Indian J.Sci.Res. 4(2) : 37-40, 2013

ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

CIRCADIAN PHASE RELATION OF SEROTONIN AND DOPAMINE INFLUENCES REPRODUCTIVE CONDITIONS IN DOMESTIC PIGEON, *Columba livia domestica*

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ABSTRACT

The aim of the present study was to learn whether circadian phase relationship of serotonin and dopamine can influence reproductive conditions in monogamous bird domestic pigeon *Columba livia domestica*. Mature birds were divided into two groups. Group I received daily injections/day of normal saline served as controls. A bird in another experimental group was injected daily with 5-HTP (5-Hydroxytryptophan) and L-DOPA (L-Dihydroxyphenylalanine) at 8-hour relationships. These doses were administered for 13 days at a dose of 5 mg /100gm bw. Observations were made 24 hour after last injection. Gravimetry of gonads, crop gland weight and hormonal estimation were recorded. Results indicate that 8hr. relationship showed stimulatory effects.. The present study yields experimental evidences that circadian phase relationships of serotonergic and dopaminergic activities in the brain do alter physiological conditions which in turn affects the seasonality of domestic pigeon.

KEYWORDS : 5-HTP, L-DOPA, Domestic Pigeon

It is suggested that hormonal rhythms are the expression of two circadian neuroendocrine oscillations, which change seasonally in their phase relations and thereby regulate seasonality (Meier et al., 1981). It was also hypothesized that hormone injections may reset two circadian neuroendocrine oscillations and temporal interaction of these oscillations determine the complex of physiological conditions. Since corticosteroids stimulate serotonin ((Sze et al., 1976) and prolactin increases dopamine synthesis (Mai et al., 1994) an attempt was made to duplicate the hormonal effects by injecting precursor chemicals for the synthesis of these neurotransmitters. Thus, 5-hydroxytryptophan (5-HTP) a rate limiting precursor for serotonin was substituted for corticosterone and L-dihydroxyphenylalanine (L-DOPA), a rate limiting precursor for dopamine was substituted for prolactin (Meier et al., 1981). Daily injections of 5-HTP and L-DOPA brought about comparable changes. In white throated sparrows, 12-hr relation between the above two neurotransmitter precursor drugs established spring condition, 8-hr relation established summer condition (Meier et al., 1981; Miller and Meier, 1983).

Reproductive biology and behavoir among pegion are on record (Richard, 1998). Both sexes in pigeon show parental care. Their nestlings feed on their crop milk. Crop milk is a semi solid, slimy material formed by the desquamation of epithelial cells of crop inner lining. Crop milk is rich in fat, protein and minerals (David, 1989). The endocrine control of its formation is regulated by prolactin (Riddle and Braucher, 1931). Pigeons have drawn attention of scientists in particulars necessitating new research on breeding and milk producing ability.

In view of interrelationship/interdependence of neural oscillators and overt rhythms and seasonal variation in circadian rhythms and their phase relationship of serotonergic and dopaminergic activity may influence seasonal reproductive condition, present study was designed in an avian species which is monogamous and produces crop milk during brooding. Since earlier studies suggest that only 8 hr circadian phase relationship of serotonergic and dopaminergic activity has inhibitory response in some species, in the present study the effect of this (8 hr) relationship was tested on reproductive organs, crop gland growth and plasma hormonal (Prolactin and Estrogen) level in domestic pigeon, *Columba livia*.

MATERIALS AND METHODS

Birds were acclimatized for two weeks in captive conditions. During quiescent phase of annual reproductive cycle female birds were separated by lateral laparotomy (Birds were anesthetized with sodium pentobarbital and a cut were made with the help of scissor, in the lower abdomen (near last ribs) of left side. Ovary was observed and cut was stitched and necessary post operational cares were made) and divided into two groups of 6 birds each.

Group I [S: S (saline control)]: Birds received normal saline (0.1ml) twice (i.e. 8 AM and 8 PM) a day.

Group II [5-HTP: L-DOPA (8 hr circadian phase

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relationship of 5-HTP and L-DOPA)]: Injection of 5-HTP (5-Hydroxytryptophan, a serotonin precursor) was given at 8 AM and L-DOPA (L-Dihydroxyphenylalanine, a dopamine precursor) at 4 PM. The doses of 5-HTP and L-DOPA were 5mg/100gm body weight, to stimulate production of serotonin and dopamine respectively. All the injections were given intraperitoneally over a period of 10 days. During the treatment period birds were maintained under LL (continuous condition of light) to avoid possible photoperiodic interference due to light: dark cycle with neuroendocrine entrainment by precursor drug injections. Photoperiodic condition was maintained in light proof room fitted with fluorescent light (300 Lux at cage level) and automatic timer. During the course of the study the birds were provided with food grains and drinking water ad libitum. Birds were anesthetized and observations were made at 45 days post treatment (after 45 days of last injections). Body weight was taken by single pan balance. Crop gland and reproductive organ (ovary) were removed and weighed. Plasma prolactin (PRL) and estrogen level were measured. For the assay of prolactin and estrogen blood collected directly from the left ventricle of heart. Hormones were assayed by RIA kit (ACS 180, USA). Crop gland was fixed in Bouins fluid for histological studies. Observations were expressed in terms of Mean ± SE. Statistical analysis was done by student's't' test (Bruning and Kintz 1977). Experiments on the animals were conducted in accordance with institutional practice and within the framework of revised animals (Scientific Procedures) Act of 2002 of government of India on animal welfare.

RESULTS AND DISCUSSION

Results indicate that the pigeons which were treated with 5-HTP and L-DOPA at 8 hr interval showed increased body weight, ovary and crop gland weight and plasma prolactin and estrogen level all were significantly increased ($p<0.001$) in comparison to control (Figure 1, 2 and 3).

The daily injections of the two neurotransmitter precursors (5-HTP and L-DOPA) probably induced a diurnal rhythm in central nervous serotonergic and dopaminergic function. The induction of such putative rhythms results, changes in reproductive function depending on the phase angle between the rhythms suggests that there may be of physiological relevance. A circadian variation in the hypothalamic concentration of neurotransmitters (serotonin and dopamine) has been reported in Japanese quail, Coturnix coturnix japonica and suggested that circadian serotonergic and dopaminergic oscillator varies as a function of reproductive status of the bird, and breeding/ non-breeding conditions may be induced experimentally by changing the phase relation of these oscillations (Tiwari et al., 2006).

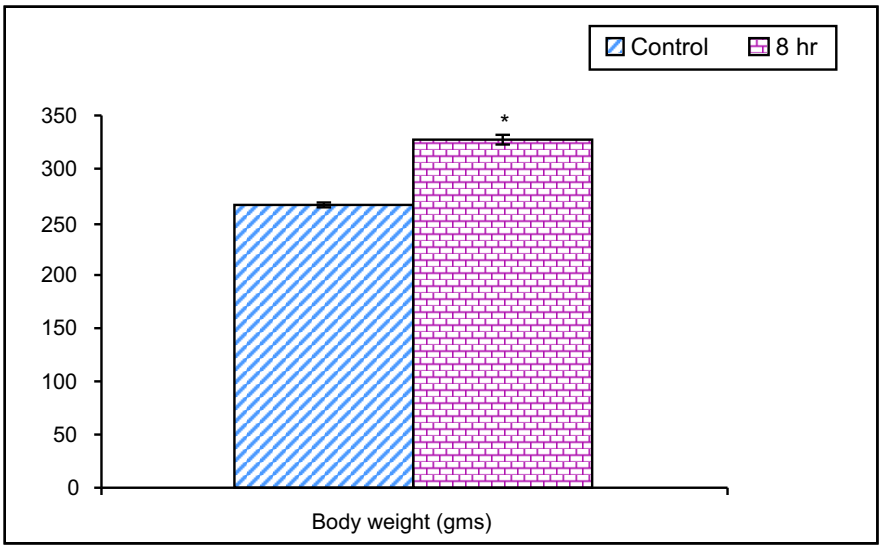

Figure 1 : Effect of 5-HTP and L-DOPA at 8 hr. interval on body weight of domestic pigeon, *Columba livia*
* Significance of difference from control ($p<0.001$), Student's't'test. Value = Mean ± S.E.

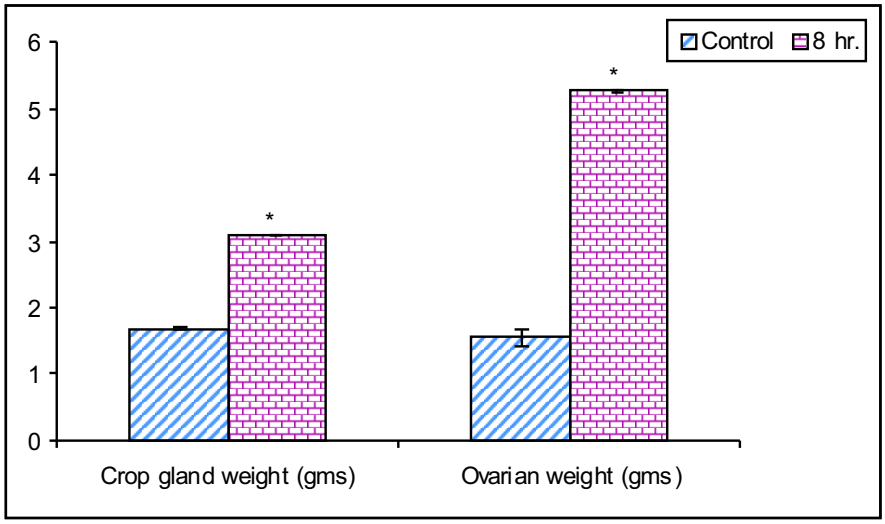

Figure 2 : Effect of 5-HTP and L-DOPA at 8 hr. interval on weight of female reproductive organs (ovary) and crop gland.
*Significance of difference from control ($p<0.001$), Student's' test. Value = Mean ± S.E.

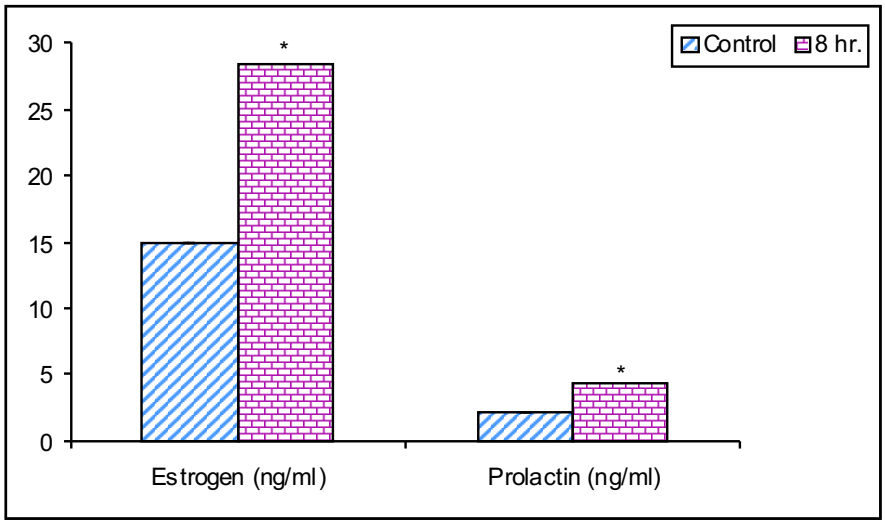

Figure 3 : Effect of 5-HTP and L-DOPA at 8 hr interval on plasma prolactin and estrogen level.
*Significance of difference from control ($p<0.001$), Student's' test. Value = Mean ± S.E.

Steroid hormones (estradiol and progesterone) are important for the action of PRL in birds. They are thought to have a priming function, possibly enhancing PRL secretion and / or enhancing sensitivity of the neural system to PRL (Schradin and Anzenberger, 1999). In doves, high levels of luteinizing hormone (LH) and follicular stimulating hormone (FSH) trigger egg lying. Incubation is triggered by high levels of progesterone and oestradiol. Incubation behavior is maintained by high levels of prolactin, which is also crucial for the growth of the crop sac and crop milk production. The amount of crop wall which in normal incubation and under prolactin dosages undergoes extremely marked proliferation with subsequent formation of crop milk. The desquamation of epithelial cell lining of crop enhances milk production (Riddle and Braucher, 1931). When the chicks no longer require crop milk prolactin levels drop. Prolactin level reaches a peak at hatching.

Testicular and ovarian development and fat deposition are stimulated or inhibited after daily injection of 5-HTP and L-DOPA at the interval of 12 or 8 h respectively in the White throated sparrow Zonotrichia albicollis (Miller and Meier,1983), the migratory Red headed bunting, Emberiza bruniceps (Chaturvedi and Bhatt, 1990), Lal

munia, Estrilda amandava (Chaturvedi et al., 1994), Indian weaver bird. In other study it has been suggested that the circadian rhythm of reproduction in Spotted munia can be reset during all the phases of reproductive cycle14 by properly timed injections of these two precursors that influence the activities of two circadian neuroendocrine oscillators. Thus, 12-hr relationship of these two neurotransmitter precursor drugs (5-HTP and L-DOPA) clearly showed stimulatory response for various metabolic and reproductive activities in other avian species including male pigeon. In contrast to these results the present study (in female pigeon) exhibited opposite effect i.e. 8 hr circadian phase relation of serotonergic and dopaminergic activity was stimulatory for reproductive conditions. Further, high level of plasma estrogen and prolactin and active crop gland and ovarian growth following 5-HTP and L-DOPA treatment at 8 hr interval (at specific time) supports the stimulatory reproductive response in female pigeon. The stimulatory response of 8 hr relation of 5-HTP and L-DOPA, in domestic pigeon Columba livia suggests that circadian phase relationship of serotonergic and dopaminergic activities in the brain might be sex dependent and differ in different avian species in regulating various metabolic/reproductive phenomena.

ACKNOWLEDGEMENT

Financial help received from UGC as PDF for women is respectfully acknowledged. Head of the Department gave Departmental facilities.

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