as daily injections of 5-HTP decreased uterine weight and serum concentrations of Thyroxine and LH in scotorefractory female Hamsters (Wilson & Meier, 1989). It has also been reported that, in Spotted munia (Prasad & Chaturvedi, 1992a, b) and Japanese quail (Philips & Chaturvedi, 1993), hypothalamo-hypophysial- gonadal axis may be affected by simultaneous injection of LH and 8-hr temporal relation of 5-HTP and L-DOPA. In weaver bird it has also been reported that plasma testosterone concentration was significantly higher in 12-hr treated birds and low concentration in that of 8-hr (Das, 1999). These reports suggest that two neurotransmitter precursors administered 12-hr apart (12-hr relationship) induced increased gonadotrophin level and consequently increased level of plasma testosterone while that of 8-hr

Fig. 1: Effect of 0-hr and 12-hr temporal relationships of 5-HTP and L-DOPA on testicular lipid profile and total plasma testosterone level in Indian garden lizard, *Calotes versicolor.* Values are Mean ± S. E.



T L : Total lipids, T G : Triglycérides, T C : Total cholestérol, T T : Total testostérone. Significance of difference from control (*P<0.001; Student's't' test and ANOVA)

treatment induced opposite effects. On the basis of the results of the present study it may be concluded that daily injections of 5-HTP and L-DOPA given zero and 12-hr apart actually affected the neuroendocrine-gonadal axis and their hormonal expression (i.e. LHRH, LH and Testosterone). Further possibly these treatments of only thirteen days reset the switch of neuroendocrine mechanism resulting in gonado-suppressive or stimulatory response. Therefore, we presume that in nature too, these exists specific phase relationship between circadian neural oscillation which change seasonally and may be the basis of specific breeding or non-breeding condition. This suggestion gets indirect support from following reports. (a) Circadian variation has been reported in brain / hypothalamic neurotransmitters (Serotonin and dopamine) which are reported to have significant neuroendocrine function affecting reproduction. Further, circadian changes of these neurotransmitters are also reported to be influenced by photoregimn / light:dark condition and hormones (Wilson & Meier, 1989; Mai *et al*., 1994; Pan, 1996; Shieh *et al*., 1997). (b) Circadian peak of neurotransmitter activities occur at different time in different breeding and physiological conditions (Morin *et al*., 1977; Khan & Joy, 1988; Oliveira *et al*, 2016, French & Muthusamy , 2016; Albrecht, 2017)

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How to Cite this Article:
Thakur SK and Prasad SK, 2017. Administration of 5-HTP and L-DOPA at specific time interval influences testicular lipid profile and plasma testosterone level in Indian Garden Lizard, *Calotes versicolor*. *Bioscience Discovery*, **8**(2):177-183.