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DEPENDENCY ON TESTOSTERONE OF PHOTOPERIODICALLY-INDUCED VERNAL FAT DEPOSITION IN FEMALE WHITE-CROWNED SPARROWS[1]

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Abstract. Vernal premigratory fat deposition in White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) is induced by increasing day length. This induction can be prevented by castration before photostimulation. We investigated whether steroid hormone replacement during short days abolishes the consequences of castration. The inhibition by ovariectomy of long-day-induced fat deposition was reversed by testosterone, but not by 5α-dihydrotestosterone or estradiol-17β. The administration of an aromatase inhibitor (ATD) simultaneously with testosterone blocked its effect. It is suggested that low levels of testosterone present in both sexes during the wintering phase are required for vernal premigratory fattening. It appears that testosterone initiates a process that leads to fat deposition after photostimulation, and that this process most likely occurs in the CNS.

Key words: Vernal migratory fattening; photoperiodism; testosterone; ovariectomy; White-crowned Sparrow; Zonotrichia leucophrys gambelii.

INTRODUCTION

Induction of vernal premigratory fat deposition by long days is prevented by castration before photostimulation in both sexes: male *Zonotrichia albicollis* (Weise 1967); male *Z. leucophrys gambelii* (Mattocks 1976); female *Z. l. gambelii* (Schwabl et al. 1988). Lesions of the posterior median eminence of the hypothalamus inhibit photoperiodically induced testicular growth and premigratory fattening in males (Stetson 1971, Yokoyama 1976). These effects of castration and lesions of the posterior median eminence on fattening are reversed by exogenous testosterone *during* photostimulation (Mattocks 1976, Yokoyama 1976), suggesting that this hormone is essential for migratory fattening of males. Field studies showed that androgens, but not estrogens are present in detectable amounts in plasma of both sexes during the wintering phase (Wingfield and Farner 1978). Thus androgens may be involved in the mechanisms that induce vernal migratory fattening in both sexes. We investigated (1) if androgens or estrogens can reverse the inhibitory effect of ovariectomy on fattening and (2) if treatment with hormone during short days *prior to* photostimulation is sufficient to facilitate long-day-induced fattening in ovariectomized females.

MATERIALS AND METHODS

Female White-crowned Sparrows (*Z. l. gambelii*) were trapped from wintering flocks at the Sunnyside Wildlife Refuge in Yakima County in central Washington (ca. 46°50′N, 120°W). They were initially housed in large outdoor aviaries on the roof of Kincaid Hall, University of Washington, Seattle (ca. 47°30′N, 122°20′W). In the first experiment, females were transferred to environmental chambers between December 14 and 23. Experimental subjects were ovariectomized under anesthesia (Equithesin) by cauterization followed by removal of all ovarian tissue. Control females (n = 9) were sham operated. Subsequently all birds were held on short days (8L: 16D) in individual cages (41 × 26 × 27 cm) with food and water available ad libitum. After about 4 weeks, castrates were assigned randomly to one of three treatment groups and implanted subcutaneously with one of the following hormones in silastic tubing (Dow Corning o.d. = 1.96 mm;

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i.d. = 1.47 mm): *T-group* (n = 10) received an implant (2 mm long) filled with crystalline testosterone (T); *T-ATD-group* (n = 9) received an implant of testosterone of the same size plus two implants (15 mm) of the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dione); *5α-DHT-group* (n = 10) received an implant (10 mm) of crystalline 5α-dihydrotestosterone. After 15 days all implants were removed; 4 days later the photoregimen was changed from 8L:16D to 20L:4D to induce long day functions. Birds were weighed at intervals of 3 to 5 days; change from initial body mass was used as an index for fat deposition, since the extent of fat deposition is well correlated with body mass (King and Farner 1959).

In the second experiment females were transferred to experimental rooms (8L:16D) and ovariectomized between November 28 and December 20. On February 11, seven ovariectomized females received testosterone implants (1 mm), six ovariectomized females received a similar implant of testosterone plus two implants of ATD (15 mm), and eight ovariectomized females received implants (7 mm) filled with crystalline estradiol-17β. Six intact controls received no treatment. Testosterone implants were removed after 5 days, those of estradiol after 10 days, and those of ATD after 17 days. On March 1 all birds were photostimulated with 20L:4D and body mass was measured about every 4 days.

Effectiveness of ovariectomy was assessed by visual inspection of the gonad after 45 days of photostimulation. Only data from females without regeneration of ovarian tissue were included in the results.

Blood samples were taken in both experiments and plasma levels of steroid hormones produced by the implants were measured by the method of Wingfield and Farner (1975).

Variation among treatment groups was compared by ANOVA for repeated measures followed by Student-Newman-Keuls test.

RESULTS

Plasma levels of the steroid hormones produced by the different implants during short days are summarized in Table 1. Small implants of testosterone of 1 to 2 mm in length produced rather high levels of androgens. Very high levels of LH after photostimulation (not shown) indicated that ovariectomy was successful.

In both experiments body mass varied significantly with time of photostimulation ($P < 0.01$) and among treatment groups ($P < 0.01$). In experiment 1 (Fig. 1) increases in body mass similar to those of controls were only produced by treatment with testosterone ($P > 0.05$), but not by treatment with 5α-DHT or testosterone plus ATD ($P < 0.05$). After 22 days of photostimulation body mass decreased in both intact controls and testosterone treated ovariectomized females.

In the second experiment (Fig. 2) results from treatment with testosterone and testosterone plus ATD were similar to those of the first experiment, i.e., testosterone reversed the inhibitory effect of ovariectomy on the photoperiodically induced increase in body mass compared to intact controls ($P > 0.05$), while this was absent by simultaneous administration of ATD ($P < 0.05$). Estradiol-treated ovariectomized females had significantly lower body mass than intact controls ($P < 0.05$).

TABLE 1. Plasma levels ($\bar{x} \pm$ SE) of hormones following implantation on short days. Numbers in parentheses refer to experiment number. nm = not measured; nd = nondetectable (<50 pg/ml).

Implant		Plasma levels (ng/ml)		
		T	5α-DHT	E_2
T	(1)	4.8 ± 1.1	0.9 ± 0.3	nm
	(2)	3.8 ±0.7	0.4 ± 0.5	nm
T-ATD	(1)	4.1 ± 0.6	0.9 ± 0.1	nm
	(2)	3.5 ± 0.5	1.3 ± 0.1	nm
5α-DHT	(1)	0.5 ± 0.1	5.0 ± 0.6	nm
E_2	(2)	nd	nd	0.9 ± 0.1

DISCUSSION

In both experiments testosterone replacement during short days reversed the inhibitory effect of ovariectomy on long-day-induced fat deposition. Neither 5α-DHT nor estradiol-17β had this effect. The action of testosterone was blocked by the strong aromatase inhibitor, ATD (Alexandre and Balthazart 1986). Small implants of testosterone produced rather high circulating levels of testosterone in our females that were treated under short days, while implants of comparable length produced circulating levels of only 0.5 to 1.0 ng/ml in photostimulated males (Matt 1982). This may be due to low clearance rates in females compared to males or to low clearance rates under short days as compared to long days. The inhibition by ATD of testosterone-facilitat-

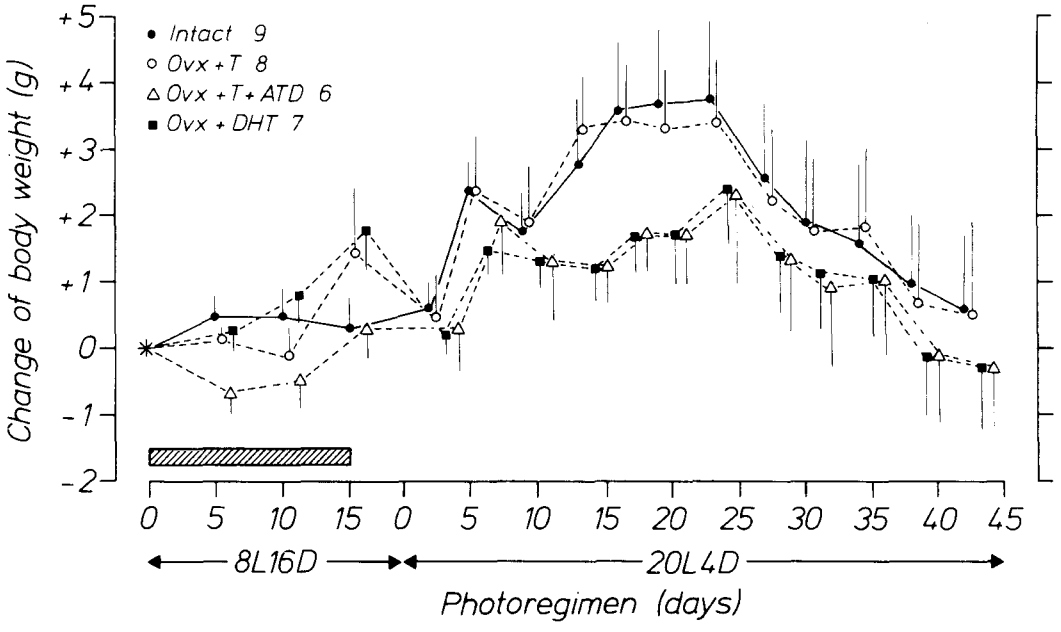

FIGURE 1. Variation of body mass ($\bar{x} \pm$ SE) of intact and hormone-treated ovariectomized females. Duration of hormone treatment is indicated by the cross-hatched bar. Sample sizes shown at treatment legend.

ed fat deposition suggests that testosterone may act via aromatization to estrogens. This suggestion is valid only if one assumes that the administration of estradiol somehow failed to reach the target site in the cell that is reached by estradiol formed by aromatization of testosterone in the cell. Alternatively testosterone may act on fat deposition through its conversion by 5β-reductase or 5α-reductase. ATD not only blocks aromatase, but also substantially inhibits 5β-reductase and, to a lesser extent, 5α-reductase (Alexandre and Balthazart 1986), and 5β-reductase activity is high in both sexes of birds during the nonbreeding season (Sharp et al. 1986). Action of testosterone on the fattening mechanism via 5α-reduction is unlikely since exogenous 5α-DHT failed to reverse the castration effect.

Prior to vernal migration, plasma levels of testosterone are similar in male and female *Z. l. gambelii*. Therefore Wingfield and Farner (1980) suggested that testosterone is involved in induction of migratory fattening in both sexes. Our results confirm that testosterone is involved in photoperiodic induction of vernal migratory fattening in females, while previous experiments confirmed this for males (Mattocks 1976; Yokoyama 1976, 1977). Thus the processes involved appear to be comparable for both sexes while suggesting a function for the secretion of androgens in nonreproductive wintering females.

We do not know whether the site of action of testosterone is in the CNS or in peripheral organs. Yokoyama's (1976, 1977) experiments with males strongly suggest a central action on the hypothalamus or pars distalis of the pituitary gland, and not on a peripheral organ such as the liver. However, the speculation that testosterone acts by enhancement of photoperiodically induced release of prolactin causing fattening is not supported, since ovariectomy does not influence long-day-induced prolactin secretion at the time of fat deposition (Schwabl et al. 1988).

From previous studies, it was concluded that only castration before but not after photostimulation prevents fat deposition (Morton and Mewaldt 1962, Weise 1967, Mattocks 1976). This implies two alternative assumptions. First, the slightly longer days during the end of January, when ovariectomy no longer is effective (Schwabl et al. 1988), stimulate testosterone production which then renders the fattening system responsive for subsequent induction by longer days. Or second, that both long days and the presence of

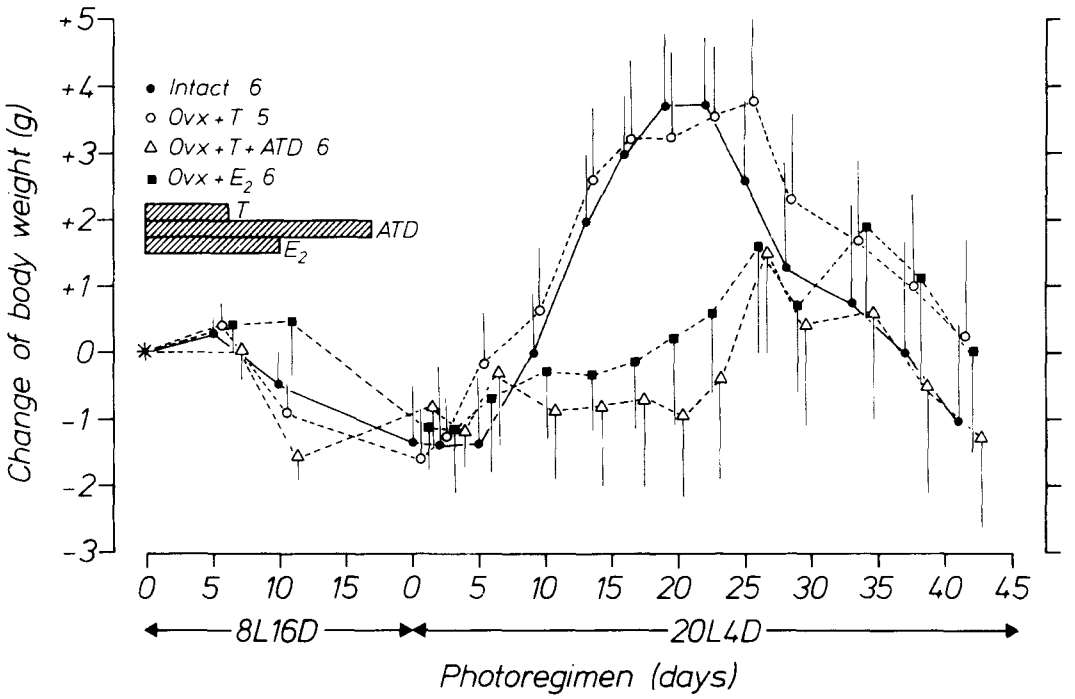

FIGURE 2. Variation of body mass ($\bar{x} \pm$ SE) of intact and hormone-treated ovariectomized females. Duration of hormone treatment is indicated by the cross-hatched bar. Sample sizes shown at treatment legend.

testosterone are required simultaneously. We now show that testosterone treatment during short days, prior to photoinduction, is sufficient to reverse the castration effect, supporting the former explanation of sequential effects. It appears that a priming mechanism by testosterone of the CNS initiates a process that leads to fat deposition during photostimulation. While many physiological and behavioral effects of steroids depend on the continued presence of hormone, in this case transient presence is sufficient to induce lasting alterations on the ability of the system to respond to subsequent photostimulation.

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