Proc. Indian natn. Sci. Acad. **B63** No. 5, pp 427-436 (1997)

Antigonadal and Antiphotoperiodic Response of Exogenous Prolaction in Photosensitive and Photostimulated Blackheaded Bunting, *Emberiza melanocephala*

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(Received on 17 April 1997; after revision 28 July 1997; Accepted on 20 August 1997)

To explore the effect of exogenous prolactin (PRL), photosensitive and photostimulated female Blackheaded buntings were given 16 daily injection (100 μg/0.1 ml) of ovine prolactin in early hours (800-900 hrs). Results obtained from exogenous administration of PRL suggest antigonadal and antiphotoperiodic action of PRL. The plasma estradiol level was also decreased significantly during PRL administration. Thus the result supports earlier findings that in strong migrants (such as Blackheaded bunting) PRL is inhibitory to body weight, ovarian weight and plasma estradiol concentration. The birds have responded to same photoperiod after the withdrawal of the PRL administration. Therefore, the present results clearly support the inhibitory role of PRL in strong migrants and extends our knowledge to regulatory function of PRL in migratory Blackheaded buntings.

Key Words : Photosensitive, Photostimulated, Antigonadal, Antiphotoperiod, Prolactin, *Emberiza melanoceplala*

Introduction

The antigonadal role of prolactin has been demonstrated earlier by several workers in various avian species (Riddle & Bates 1933, Bailey 1950, Lofts & Marshall 1956, Thapliyal & Saxena 1964, Meier & Dasseau 1968, Meier 1969, Tewary et al. 1983, 1984). On the other hand, in some avian species prolactin has shown to have no effect on gonads (Laws & Farner 1960, Hamner 1968, Meier & Dasseau 1968, Jones 1969). Meier and Dasseau (1968)have shown that prolactin has no effect on gonadal regression in a strong migratory species, *Z. leucophrys gambelii*. It has also been reported that there is no strong correlation between PRL and migratory fattening in some migratory species (Boswell et al. 1995, Hall et al. 1987, Schwabl et al. 1988, Silverin et al. 1989). Later on, Tewary et al. (1983, 1984) have suggested the

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antigonadal role of prolactin in a migratory finch. Earlier, it was thought that the antigonadal role of PRL was due to suppression of follicle stimulating hormone (Marshall 1961). It has been reported previously that the elevated circulating PRL may play role in suppression of Luteinizing hormone (LH) secretion (Sharp et al. 1988, Halawani et al. 1983, Zadworny et al. 1987). Recently, Halawani et al. (1991) have suggested the delay in photo induced sexual maturity due to exogenous PRL administration in Turkey. Whereas the relationship between exogenous PRL and LH release is well established with regard to the suppressive action of PRL on LH release but situation with regard to the plasma estradiol,ovarian growth and body mass is relatively unknown. Thus, the present study was aimed to explain whether exogenous prolactin has any impact on plasma estradiol, body weight and ovarian weight in photosensitive and photostimulated migratory Blackheaded bunting.

Materials and Methods

The Blackheaded bunting, i.e. *Emberiza melanocephala* is a palaearctic species which visits India during winter season. It arrives in Varanasi (India, 25° 18′ N, 83°01′E) during September/October and returns to their breeding grounds (West Asia and East Europe) during March/April (Ali & Ripley 1983). Blackheaded bunting exhibits annual cyclic pattern relating to reproduction in four different phases, such as reproductive phase (May-June), regressive phase (July-August), quiescent phase (September-January) and progressive phase (February-April) (Kumar 1981). Adult female Blackheaded buntings were captured locally during December/January, when they are in plenty. They were brought to outdoor aviary and acclimatized for about two weeks. Acclimatized buntings maintained under short photoperiods (8L/16D) for two months to ensure the photosensitivity. Photosensitive buntings were weighed and laparotomized to ensure that they had maintained regressed ovaries (4-5 mg) and body weight(19-21 g). With these photosensitive female Blackheaded buntings, following two experiments were conducted for 35 days for assessment of incorporation of circadian rhythm in photoperiodic control machanism of Blackheaded bunting for control of its reproductive and associated migratory events.

Experiments I : In this experiment 12 birds were used.They were divided into two groups (6 each) and exposed to long photoperiods (15L/9D)for 35 days. Beginning on day 1, one group received 16 consecutive daily injection of 100 μg/0.1 ml of prolactin made in normal saline. The other group (control) received 0.1 ml normal saline (NS) from day 1 to day 16. On Day 16, birds were weighed and laparotomized and two birds from both group were weighed, killed and their blood plasma was collected and stored at -20°C for estradiol assay. After day 17, the birds in both groups (4 birds/ group) remained on long photoperiods (15L/9D) without injection till Day 35. On day 35, birds were weighed, killed and ova-

ries were dissected out and weighed. Blood was taken and plasma was collected and stored at - 20°C for assay of estradiol.

Experiment II : This experiment was planned to determine whether PRL affects the photoperiodic response of buntings when they are continuously photostimulated. Photosensitive birds were exposed to continuous light (24L/0D) for 30 days. Birds were laparotomized, ovarian weight was assessed *in situ*, and blood plasma was collected. They were divided into two groups (6birds/group). Like experiment I, one group received 100 μg/0.1 ml of PRL injection and other (control) 0.1 ml of normal saline from day 1 to day 16. On day 16, birds were weighed and laparotomized. Ovarian weight *in situ* was assessed. Blood plasma was collected. From day 17th to day 35th birds of both groups remained in continuous light (24L/0D). On day 35, all birds were weighed, killed and ovarian weight was taken. Blood plasma was collected and stored.

Food and water were given *ad libitum*. Birds were kept in wirenet cages and placed in photoperiodic chambers which were illuminated by a 20 Watt fluorescent tube providing light at an intensity of about 400 lux at perch level. At each laparotomy, ovarian weight was assessed by comparing the size of ovary *in situ* with a standard set of gonads of known weights. The error by this method is less than ± 20% (Tewary et al. 1983). The blood was taken after killing the birds. The blood was centrifuged at 4°C, and plasma was collected in eppendorf tubes and stored at - 20°C for assay of estradiol. The plasma estradiol concentration was measured by using Estradiol Direct Radioimmuno assay kit (Biotecx Laboratories, Inc 6023, South loop East Huston, Tx. 77033). The statistical analysis was done using analysis of variance test (ANOVA) and students "t" test.

Results

Experiments I: The sixteen daily administration of exogenous PRL in female Blackheaded bunting was inhibitory to body weight, ovarian weight and plasma estradiol concentration (figure 1&3A) under long photoperiod (15L/9D). But withdrawal of PRL for 20 days in long photoperiods (15L/9D) buntings showed significant elevation in body weight ($P<0.01$), ovarian weight ($P<0.001$) and plasma estradiol concentration ($P<0.001$). Control group showed significant increase in body weight ($P<0.05$), ovarian weight on 16th day, and for further 20 days they were kept without 0.1ml normal saline injection and here also an increased body weight ($P<0.05$), ovarian weight ($P<0.01$) and plasma estradiol concentration. While comparing the data between NS treated group with PRL treated group on Day 16, a significant increase in body weight ($P<0.01$), ovarian weight ($P<0.01$) and plasma estradiol concentration ($P<0.001$) in NS-treated group was observed but in PRL-treated group, there was no increase in body weight, ovarian weight and plasma estradiol (non-detectable) level. After withdrawal of PRL & NS for further

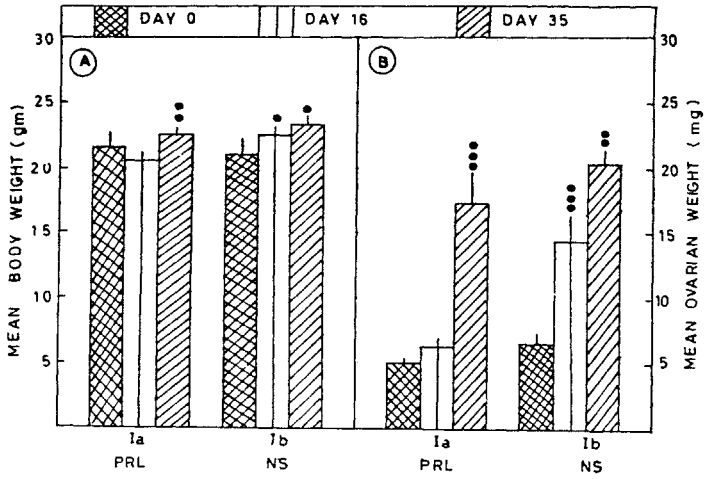

Figure 1. Effect of exogenous PRL on body weight (A) and ovarian weight (B) of female Blackheaded bunting under long photoperiod (15L/9D). Experimental group Ia received PRL for sixteen days which caused inhibition of body and ovarian weight, while after withdrawal of PRL injection for 20 days i.e. on day 35, a significant rise in body weight and ovarian weight was observed. Like experimental group Ia, the control group Ib received normal saline and resulted in significant increase in body and ovarian weight on day 16 & day 35. Vertical bars showing SEM (Standard Error of Mean) • $P < 0.05$, •• $P < 0.01$ and ••• $P < 0.001$.

20 days, i.e. on day 35, no significant increase in body weight and plasma estradiol concentration was marked but significant increase ($P<0.01$) in ovarian weight in NS-treated group compared to PRL-treated group.

Experiment II: On day 16, the PRL injected group showed significant decrease in body weight ($P<0.01$), ovarian weight ($P<0.001$) and plasma estradiol concentration ($P<0.001$) (figures 2 & 3). When this group was kept for another 20 days without injection in continuous light they showed significant increase in body weight ($P<0.05$), ovarian weight ($P< 0.01$) and plasma estradiol concentration ($P < 0.001$). In control group which received normal saline (0.1 ml/day) no significant effect was observed but the plasma estradiol concentration increased significantly ($P < 0.05$) on 16th day. After withdrawal of normal saline injection in this group on day 35, significant decrease ($P < 0.01$) in plasma estradoil level was observed but the plasma estradiol concentration increased significantly ($P< 0.005$) on 16th day. After withdrawal of normal saline injection in this group on day 35, significant decrease ($P < 0.01$) in plasma estradiol level was observed. No significant effect was observed in body and ovarian weight. While comparing the reslts of PRL treated group with NS treated group (figure 2 IIa vs. IIb & figure 3 IIa vs. IIb) on day 16, there was significant increase in body weight ($P<0.05$), ovarian weight ($P<0.01$) and plasma estradiol concentration was observed in NS-treated group and on day 35, there was no significant difference between PRL-treated and NS-treated group but plasma estradiol concentration of NS-treated group was significantly higher ($P<0.01$) than PRL-treated group.

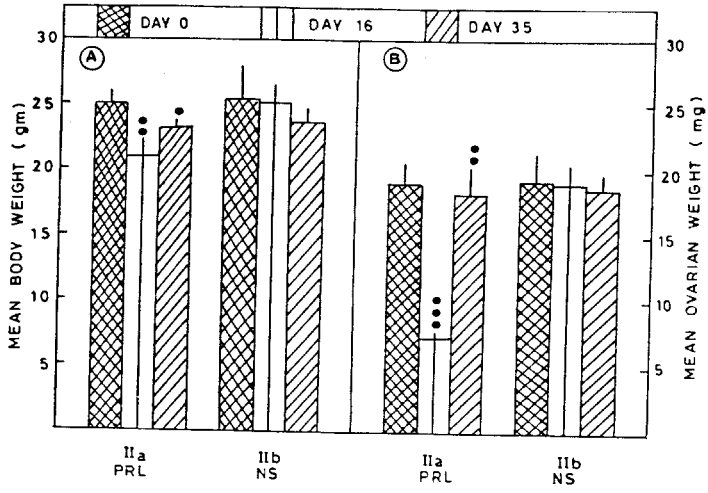

Figure 2. Effect of exogenous PRL on body weight (A) and ovarian weight (B) of female Blackheaded bunting under continuous light (24L/0D). Experimental group (Ia) received PRL and control group (IIb) received normal saline for sixteen days. On day 16, IIa group showed significant decrease in body and ovarian weight. Withdrawal of PRL injection for further 20 days caused significant increase in body and ovarian weight. No significant effect on body and ovarian weight was marked in control group (IIb). Vertical bars showing SEM (Standard Error of Mean) • $P < 0.05$, •• $P < 0.01$ and ••• $P < 0.001$

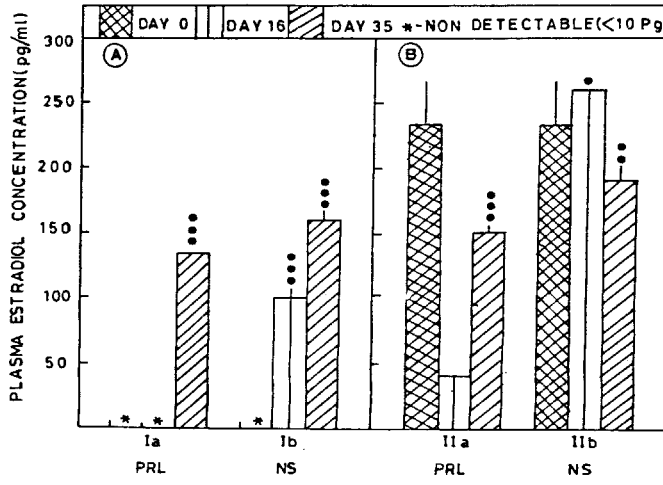

Figure 3. Effect of exogenous PRL on plasma estradiol level (pg/ml) of female Blackheaded bunting under photosensitive (A) and photostimulated (B) condition. The administration of exogenous PRL for sixteen days caused inhibition of plasma estradiol, in Ia group where plasma estradiol was non-detectable (< 10pg/ml) while in IIa group PRL caused significant decrease in plasma estradiol level. On day 35, significant increase in plasma estradiol concentration in Ia and IIa group was observed. The control group, significant rise in plasma estradiol level was marked on day 16, on day 35, Ib showed significant higher level of plasma estradiol and IIb exhibited slight decrease in plasma estradiol level. Vertical bars showing SEM (Standard Error of Mean) •$P < 0.05$, •• $P < 0.01$ and ••• $P < 0.001$.

While interpreting the result obtained in both experiments two point become very clear : (a) In Experiment I, where photosensitive birds were used, PRL-inhibited the increase in body weight, ovarian weight and plasma estradiol concentration in long photoperiodic (15L/9D) condition, on the other hand NS-treated group significant increase in body weight, ovarian weight and plasma estradiol concentration on day 16. While withdrawal of PRL injection for 20 days in same photoperiodic condition caused increase in body weight, ovarian weight and plasma estradiol concentration, (b) In Experiment II, where the photostimulated birds were used, PRL caused inhibition of body weight, ovarian weight and plasma estradiol concentration on day 16. After withdrawal of PRL injection for further 20 days it resulted in significant increase in body weight, ovarian weight and plasma estradiol concentration. Thus, the result clearly indicates the antiphotoperiodic and antigonadal role of PRL in Blackheaded bunting.

Discussion

The results obtained by us are consistent with earlier reports in several avian species that exogenous prolactin is antiphotoperiodic and antigonadal (Meier & Dasseau 1968). The sixteen daily injection of prolactin (100 μg/0.1 ml) in photosensitive as well as in photostimulated buntings caused a significant decrease in plasma estradiol level which supports the antigonadal role of prolactin in Blackheaded bunting. The withdrawal of injection caused significant elevation in plasma estradiol level. In some avian species such as *Zonotrichia leucophrys gambelii* (Law & Farner 1960), *Junco hyemalis* (Meier & Dasseau 1968), prolactin was shown to have progonadal effect. The possible explanation about progonadal action of PRL in above species was suggested by Tewary et al. (1983)—that it could be due to administration of PRL during different phases of the reproductive cycles of the species investigated. In this present investigation photosensitive buntings (pretreated with 8L/16D (short photoperiod) for 8 weeks were used while others have carried their experiments on wild birds captured and used directly from the nature during spring (Meier & Dasseau 1968). Thus, the lack of inhibitory action of exogenous prolactin in strong migrant is due to an existing maximal antigonadal effect of endogenous prolactin in spring so that the exogenous prolactin have no additional effect. The effect of exogenous PRL administration on migratory fattening and their progonadal impact appears to be dependent upon duration of the injections, (Meier & Fivizzani 1980, Vleck et al. 1980, Meier & Dasseau 1968). Meier and Davis (1967) have suggested that both photosensitive as well as photorefractory states may in part depend upon time of day when prolactin is released. Mid day injections of prolactin induce fattening but the early injections cause the loss of body weight and fat stores. On the other hand the antiphotoperiodic effect of prolactin in female Blackheaded bunting was due to time of PRL administration (PRL injec-

tions were used in early hours, 08-09 hrs) and variation in circadian component which operates therein. The present result is thus consistent with earlier findings and creates an evidence in support of antigonadal and antiphotoperiodic role of prolactin. The decrease in plasma estradiol level during prolactin administration supports the earlier concepts that prolactin inhibits the photoperiodic induction of gonadal growth which could be due to suppression of follicle stimulating hormone (Nalbandov 1946, Marshall 1961, Meier 1969). Zadworny et al. (1988) have suggested that in Japanese Bantam the concentration of estradiol was decreased when the circulating level of PRL were high and thus increased level of PRL may provide a mechanism by which the Japanese Bantam can inhibit the ovarian growth. Similarly, rise in circulating PRL was initiated by photoinduced gonadal recrudescence in female Turkeys (Burke & Dennison 1980, Halawani et al. 1995). It has been also observed that increased plasma PRL accompanies gonadal regression in long days (Dawson & Goldsmith 1983, Goldsmith & Nicholls 1984a).

However, further investigation is needed to understand the role of prolactin and its interrelationship between reproduction, hyperphagia and migratory restlessness. The role of circadian component involved during prolactin administration in photosensitive and photostimulated buntings cannot be ruled out.

Acknowledgments

Financial supports from the Council of Scientific & Industrial Research, New Delhi (Grant No. 38(808)93 EMR-II) to PDT is gratefully acknolwedged.

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