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Reproductive Phase Dependent Sensitivity of Pineal-Biochemical Constituents of Indian Palm Squirrel, *Funambulus pennanti* to Testosterone Propionate Treatment

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ABSTRACT—The pineal gland (PG) possesses receptor proteins capable of binding androgen with high affinity and specificity. However, the sensitivity of PG to exogenous testosterone (T) in term of biochemical constituents during different reproductive phases is still unknown. Hence, an attempt was made to study the effect of testosterone propionate (TP; 20 µg/animal/day; i.m. injection) on the biochemical constituents of PG i.e. protein, cholesterol, serotonin and plasma melatonin (MEL) level of the subtropical zone rodent, *Funambulus pennanti* during reproductive active (RAP) and inactive phases (RIP). During RAP, TP increased the plasma MEL level and prostate gland weight while, it had no effect on other biochemical constituents of PG, plasma T level and testes weight. It may be suggested that during the RAP, exogenous TP initiates the negative feed back mechanism at leydig cell level. Further, TP is known to affect directly the MEL synthesis by increasing the activity of PG. During RIP, TP decreased the PG weight, plasma MEL level, serotonin and protein content and increased the testes, accessory sex organ's weight and plasma T level. This decrease in PG activity may be due to the decrease in enzyme activity by TP which in turn inhibited the MEL synthesis and initiated the gonadal function.

Therefore, in this squirrel PG biochemical constituents presented a reproductive phase dependent variation modulated by T. Further, active PG of RIP appeared to be more sensitive to exogenous TP treatment in reducing the biochemical constituents and increasing MEL synthesis.

INTRODUCTION

During the past decade, great effort has been made to understand the mechanism of MEL action on target cell including binding studies and cell response analyses. The inhibitory action of MEL on androgen synthesis was first reported by Ellis (1972). Studies have identified the MEL binding sites on the vas deferens and testes (Skene *et al.*, 1993; Ayre and Pang, 1994). Moreover, the direct effect of MEL on T secretion seems to be a robust result in squirrel, hamster, rat, mouse etc. (Haldar and Saxena, 1990; Lukaszyk, 1991; Persengiev and Kehajova, 1991; Niedziela and Lukaszyk, 1993; Niedziela *et al.*, 1995; Valenti *et al.*, 1995). Recent reports suggest that MEL may block the effect of forskolin which is a potent stimulator of T secretion by acting selectively through adenylate cyclase of intact leydig cells (Arendt, 1995). On the other hand, 5 α -dihydrotestosterone (5 α -DHT) receptor has also been detected on the PG (Haldar and Gupta, 1990). However, the literature suggest that there is a total lack of information on the androgen mediated biochemical changes in PG other than hydroxy-o-methyl-transferase (HIOMT), Mono amine oxidase

(MAO) and MEL (Vacas and Cardinali, 1979). Recently, we presented the data proving that some biochemical constituents such as protein, cholesterol and serotonin act as markers of PG activity of *Funambulus pennanti* (Sarkar and Haldar, 1997).

The Indian Palm squirrel, *F. pennanti* is a seasonal breeder, arboreal in habitat and partial hibernator. The sexual cycle in male (Haldar and Saxena, 1988) presents a clear biphasic annual testicular cycle characterized by a short period of quiescence with total arrest of spermatogenesis during October-November. On the other hand, decline in testicular activity is noted from March followed by inactive phase during April-June. Hence, the present study was aimed to note the changes in different pineal biochemical constituents of PG and its sensitivity to the exogenous administration of TP in a seasonal breeder, *F. pennanti* during active and inactive phases of reproductive cycle.

MATERIALS AND METHOD

The experiments were performed during April (reproductive active phase) and November (reproductive inactive phase). Fifty adult male squirrels weighing 110±10 g were obtained from the local supplier at the vicinity of Varanasi (Long. 83°, 1' East; Lat.: 25°, 18' North) and used after 15 days of acclimatization in laboratory condition (equivalent to natural condition i.e. daylength ~ 12.45 hr.; temp. ~

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35.3°C; RH ~ 55.4% in the month of April and daylength ~ 10.3 hr.; temp. ~ 29°C; RH ~ 62% in the month of November). The squirrels had an access to food consisting of soaked gram seeds (*Cicer arietinum*) and water *ad libitum*. Twenty five adult squirrels received TP (SIGMA, St. Louis, USA; 20 µg/day/animal; Haldar and Vidhu, 1997) daily (intramuscular injection; morning hrs. ~ 1000-1030 hr) injections for thirty consecutive days during both the reproductive active and inactive phases. The control group of twenty five squirrels received equal volume of vehicle i.e. sesame oil and propane 1,2 diol and methanol; 10:0.1 V/V, respectively. After thirty days, squirrels were bled from the subclavian vein at 2300 hr (for MEL radio-immunoassay), centrifuged at 800 × g and 2°C for 20 min to separate out the plasma and stored at -20°C. The squirrels were sacrificed (0900-1100 hr) next day by decapitation and body weight noted. The trunk blood was centrifuged to separate the plasma and stored at -20°C till the RIA of T. The PG were quickly removed on ice and stored at -20°C till the estimation of biochemical constituents.

Protein was estimated by homogenizing the PG in 0.9% NaCl following the method of Lowry *et al.* (1951), total cholesterol was estimated by homogenizing the PG in acetone: ether mixture (3:1) following the method of Sacket (1925). The spectrofluorometric estimation of serotonin was done by homogenizing the PG in 0.1N HCl: 0.5% ascorbic acid mixture following the method of Quay (1963). The radioimmunoassay (RIA) of MEL was performed by the method of Rollag and Niswender (1976) using ³H melatonin (Amersham, U.S.A.) and antibody (Guild antisera-sheep antimelatonin Batch no. G/S/704-8483, Surrey, Guildford, U.K.). The RIA for plasma level of T was done following the kit of Immunochem. Corporation, Carson, California (Covalent Coat TM, Direct Testosterone Ks, Kit). The details of RIA of MEL and T were presented in Table 1.

The results of the effect of TP on the PG weight, reproductive organ's weight and biochemical constituents were expressed by Mean ± SEM. The data was analyzed with the help of Student's *t* test and two way ANOVA (Brunner and Knitz, 1977; Table 2).

RESULT

Treatment of TP during reproductive active phase

TP increased the plasma MEL level, while the PG weight and its protein, cholesterol and serotonin content had no change (Fig. 1, Table 2). Moreover, TP had no significant effect on testes and seminal vesicle weight and plasma T level during reproductive active phase, while it increased the prostate gland weight (Fig. 2, Table 2).

Treatment of TP during reproductive inactive phase

TP decreased the PG weight, protein, serotonin content and plasma MEL level, whereas no significant change was observed in the cholesterol content (Fig. 3, Table 2). Moreover, TP injection significantly increased the testes and accessory sex organ's weight and plasma T level (Fig. 4, Table 2).

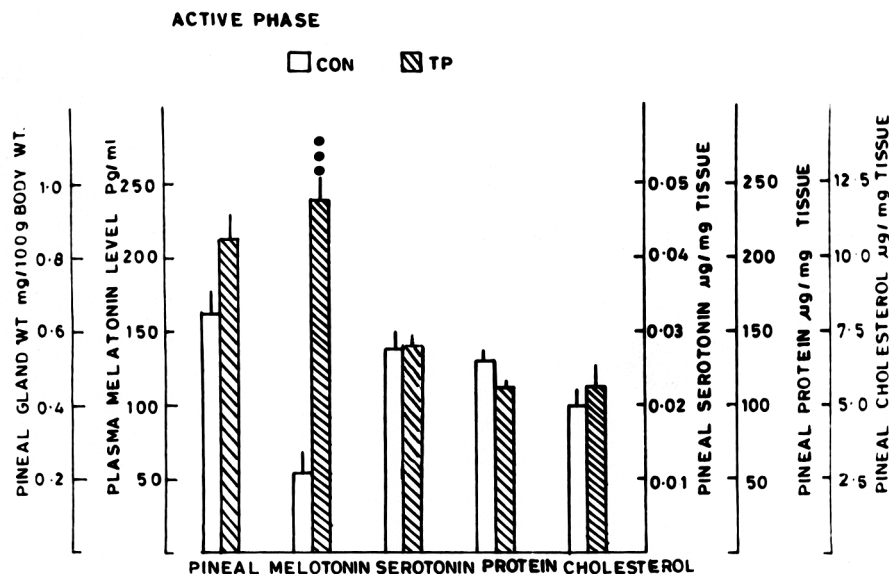


Fig. 1. Effect of TP (20 µg/animal/day) on the pineal gland weight, pineal protein, cholesterol, serotonin content and plasma melatonin level during reproductive active phase in *F. pennanti*. In all figures vertical bars represent standard error. Significance of difference: *** P < 0.001

Table 1. Characteristics of Radioimmunoassay

Hormone	Slope	Correlation of Coefficient (r)	Interassay Coefficient of variation (%)	Intraassay Coefficient of variation (%)	Detection Limit (pg/ml)	Correlation Coefficient of added and estimated value (r)
Melatonin	-1.0693	-0.887	9.0	15.0	5	0.999
Testosterone	-2.0307	-0.996	7.2	8.3	6	0.999

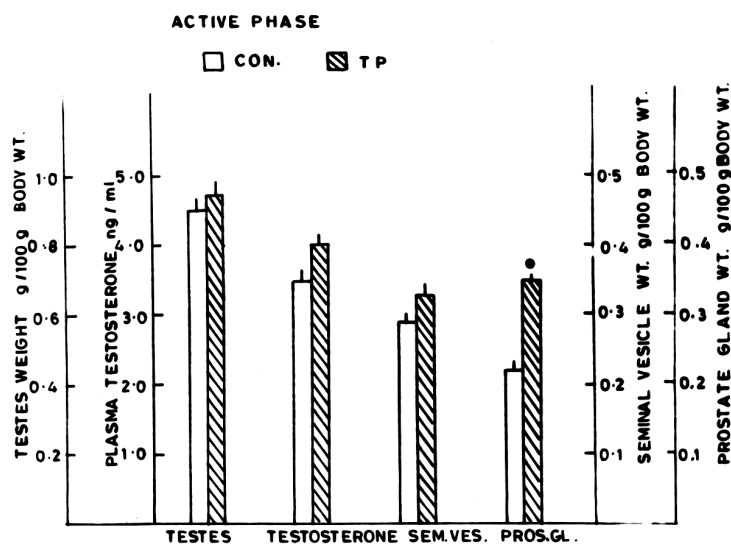


Fig. 2. Effect of TP (20 µg /animal /day) on the testes, seminal vesicle, prostate gland weight and plasma testosterone level during reproductive active phase in *F. pennanti*. Significance of difference: • $P < 0.05$

Table 2. Summary of Two way analysis of variance (ANOVA) of the data on the effect of TP on pineal, testes, seminal vesicle and prostate gland weight and testosterone level protein, cholesterol, serotonin and melatonin level.

Parameters	Treatment	Phase	Interaction Treatment x Phase
Pineal wt.	10.74 $P < 0.01$	1.64	5.4 $P < 0.05$
Testes wt.	3.94	162.90 $P < 0.001$	3.60
Sem. ves. wt.	7.2 $P < 0.025$	229.8 $P < 0.001$	0.56
Pros. gl. wt.	5.75 $P < 0.05$	9.2 $P < 0.01$	0.058
Testosterone	10.54 $P < 0.01$	50.01 $P < 0.001$	3.40
Protein	8.62 $P < 0.025$	9.45 $P < 0.01$	5.79 $P < 0.05$
Cholesterol	1.83	4.99 $P < 0.05$	1.05
Serotonin	7.60 $P < 0.025$	74.55 $P < 0.001$	0.094
Melatonin	10.25 $P < 0.01$	50.02 $P < 0.001$	3.6

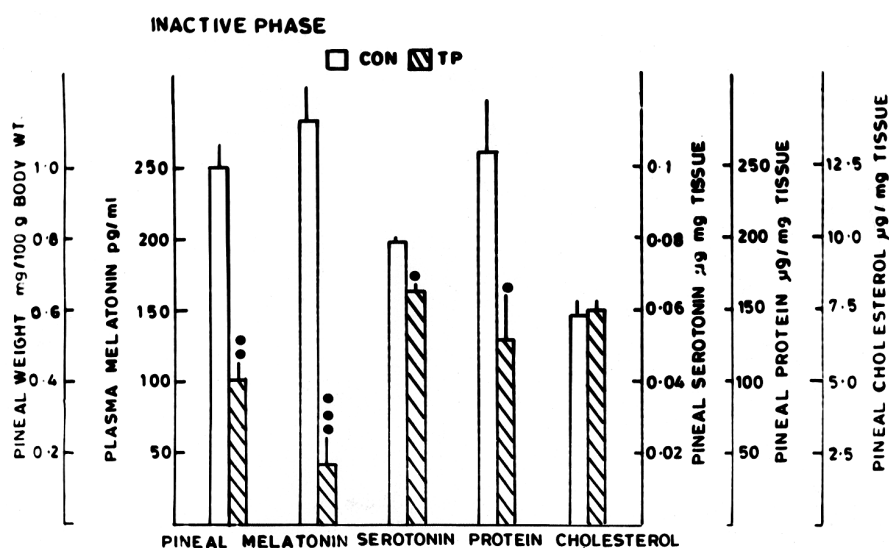


Fig. 3. Effect of TP (20 µg /animal /day) on the pineal gland weight, pineal protein, cholesterol, serotonin content and plasma melatonin level during reproductive inactive phase in *F. pennanti*. Significance of difference: • $P < 0.05$; •• $P < 0.01$; ••• $P < 0.001$

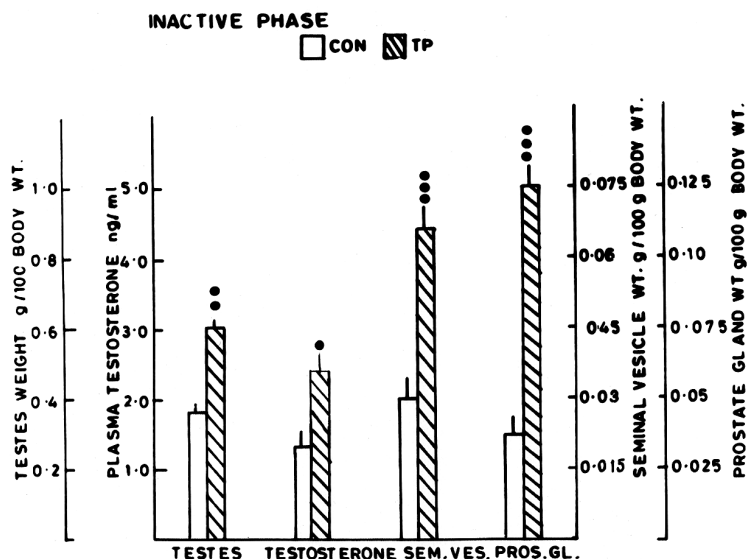


Fig. 4. Effect of TP (20 µg/animal/day) on the testes, seminal vesicle, prostate gland weight and plasma testosterone level during reproductive inactive phase in *F. pennanti*. Significance of difference: • $P < 0.05$; •• $P < 0.01$; ••• $P < 0.001$

DISCUSSION

We found that exogenous injection of TP during reproductive active phase increased the plasma MEL level, without affecting the PG biochemical constituents (protein, cholesterol and serotonin content; Fig. 1) and T level in plasma. During reproductive active phase when endogenous T is already high, the exogenous TP could not elevate it more. From the results it appears that exogenous TP might have initiated the negative feed back at the leydig cell. Further, TP is known to effect directly the MEL synthesis by increasing the activity of neuroendocrine PG (Haldar and Gupta, 1990; Vacas and Cardinali, 1979). In mammals, T influences the incorporation of labeled leucine into PG proteins (Cardinali *et al.* 1976, Nagle *et al.*, 1975), decrease the HIOMT activity and MAO activity (Haldar and Gupta, 1990; Vacas and Cardinali, 1979). However, in the present study such an effect specially on protein content was not observed during the reproductive active phase. Therefore, we extended our study to note the effects during the reproductive inactive phase. This is because in *F. pennanti* it is known that administration of TP can maintain the testicular activity in intact as well as in pinealectomized animals and presents a phase dependent effect on testes and accessory sex organ's weight (Haldar and Vidhu, 1997). Further, it has been reported that the basal level of T and MEL remained high and low respectively, during reproductive active phase (Haldar, 1997).

During inactive phase of reproductive cycle, TP showed an inhibitory effect on PG weight, protein, serotonin content of PG and plasma MEL level (Fig. 3), which was quite similar in comparison to an inactive state of PG biochemical constituents recorded earlier during study of annual cycle by us (Sarkar, 1996). This decrease of PG activity following TP administration noted in *F. pennanti* may be due to the decrease

in enzyme activity (HIOMT) as it has been reported for castrated rats that TP apparently exerts a dose dependent effect on HIOMT activity of PG (Houssay and Barcelo, 1972). They have suggested that the administration of TP (30-100 µg/day) for 21 days depressed enzyme activity but the effect can be restored to the HIOMT level of intact control with the dose 0.1-1 mg/day for 3 days, whereas higher doses (5 mg/day) depressed enzyme activity. It helps to extend our discussion with support that during reproductive active phase higher plasma TP level might have switched on HIOMT activity of PG hence, an increase in the MEL level was noted.

Therefore, it may be suggested that inactive PG responded less to TP in term of biochemical constituents. TP can interfere the functioning and activity of the PG and plasma MEL level directly via 5 α -DHT receptor (Haldar and Gupta, 1990) or by influencing the hypothalamo-hypophyseal-gonadal axis (via LH-RH; Reiter *et al.*, 1968).

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