Effects of Administration of Adrenocorticotropin, Corticosteroids and Adrenal Inhibitor Metyrapone on Adrenocortical, Pineal and Gonadal Activity in Nocturnal bird, Athene brama and Diurnal bird, Perdicula asiatica

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Source: Zoological Science, 18(1) : 81-90

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.81

ZOOLOGICAL SCIENCE **18**: 81–90 (2001)

Effects of Administration of Adrenocorticotropin, Corticosteroids and Adrenal Inhibitor Metyrapone on Adrenocortical, Pineal and Gonadal Activity in Nocturnal bird, *Athene brama* and Diurnal bird, *Perdicula asiatica*

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ABSTRACT—Present study showed effects of adrenal gland modulation on adrenocortical, pineal and gonadal activity in two Indian tropical male and female birds belonging to nocturnal (Spotted owlet, *Athene brama*) and diurnal (Jungle bush quail, *Perdicula asiatica*) habitats. Administration of adrenocorticotropin (ACTH) to both sexes of nocturnal or diurnal bird during regressive or active and inactive phases of the reproductive cycle elevated adrenal and gonadal weights when compared to vehicle control groups. Pineal mass showed no change for the ACTH treatment in the regressive phase while it decreased significantly in the inactive phase. Administration of ACTH elevated the adrenocortical function during the inactive phase in both species, which becomes evident from the increase in adrenal lipids and plasma corticosterone level. Conversely, ACTH treatment during peak adrenal activity (regressive phase in owlet and active phase in quail) decreased the adrenal free cholesterol (FC) while increased adrenal phospholipids (PL) and esterified cholesterol (EC) levels in both species. However, plasma corticosterone levels after the ACTH treatment did not show any change. Administration of corticosterone and dexamethasone to both sexes of nocturnal birds during the regressive phase decreased the gonadal weights while the adrenal gland mass showed no change. Dexamethasone treatment alone reduced the pineal gland mass in females. In diurnal birds, corticosteroids produced no change in adrenal, gonadal and pineal gland weights. However, corticosteroids elevated PL and EC in both species while adrenal FC was unaltered in the nocturnal bird but decreased in the diurnal bird. Administration of adrenal gland inhibitor, metyrapone, to both sexes of the diurnal bird reduced adrenal and gonadal weights and adrenal lipids while it elevated the pineal gland mass. However, in nocturnal birds, the treatment altered only adrenocortical function.

These results indicated that adrenal gland modulation possibly evoked a feedback response from the gland depending on the phase of reproductive (or adrenal) cycle and through this it altered pineal and gonadal activity differentially. Modulation of adrenal activity by ACTH produced a profound reduction in the pineal gland mass during the inactive phase of the reproductive cycle in both sexes of nocturnal and diurnal bird. Changes induced in the adrenocortical function also reflected to some extent on gonadal activity in both regressive/active and inactive phases of reproductive cycle in both species.

INTRODUCTION

The annual/seasonal changes in adrenal mass and cytology, plasma levels of corticosterone or adrenal activity have been reported in a number of bird species and most of these studies correlated the cyclic adrenal function with the annual reproductive cycle (Fujita, 1961; Lorenzen and Farner, 1964; Etches, 1979; Silverin, 1979; Sudhakumari, 1995; Haldar *et al.*, 2000). Recently, a role for pineal gland in the regulation of adrenal function was demonstrated in some bird species (Zemen *et al.*, 1993; Sudhakumari and Haldar, 1997; Haldar *et al.*, 2000) as in mammals (Reiter *et al.*, 1966; Vaughan *et al.*, 1972; Sewerynek and Lewinski, 1989). Administration of melatonin and surgical removal of pineal gland showed an inhibitory influence of pineal on adrenocortical (Zemen *et al.*, 1993; Sudhakumari and Haldar, 1997) and gonadal (Haldar and Ghosh, 1990; Chakraborty, 1993; Sudhakumari, 1995; Haldar and Rai, 1997; Sudhakumari and Haldar, 1997) function in birds. Experimental investigations by modulating adrenal activity to study adrenal-gonadal relationship are limited

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to some bird species and mostly focused on domestic hen (Greenman and Zarrow, 1961; Bhattacharya and Ghosh, 1970; Etches and Cunningham, 1976; Silverin, 1979; Rzasa *et al*., 1983; Etches *et al*., 1984a,b; Petitte *et al*., 1984; Chaturvedi and Suresh, 1990). However, there are no reports on the influence of hypo/hyper activity of adrenal on pineal gland together with gonad. Most of the earlier studies suggested that corticosterone or adrenal gland is gonado-inhibitory in many bird species (Greenman and Zarrow, 1961; Bhattacharya and Ghosh, 1970; Silverin, 1979; see Etches *et al*., 1984a; Chaturvedi and Suresh, 1990). Adrenocorticotropin (ACTH) and corticosterone are reported to alter the responsiveness of ovarian tissues to gonadotropins and also alter luteinizing hormone (LH) secretion in domestic hen (see Etches *et al*., 1984a). In our annual cyclic study, in the nocturnal bird, the peak gonadal activity preceded the seasonal rise in adrenal activity (Sudhakumari, 1995; Haldar *et al*., 2000) while in the diurnal bird both gonadal and adrenal peak activities coincided (Sudhakumari, 1995). Studies in pied flycatcher, a migratory bird, showed that gonadal regression occurs when adrenal activity reached a maximum (Silverin, 1979). Although the seasonal studies in the nocturnal bird tend to indicate an inhibitory role for adrenal on gonadal function, while in the diurnal bird such a distinction was not evident. However, difference in the seasonal adrenal activity in nocturnal and diurnal bird warranted a differential interaction of adrenal-gonadal activity in these bird species (Sudhakumari, 1995; Haldar *et al*., 2000). Incidentally, the inhibitory effect of pineal on adrenal-gonadal activity is almost similar in nocturnal and diurnal birds (Sudhakumari, 1995; Haldar and Rai, 1997; Sudhakumari and Haldar, 1997). Studies on the regulation/modulation of adrenal function may throw some light to derive its relationship with other major physiological mechanisms including reproduction and also its own feedback response. In this regard, reports on adrenocortical feedback or drugs known to alter adrenocortical function and its impact on adrenal–gonadal-pineal relationship together are scarce in birds. To this end, we studied the adrenocortical function/response and correlated with gonadal and pineal mass (activity) in two different habitat (nocturnal and diurnal) birds after the administration of ACTH, corticosterone and dexamethasone, a corticosteroid analogue, and metyrapone, an inhibitor of adrenal function, during two different phases of reproductive cycle.

MATERIAL AND METHODS

Males and females of nocturnal and diurnal birds belonging to tropical India were purchased from the local bird dealers of Varanasi City (Latitude 25°C, 18'N, Longitude83°C, 01'E) of Uttar Pradesh State, India.

(A) Adult nocturnal bird, *Athene brama* (Order: Strigiformes) of both sexes weighing 120–125g body weight (BW) were used in the present study. It is an omnivorous bird without any sexual dimorphism even during the peak reproductive phase. Usually the males weigh lighter than females. The reproductive cycle of *A. brama* can be divided into four phases, inactive (September-November), recrudescence (December-February), active (March-April) and regressive (May-August) phases. In this bird, pairing is once in the reproductive active phase (March-April). They lay eggs, 2–3 in number and incubation of eggs, 22 days, is completed within April.

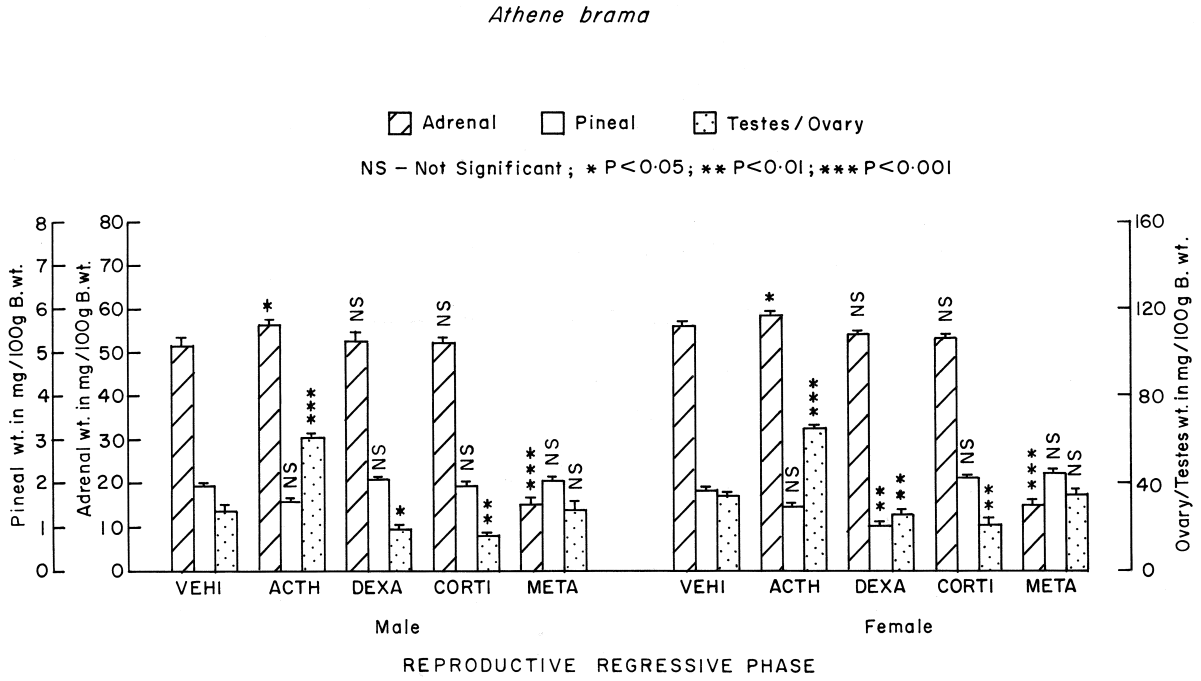

Fig. 1. Effects of administration of adrenocorticotropin (ACTH), corticosterone (CORTI), dexamethasone (DEXA) and metyrapone (META) on adrenal, pineal and gonadal weights of both sexes of the nocturnal bird *Athene brama* during the reproductive regressive phase. VEHI represents the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

(B) Adult diurnal bird, *Perdicula asiatica* (Order: Galliformes) of both sexes weighing 45-50g BW were used in the present study. It is a herbivorous (occasionally omnivorous), showing distinct sexual dimorphism during recrudescence and active phases of the reproductive cycle. Unlike the male, a dark patch below the neck is evident in females during gonadal recrudescence and active phases. The reproductive cycle of *P. asiatica* can be divided into four phases, inactive (November-December), recrudescence (January-April), active (May-June) and regressive (July-October) phases. In this bird, pairing and mating takes place in May. In June, they lay eggs, 4–6 in number and incubation of eggs (14–16 days) is also completed within that month.

Interestingly, in the diurnal bird, both adrenal and gonadal cycles run in parallel while in the nocturnal bird the gonadal activity precede the adrenal. Both nocturnal and diurnal birds were kept separately in an open-air fenced aviary exposed to all changes in normal environmental conditions during acclimation. The nocturnal birds were fed with fresh meat, rodents while the diurnal birds were fed with millet seeds (*Pennisetum typhoides*) and provided water *ad libitum.* Similar type of feed was provided during the course of experiments too. The birds were acclimated for a fortnight before administration of different substances/drugs. Feeding habits were found to be normal and no mortality was observed in both sexes of both the species during acclimation and experimentation.

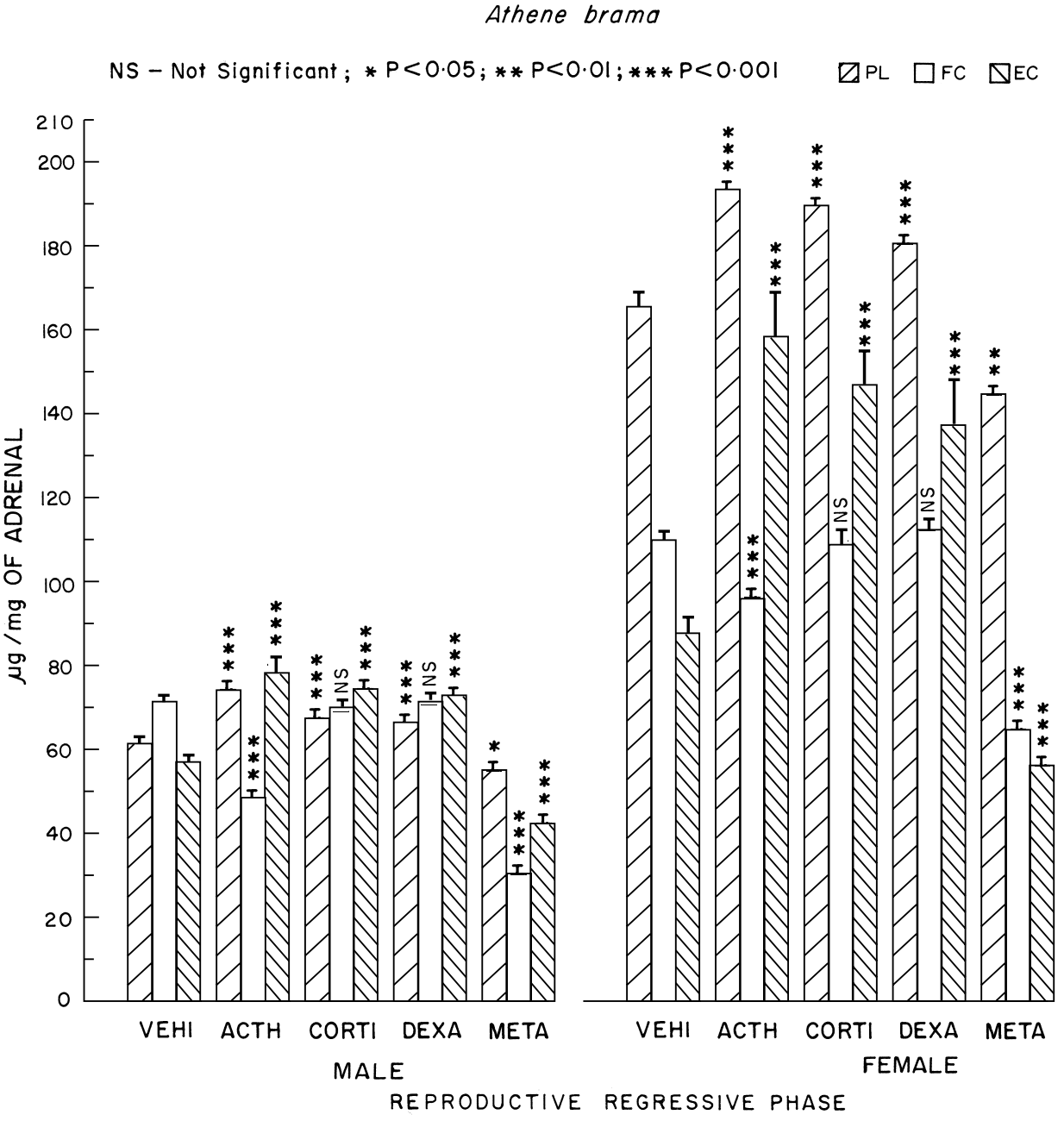

Fig. 2. Effects of administration of adrenocorticotropin (ACTH), corticosterone (CORT), dexamethasone (DEXA) and metyrapone (META) on adrenal lipids [phospholipids (PL), free (FC) and esterified cholesterol (EC)] of both sexes of the nocturnal bird *Athene brama* during the reproductive regressive phase. VEHI represents the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

The following experiments were performed in the nocturnal bird, *A. brama* at two different reproductive phases with duration of 15 days.

Birds of both sexes were divided into five groups and injected with different substance during late regressive phase (June), when the adrenal activity was at its peak. *Group 1*: Fifteen birds each for both sexes were injected with normal saline (0.9% NaCl, w/v) and it served as the vehicle control. *Group 2*: Fifteen birds each for both sexes were injected (subcutaneous) with 0.25 IU/0.1 ml/day of ACTH (Acthar Gel, Rhone-Poulenc Pharm. Inc., PA, USA). *Group 3*: Fifteen birds each for both sexes were injected (subcutaneous) with metyrapone (ICN, Costa-Mesa, CA, USA) at 20μg/0.1ml/day. *Group 4*: Fifteen birds each for both sexes were injected (subcutaneous) with dexamethasone (Sigma, St. Louis, MO, USA) at 20μg/0.1ml/day. *Group 5*: Fifteen birds each for both sexes were injected (subcutaneous) with corticosterone (Sigma, St. Louis, MO, USA) at 20μg/0.1ml/day.

In the reproductive (and adrenal) inactive phase (October), birds of both sexes were divided into two groups. *Group 1*: Fifteen birds each for both sexes were injected with normal saline for 15 days and it served as the vehicle control. *Group 2*: Fifteen birds each for both sexes were injected (subcutaneous) with 0.25 IU/0.1 ml/day of ACTH for 15 days.

Similarly the diurnal bird, *P. asiatica*, belonging to both sexes, were subjected to following experiments but with duration of 30 days.

Table 1 shows the changes in plasma corticosterone profiles (ng/ml) after ACTH treatment for 15 days in regressive and inactive phases of reproductive cycle in the nocturnal bird, *Athene brama*.

Reproductive Phase	Treatment	Male	Female
Regressive	Vehicle (saline)	86.3 ± 3.2	92.4 ± 3.4
	ACTH (0.25IU/0.1ml/day)	82.1 ± 1.9 ^{NS}	88.9 ± 2.1 ^{NS}
Inactive	Vehicle (saline)	58.5 ± 1.2	62.2 ± 1.4
	ACTH (0.25IU/0.1ml/day)	79.2 ± 2.1*	82.1 ± 2.2*

Data indicate mean ± SEM (n=15). *Means P<0.001 significance level (Student's t test) when compared to the Vehicle (Control). ^{NS} means not significantly different from Vehicle.

In the reproductive and adrenal active phase (June) Birds of both sexes were divided into five groups. *Group 1*: Fifteen birds each for both sexes were injected with normal saline and it served as the vehicle control. *Group 2*: Fifteen birds each for both sexes were injected (subcutaneous) with 0.25 IU/0.1 ml/day of ACTH. *Group 3*: Fifteen birds each for both sexes were injected (subcutaneous) with metyrapone at 10μg/0.1ml/day. *Group 4*: Fifteen birds each for both sexes were injected (subcutaneous) with dexamethasone at 10mg/0.1ml/day. *Group 5*: Fifteen birds each for both sexes were injected (subcutaneous) with corticosterone at 10μg/0.1ml/day.

In the reproductive (and adrenal) inactive phase (October), birds of both sexes were divided into two groups. *Group 1*: Fifteen birds each for both sexes were injected with normal saline for 30 days and it served as the vehicle control. *Group 2*: Fifteen birds each for both sexes were injected (subcutaneous) with 0.25 IU/0.1 ml/day of ACTH for 30 days.

After the completion of experiments, birds were weighed and sacrificed by decapitation during 10:00–11:00hr. Ovaries/testes, adrenal and pineal glands were removed, freed from adherent tissues and blotted on a filter paper and weighed in a micro-electrical balance to calculate adrenal, pineal and gonado-somatic index, which is expressed as the mean glandular weight in mg per 100g BW.

Blood collected from the ACTH and the saline (vehicle control) injected birds in heparinized tubes was centrifuged at 800xg at 4°C to separate out plasma. The plasma samples were stored briefly at –20°C until assayed for plasma corticosterone by RIA for ACTH and vehicle control groups. Plasma corticosterone was estimated by using [1,2,6,7-^{3}H (N)] corticosterone (Sp. Act. 70–100 Ci / mmol) purchased from NEN, Boston, MA, USA and the antiserum to corticosterone was obtained as a gift from Dr. J.K. Datta, formerly at NIHFW, New Delhi, India. The basic RIA methodology illustrated by Abraham (1974) and plasma extraction procedure were similar to the protocol described previously by Senthilkumaran and Joy (1994). The intra- and inter-assay variations of the RIA were 3.2% and 2.4% (n=10), respectively. The sensitivity of the RIA was 10 pg/ml. The recovery of standard in the RIA ranged between 87 to 90%. The antiserum to corticosterone did not show any significant cross-reactivity (<0.01)

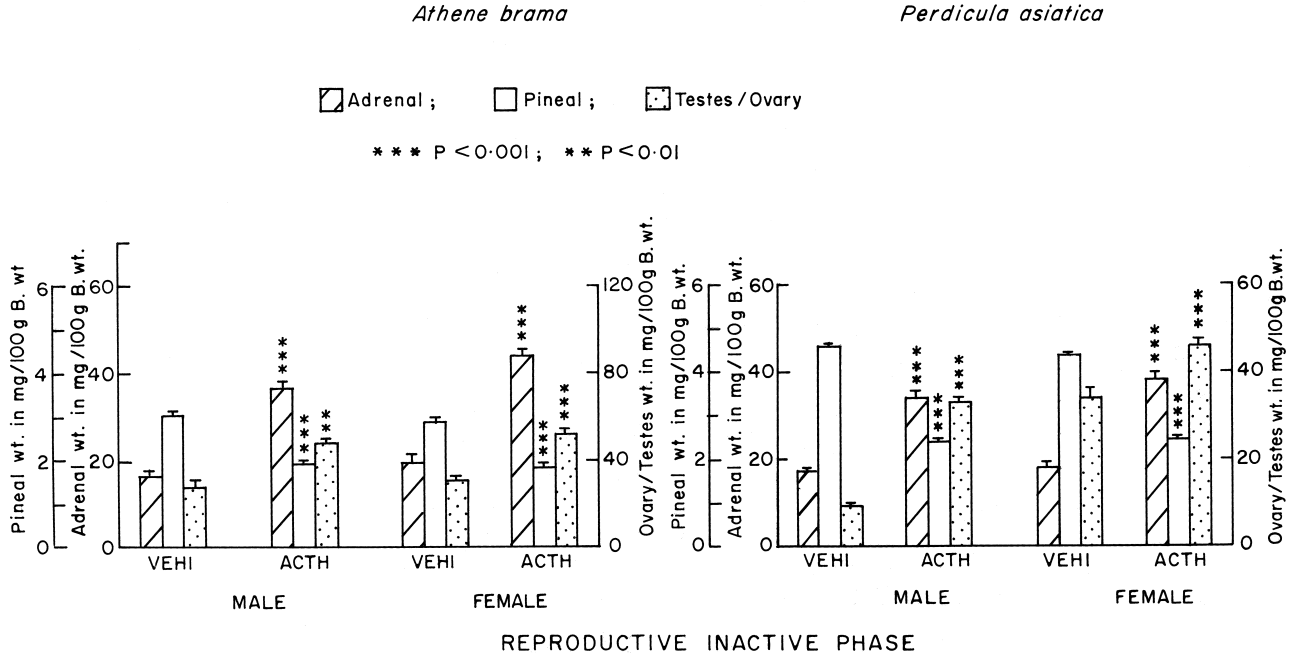

Fig. 3. Effects of administration of adrenocorticotropin (ACTH) on adrenal, pineal and gonadal weights of both sexes of nocturnal *Athene brama* and diurnal *Perdicula asiatica* during the reproductive inactive phase. VEHI the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

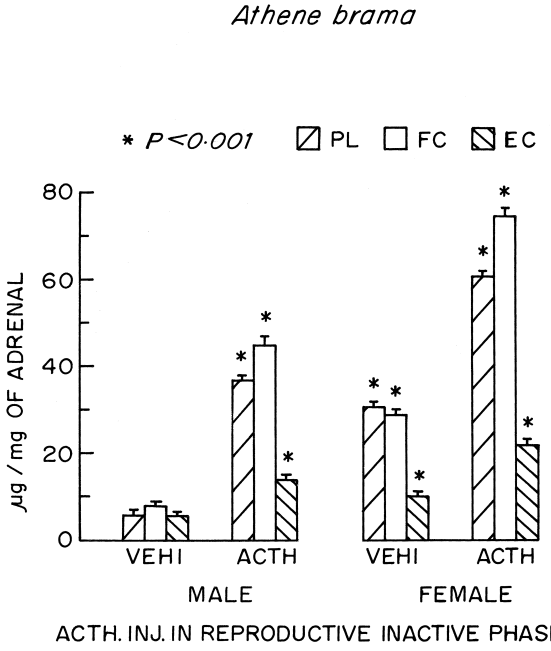

Fig. 4. Effects of administration of adrenocorticotropin (ACTH) on adrenal lipids [phospholipids (PL), free (FC) and esterified cholesterol (EC)] of both sexes of the nocturnal bird *Athene brama* during the reproductive inactive phase. VEHI represents the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

with other corticosteroids. All adrenal samples after the administration of different substances/drugs were processed for the analysis of lipids, in which phospholipids (PL), free (FC) and esterified cholesterol (EC) were estimated by adopting the method of Folch *et al.* (1957), using thin-layer chromatography (TLC). Recovery of lipid standards in the assay was more than 90%. Details of this method described previously by Kirubagaran and Joy (1995). Prior to TLC assay of adrenal lipids, the adrenal gland tissues were stored briefly at –20°C.

Statistical analysis

All data were expressed as means ± standard error mean (SEM). Multiple Student's *t* test was employed to compare the experimental groups with Vehicle (saline) control groups. Data after ACTH treatment at two different phases were analyzed by two-way analysis of variance (two-way ANOVA). Differences were considered significant from P<0.05.

RESULTS

Birds belonging to both sexes of *A. brama* and *P. asiatica* treated with normal saline showed changes on adrenal, pineal and gonadal weights, adrenal lipids and plasma corticosterone profiles in accordance to their breeding cycle or phases (see Discussion), during which experiments were conducted.

Effects of administration of ACTH, corticosterone, dexamethasone and metyrapone on *A. brama* during regressive and/or inactive phases of the reproductive cycle

Both sexes of nocturnal birds, when administrated with

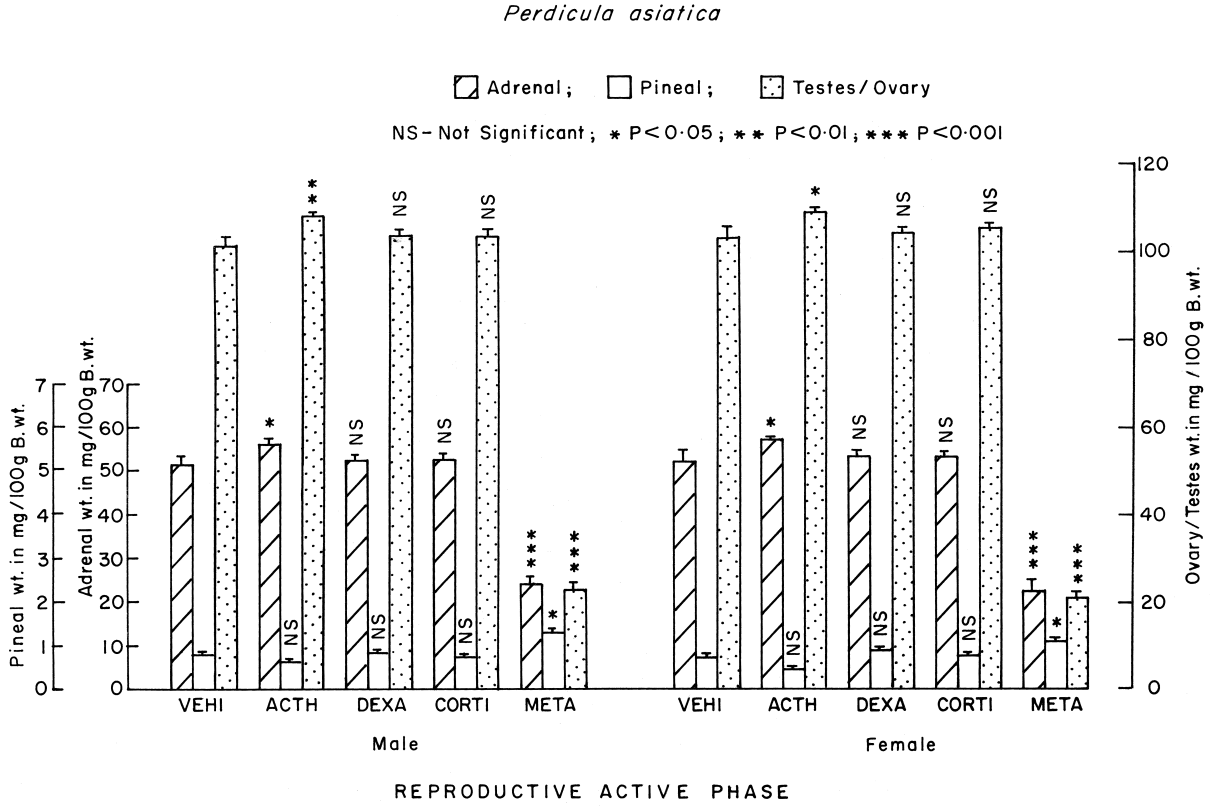

Fig. 5. Effects of administration of adrenocorticotropin (ACTH), corticosterone (CORTI), dexamethasone (DEXA) and metyrapone (META) on adrenal, pineal and gonadal weights of both sexes of the diurnal bird *Perdicula asiatica* during the reproductive active phase. VEHI represents the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

ACTH for 15 days during late regressive phase significantly increased adrenal and gonadal weights (Fig. 1). However, the pineal gland mass showed no change when compared to vehicle control groups. Administration of ACTH during late regressive phase increased adrenal PL and EC significantly while FC content (Fig. 2) reduced significantly. However, plasma corticosterone (Table 1) level was unaltered in ACTH injected bird during the regressive phase. On the other hand, administration of ACTH during inactive phase of the reproductive cycle decreased pineal mass (Fig. 3) significantly. However, both adrenal and gonadal weights (Fig. 3) elevated significantly like that of the response after ACTH injection in late regressive phase. Administration of ACTH during inactive phase significantly elevated all three adrenal lipid fractions (PL, FC and EC) (Fig. 4) and also the plasma corticosterone (Table 1) level. Two-way ANOVA (P<0.001) of the data on ACTH administration indicated that treatment and season are more effective for adrenal and gonadal activity while ACTH treatment alone is more effective for pineal mass.

Both sexes of nocturnal birds, when administrated with corticosterone for 15 days during late regressive phase significantly decreased gonadal weights (Fig. 1). However, adrenal and pineal gland masses showed no change when compared to vehicle control groups. Administration of corticosterone elevated adrenal PL and EC significantly while the FC level (Fig. 2) unaltered in both sexes when compared to vehicle control groups.

Both sexes of nocturnal birds, when administrated with dexamethasone for 15 days during late regressive phase significantly decreased gonadal weights (Fig.1). However, the adrenal gland mass showed no change when compared to vehicle control groups. In female birds, administration of dexamethasone decreased pineal gland weight (Fig.2) significantly while in males there was no change when compared to the control group. Similar to corticosterone treatment, administration of dexamethasone elevated adrenal PL and EC significantly while the FC level (Fig.2) was unaltered in both sexes when compared to vehicle control groups.

Both sexes of nocturnal birds, when administrated with metyrapone for 15 days during late regressive phase significantly decreased the adrenal weight (Fig.1). However, gonadal and pineal gland masses (Fig.1) showed no change when compared to vehicle control groups. However, administration of metyrapone reduced all three adrenal lipid fractions (PL, FC and EC) significantly in both sexes (Fig.2) when compared to vehicle control groups.

Effects of administration of ACTH, corticosterone, dexamethasone and metyrapone on *P. asiatica* during active and/or inactive phases of the reproductive cycle

ACTH treatment produced similar results in diurnal birds like that of the nocturnal bird. Administration of ACTH for 30

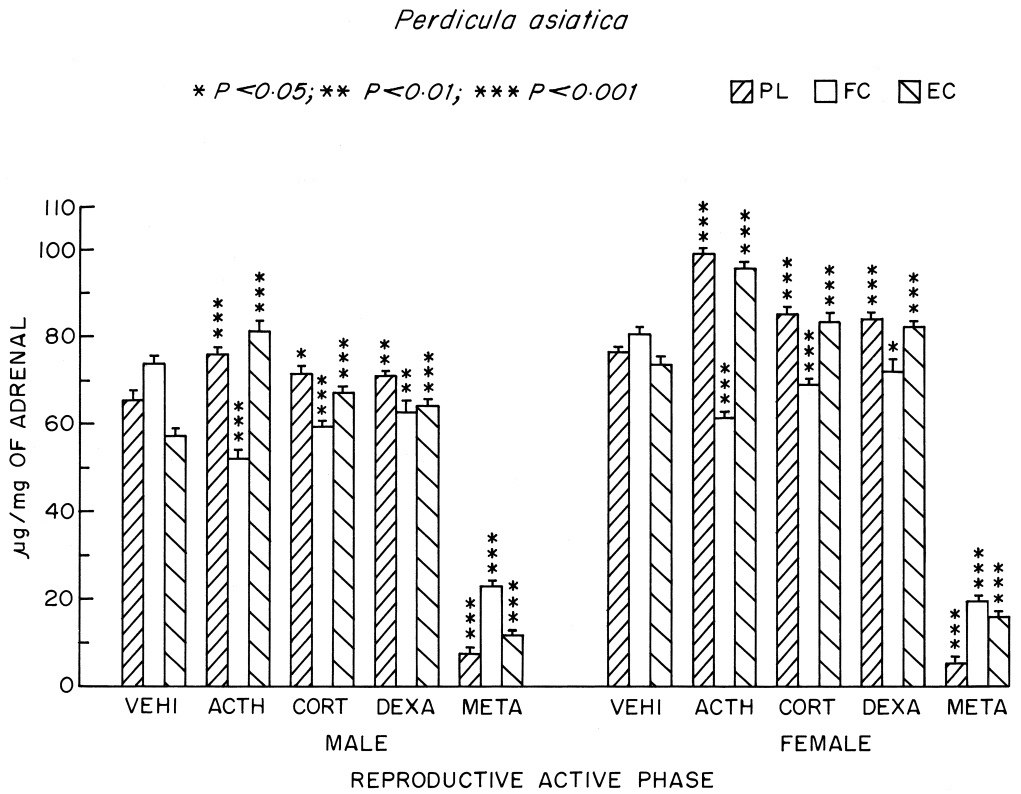

Fig. 6. Effects of administration of adrenocorticotropin (ACTH), corticosterone (CORTI), dexamethasone (DEXA) and metyrapone (META) on adrenal lipids [phospholipids (PL), free (FC) and esterified cholesterol (EC)] of both sexes of the diurnal bird *Perdicula asiatica* during the reproductive active phase. VEHI represents the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

days to both sexes of diurnal birds during active phase significantly increased adrenal and gonadal weights (Fig. 5) with no change in the pineal gland mass. Administration of ACTH during active phase increased PL and EC and decreased FC content (Fig. 6) significantly without altering plasma corticosterone (Table 2) level. Administration of ACTH during inactive phase of the reproductive cycle decreased pineal mass and elevated adrenal and gonadal weights (Fig. 3), adrenal lipids (Fig. 7) and plasma corticosterone (Table 2) level significantly. Two-way ANOVA ($P < 0.001$) of the data on ACTH administration indicated that treatment and season are more effective for adrenal and gonadal activity while ACTH treatment alone is more effective for pineal mass.

In contrast to nocturnal birds, both sexes of diurnal birds, when administrated with corticosterone or dexamethasone for 30 days during active phase produced no change in adrenal, gonadal and pineal weights (Fig. 5). However, administration of corticosterone or dexamethasone significantly elevated adrenal PL and EC while the FC level (Fig. 6) decreased in both sexes when compared to vehicle control groups.

Unlike the nocturnal birds, administration of metyrapone for 30days to both sexes of diurnal birds during active phase significantly decreased adrenal as well as gonadal weights (Fig. 5), while the pineal gland mass (Fig. 5) elevated significantly in both sexes when compared to vehicle control groups. As observed in nocturnal birds, administration of metyrapone to both sexes of diurnal birds significantly reduced adrenal lipids (Fig. 6) when compared to vehicle control groups.

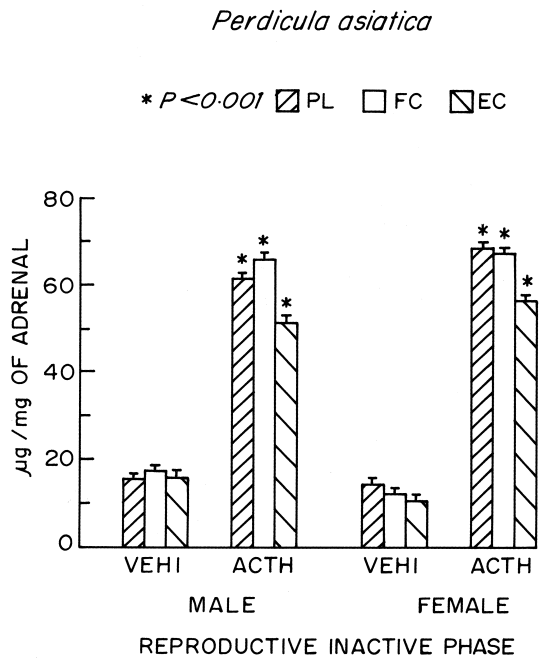

Fig. 7. Effects of administration of adrenocorticotropin (ACTH) on adrenal lipids [phospholipids (PL), free (FC) and esterified cholesterol (EC)] of both sexes of the diurnal bird *Perdicula asiatica* during the reproductive inactive phase. VEHI represents the vehicle control (saline treated) group. Vertical bars represent mean ± SEM (n=15). Other explanations for abbreviations are in the figure inlet.

Table 2 shows the changes in plasma corticosterone profiles (ng/ml) after ACTH treatment for 30 days in active and inactive phases of reproductive cycle in the diurnal bird, *Perdicula asiatica*.

Reproductive Phase	Treatment	Male	Female
Active	Vehicle (saline)	59.6 ± 2.4	62.2 ± 2.8
	ACTH (0.25IU/0.1ml/day)	56.9 ± 2.3 ^{NS}	58.7 ± 3.3 ^{NS}
Inactive	Vehicle (saline)	27.5 ± 1.1	27.7 ± 1.0
	ACTH (0.25IU/0.1ml/day)	42.3 ± 1.6*	41.9 ± 1.5*

Data indicate mean ± SEM (n=15). *Means $P<0.001$ significance level (Student's *t* test) when compared to the Vehicle (Control). ^{NS} means not significantly different from Vehicle.

DISCUSSION

Present study explains the response of the adrenal gland to its modulators such as ACTH, corticosterone, dexamethasone (a synthetic corticosteroid analogue) and adrenal gland inhibitor metyrapone in both sexes of diurnal and nocturnal birds. Furthermore, adrenal hypo/hyper activity-induced changes in gonadal and pineal gland mass were observed in both nocturnal and diurnal birds on a comparative basis. Changes in the adrenal lipids and/or the plasma corticosterone level after the treatment of ACTH, corticosteroids and metyrapone indicate a probable feed back effect on the gland, however, based on the adrenal or reproductive activity (cycle) of the bird. The data showed differential effects of these compounds on the pineal gland mass. Interestingly, the gonadal activity changed in parallel to the adrenal for the ACTH treatment in both species while the administration of corticosteroids showed an antagonistic effect on the gonadal activity only in the nocturnal bird.

The changes in adrenocortical function, pineal and gonadal mass in saline (vehicle) treated control were very similar to the intact control in the respective phase of diurnal and nocturnal bird (see Sudhakumari, 1995; Haldar *et al*., 2000). Hence the vehicle group was used as a control to compare the experimental observations. Studies modulating adrenocortical function also employed similar control. Further the dosages of ACTH, corticosteroids and metyrapone used in the present study were selected in accordance to the previous reports (Silverin, 1979; Chaturvedi and Suresh, 1990). In the present study, administration of ACTH in both sexes of nocturnal and diurnal bird elevated the gonadal mass in two different reproductive phases studied. In red headed bunting, it was shown that administration of ACTH in the reproductive recrudescence phase significantly decreased testicular volume and weight while in the regressive phase the testicular volume and weight attained like full breeding condition (Chaturvedi and Suresh, 1990). On the other hand, a high dose of ACTH treatment didn't evoke any effect on testicular weight in a migratory bird (Silverin, 1979). Following the report of Etches *et al.* (1984a), Chaturvedi and Suresh (1990) explained the elevation of testicular activity after ACTH treatment may be through gonadotropin mediated phenomenon. A number of reports support this assumption and in domestic

hen it has been reported that ACTH have an impact on tonic release of LH and ovulation (Etches and Cunningham, 1976; Peczeley and Daniel, 1979; Etches and Croze, 1983; Etches *et al.*, 1984b). In the present study too, such a contention can be considered for the elevation of gonadal mass after ACTH treatment in the peak adrenal phase. It may also be worth conceivable that ACTH treatment during peak adrenal activity might have reduced corticosterone release. Administration of ACTH significantly reduced FC pool, a precursor for steroidogenesis, in both nocturnal and diurnal birds and marginally lowered plasma corticostrerone release. The changes in adrenocortical lipids support the concept of feedback response of the gland. A low FC and a high EC levels in ACTH injected bird, when the adrenal activity is at its peak, indicated a decreased conversion of cholesterol for steroidogenesis. A slight increase in the adrenal gland mass with a reduction in adrenocortical function after ACTH treatment is unclear at present. Deviche (1983) and Peczely (1976) reported that morphological characteristics correlate more poorly with adrenal potential for glucocorticoid synthesis and secretion in birds. In birds, it has been reported that adrenal steroids inhibit gonadal activity (Greenman and Zarrow, 1961; Bhattacharya and Ghosh, 1970; Wilson and Follett, 1975; Silverin, 1979; Rzasa *et al.*, 1983; see Etches *et al.*, 1984a; Chaturvedi and Thapliyal, 1980; Chaturvedi and Suresh, 1990). ACTH-induced rise in gonadal mass is possibly through decreased FC pool/corticosterone (present study) or increased LH release, as suggested by Etches *et al.* (1984a), which remains open. Conversely, ACTH treatment in reproductive (and adrenal) inactive phase significantly elevated corticosterone and adrenal lipids in both species, indicating elevated adrenal activity. This is in accordance with earlier reports on this line (Nagra *et al.*, 1960; Peczeley, 1972; Walsh *et al.*, 1984). Elevation of gonadal mass in parallel with adrenocortical function in inactive phase after ACTH treatment may be due to simultaneous reduction in pineal activity (see below). In these birds and in Japanese quail, it has been demonstrated that pineal inhibits adrenal and/or gonadal function by modulating their hormone production (Haldar and Ghosh, 1990; Zeman *et al.*, 1993; Haldar and Rai, 1997; Sudhakumari and Haldar, 1997; Haldar *et al.*, 2000). Further, Chaturvedi and Suresh (1990) suggested that increasing or increased level of adrenal steroids required during progressive and early breeding phases of the gonadal cycle in some bird species. Hence it may also be considered as a cumulative phenomena. To our knowledge present study was first of its kind to demonstrate differential effects of ACTH not only on adrenocortical function but also on gonadal and pineal activity (see below) in nocturnal and diurnal birds.

ACTH treatment induced a major reduction (by 40 to 50%) in the pineal gland mass in the reproductive inactive phase of both owl and quail, indicating anti-pineal activity of ACTH. To our knowledge, there are no reports to compare our results. It has been reported that pineal gland exert an inhibitory effect on adrenal function including steroidogenesis in birds and mammals (Reiter *et al.*, 1966; Vaughan *et al.*, 1972; Sewerynek and Lewinski, 1989; Zeman *et al.*, 1993; Sudhakumari and Haldar, 1997). Hence ACTH through an unknown mechanism decreased the hyper pineal activity, at that phase, to elevate the inactive adrenal or a direct action of ACTH on adrenal brought a secondary effect on pineal. Further studies are warranted to understand the mode of action of ACTH to reduce pineal mass vis-à-vis pineal activity. However, absence of any change in the pineal activity after ACTH treatment in the peak adrenal phase may be due to its attainment of nadir.

Administration of corticosterone and dexamethasone decreased the gonadal weight in the nocturnal bird. It is in agreement with number of previous reports in bird species and favors gonado-inhibitory role of corticosteroids (Greenman and Zarrow, 1961; Bhattacharya and Ghosh, 1970; Wilson and Follett, 1975; Chaturvedi and Thapliyal, 1980; Chaturvedi and Suresh, 1990). Administration of corticosteroids in the nocturnal bird elevated the EC without altering the FC level. This indicates that the free flow of FC for corticosteroid biosynthesis prevailed in addition to administered corticosteroids. These observations tend to support the attainment of hyper-adrenal activity in the nocturnal bird. Increased adrenocortical function might have inhibited gonadal activity (references as above). It has been reported that corticosteroid including synthetic analogues like dexamethasone is reported to affect the LH level and inhibit ovulation (see Etches *et al.*, 1984a; Rzasa *et al.*, 1983). Furthermore, in our annual cyclic study we had shown that the regression in the gonadal activity occurs when the adrenal activity attain its peak in this species (Sudhakumari, 1995; Haldar *et al.*, 2000). Similarly in a migratory bird testicular regression is followed by the rise in adrenal activity (Silverin, 1979). In contrast, in the diurnal bird, corticosteroids did not evoke any change in adrenal and gonadal masses. It may be possible that the dosage and duration was insufficient to elevate adrenal activity. An elevation in EC with a reduction in FC level in the diurnal bird, but not in the nocturnal bird, also indicate a counter feedback response to reduce corticosterone release in the former than the latter. Furthermore, unlike the nocturnal bird, in nature, the adrenal gonadal cycles run parallel in the diurnal bird (Sudhakumari, 1995). Hence a remarkable elevation in corticosteroids level might have been required to bring hyper-adrenal activity to block gonadal function in the diurnal species. Unlike the differential gonadal response in diurnal and nocturnal birds, pineal mass did not change after corticosteroids treatment in both nocturnal and diurnal bird except for a marginal decline in the pineal gland mass in female owls after the administration of dexamethasone. There are no other studies so far in this aspect to compare our results. However, the effect of dexamethasone in females and not in males needs further experimental evidences. In white crowned sparrows, Asthmier *et al.* (1994) also reported a female specific action of dexamethasone on ACTH-induced feedback suppression, through glucocorticoid receptor mediated mechanism.

Metyrapone treatment produced inhibitory effect on adrenocortical function including adrenal gland weight in both nocturnal and diurnal birds, as observed in many bird species (Nagra *et al.*, 1963; Frankel *et al.*, 1967; Bhattacharya and

Ghosh, 1970). Metyrapone is a specific adrenal or corticosteroid inhibitor and it interferes at the level of corticosteroid biosynthesis (Krugers *et al*., 2000). Interestingly, metyrapone treatment decreased the gonadal weight and elevated the pineal mass only in the diurnal bird and not in the nocturnal bird. In the diurnal bird, a reduction in the gonadal activity might have been through adrenal inactivation as adrenal and gonadal activity runs in parallel during the annual reproductive cycle (Sudhakumari, 1995). In some migratory birds increased adrenal gland function along with reproductive activities have been shown to cope with possible emergency conditions such as mate guarding, territorial defence, nesting, feeding and vigilant monitoring for nesting predators (see Asthemier *et al*., 1994). Some of these behaviors may also be present in jungle bush quail as adrenal activity coincides with gonadal cycle. In addition, in domestic hen, it was shown that corticosterone can induce ovulation (Etches and Cunmingham, 1976) by tonic LH release (Etches and Croze, 1983) if mature follicle is present in the ovary, suggesting a positive regulation of adrenal on ovarian function (Etches *et al*., 1984b). Alternatively, chronic infusions of corticosteroids can block ovulation if the follicles are not sensitized for LH (see Etches *et al*., 1984a). In the present study, administration of corticosteroids did not reduce gonadal mass in quail, indicating a positive adrenal-gonadal relationship. Nevertheless, an increased pineal gland activity might have also contributed for decreased gonadal function or else these two factors together brought this effect. In birds inhibitory influence of pineal gland on gonadal function is well documented (Haldar and Ghosh, 1990; Zeman *et al*., 1993; Haldar and Rai, 1997; Sudhakumari and Haldar, 1997). In the nocturnal bird, since gonadal regression started already during the peak adrenal activity, a blockade of adrenal activity at that period might not have evoked any response on gonads and also the duration of treatment and dosage might also be responsible. Though metyrapone treatment altered adrenocortical function in owl, no response in gonadal activity further indicate the cessation of gonadal response due to the decline in gonadal steroids/function in that phase (Sudhakumari, 1995; Haldar *et al*., 2000). However, in some bird species during juvenile or early regressed gonadal conditions treatment of metyrapone elevated gonadal activity (Bhattacharya and Ghosh, 1970; Chaturvedi and Suresh, 1990).

In summary, present study demonstrates a feed back response of the adrenal gland to its own modulators, which becomes evident from the changes on adrenal lipids, including cholesterol and/or plasma corticosterone level. Adrenal gland modulation altered gonadal activity in two reproductive phases studied. The effect is mostly parallel with adrenal gland function, at least, for ACTH treatment in both species. Conversely, administration of corticosteroids exhibited antagonistic effect on gonadal activity only in the nocturnal bird. Interestingly, administration of metyrapone decreased the gonadal mass only in the diurnal bird, indicating a positive adrenal-gonadal relationship. Therefore, a distinct difference in the regulation of gonadal activity by adrenal do exists in the nocturnal and diurnal birds. Annual changes in adrenal and gonadal activity also support such a contention, since gonadal activity precede adrenal in owl and it coincides in quail (Sudhakumari, 1995; Haldar *et al*., 2000). Additionally, adrenal modulation by ACTH produced a profound inhibitory effect on the pineal mass, especially when the pineal activity is at its peak (reproductive inactive phase). This data indicate that adrenocortical modulation by ACTH may counter pineal gland inhibition on adrenal-gonadal function. In view of the previous reports (Ralph, 1981; Vivien-Roels, 1985; Haldar and Ghosh, 1990; Chakraborty, 1993; Zeman *et al*., 1993; Sudhakumari, 1995; Haldar and Rai, 1997; Sudhakumari and Haldar, 1997; Haldar *et al*., 2000) demonstrating the inhibitory influence of pineal on adrenal-gonadal function and together with the present study, it can be suggested that an interaction of adrenal-gonadal-pineal activity may occur to execute their regulation and function during the annual cycle.

ACKNOWLEDGEMENTS

We are grateful to Dr. B. Senthilkumaran for his technical help, valuable criticisms and suggestions. We are thankful to Prof. Dr. K.P. Joy, Department of Zoology, Banaras Hindu University for providing his laboratory facilities to perform TLC. Our thanks are also due to Dr. J.K. Datta, formerly at NIHFW, New Delhi for his technical help.

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(Received July 4, 2000 / Accepted September 12, 2000)