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Hormonally Mediated Lordosis in Female Rats: Actions of Flutamide and an Aromatization Inhibitor

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GLADUE, B. A., G. P. DOHANICH AND L. G. CLEMENS. *Hormonally mediated lordosis in female rats: Actions of flutamide and an aromatization inhibitor.* PHARMAC. BIOCHEM. BEHAV. 9(6) 827–832, 1978.—Agents which interfere with androgen metabolism or receptor binding diminished androgen-induced, but not estrogen-induced lordotic behavior in female rats. The non-steroidal antiandrogen, flutamide, reduced the amount of lordosis displayed by females receiving 250 μg testosterone propionate (TP) plus progesterone (P). Flutamide (10 mg) did not affect the lordotic behavior of animals receiving estradiol benzoate (0.5 μg or 0.25 μg EB) plus P. Similarly, the aromatization inhibitor 1,4,6-androstatriene-3,17-dione (ATD; 10 mg) blocked TP plus P induced lordosis but failed to alter EB (10 μg) plus P induced lordosis. These results suggest that androgen-induced lordosis occurs after the testosterone molecule becomes bound to a receptor and is enzymatically converted to estrogenic metabolites.

Lordosis Flutamide ATD Aromatization

THE ABILITY of ovarian steroids to stimulate sexual receptivity in the female rat is well documented [3, 6, 11, 34]. Treatment of ovariectomized rats with estrogen will restore the ability to display the sexually receptive behavior pattern, lordosis. The addition of progesterone augments the action of estrogen, producing a highly receptive female [3,29]. Some androgens are also capable of inducing the feminine pattern of sexual behavior in female rats [1, 24, 31, 32], specifically those androgens capable of conversion to estrogens, the so-called aromatizable androgens [2, 16, 30]. Administering the anti-estrogen CI-628 to testosterone-treated female rats interferes with testosterone induced lordosis [31]. The anti-estrogen CI-628, which acts by interfering with the ability of estrogen to bind to its particular cellular receptor, prevents estrogen from entering the nucleus and acting on the nuclear material to affect changes in behavior [14]. Such findings support the concept that androgen induced lordosis results from conversion of androgen to an active estrogenic metabolite.

The enzymatic conversion of testosterone to estradiol can be competitively inhibited by the steroid compound 1,4,6-androstatriene-3,17-dione (ATD). Such inhibition has been shown in placental [25] and neural tissue preparations [14]. Further, ATD has been shown to affect behavioral consequences of testosterone conversion to estradiol in the adult male rat [4,19] and in the neonate ([18,28], Clemens and Gladue, in press). The effects of such an aromatization inhibitor have yet to be assessed regarding testosterone-induced lordosis behavior in the female rat.

The purpose of this study is to examine the effects of the aromatization inhibitor ATD on androgen and estrogen-induced lordosis in the female rat, and to compare the effectiveness of ATD with the non-steroidal anti-androgen flutamide (SCH) on hormone-induced lordosis. While flutamide decreased testosterone mediated development in androgen-dependent target tissues and decreased ejaculation in the adult male rat [9, 10, 27], actions of flutamide on feminine components of sexual behavior have yet to be reported. Flutamide is reported to lack the progestagenic, estrogenic and anti-estrogenic [20] side effects known for the steroidal antiandrogen, cyproterone acetate. Cyproterone acetate has potent progestagenic [21] as well as behaviorally effective antiestrogenic properties [16]. Since flutamide blocked androgen-induced masculinization and defeminization in the rat and since these developmental processes [5,8] are sensitive to estrogens and aromatizable androgens (Clemens and Gladue, in press, [18, 26, 28]) it was decided to examine some of the hormonal actions of flutamide regarding estrogen and testosterone-induced female sexual behavior.

GENERAL METHOD

Animals and Surgery

Long-Evans strain female rats used in these experiments were obtained as adults from a commercial breeder (Charles River, Wilmington, MA). Animals were housed 4–6 rats per metal cage (18×36×50 cm) under standard laboratory conditions with food and water available ad lib. Animals were maintained on a reversed light-dark cycle of 14 hr light with lights off at 1100 hr EST. Two-weeks after arrival in the laboratory animals were bilaterally ovariectomized under ether anaesthesia at approximately 90 days of age.

Behavioral Procedure

Behavior testing for the display of lordosis began 7–10 days after ovariectomy. Initially, all animals were screened for their ability to display lordosis in response to estradiol benzoate (EB) and progesterone (P) administration. Females were given injections (IM) of 1 μg EB 48 and 24 hr prior to testing with the addition of P (500 μg) 4 hr before the start of behavioral testing. Since primary interest focused on the ability of certain treatments to interfere with hormone-mediated lordosis behavior, only those animals showing a substantial response (LQ's greater than 70) on two out of three initial screening tests, were selected for use in this study. Over 95% of the animals obtained from the supplier met this criteron. Following pre-selection tests, animals were allowed a one-week period without treatment or testing.

Testing consisted of the female being placed in a Plexiglas testing arena (45×50×58 cm) with a sexually vigorous stud male. Hormone treated females were tested for the display of lordosis in response to mounting by the male. Lordosis is characterized by a deep ventral arching of the back with dorsal elevation of the head and perineal region and lateral tail deflection. This response pattern is a main feature of sexually receptive behavior in the rat. Lordosis behavior was quantified for each animal using the lordosis quotient: LQ=frequency of lordosis in response to 10 mounts/10 mounts × 100%. All behavioral results reported here are a result of this basic testing procedure.

Hormones and Anti-Hormones

Estradiol Benzoate (EB) and progesterone (P) were dissolved in sesame oil and administered in a 0.1 cc volume. The antiandrogen flutamide (SCH) (Schering Corp., NJ) and the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD, Steraloids Inc., Rawling, NY) were dissolved in propylene glycol (PROP). Dosages and concentrations of all compounds are given in their respective experimental method sections. All injections were administered IM in the thigh region.

EXPERIMENT 1

Since the objective of these studies was to determine whether the actions of TP upon lordosis could be eliminated by altering androgen receptor availability and testosterone metabolism, it was first necessary to determine whether flutamide had a direct influence upon estrogen-induced lordosis. Cyproterone acetate, a steroidal antiandrogen, has been shown to reduce sexual receptivity in ovariectomized females treated with estrogen and progesterone [16]. Apparently cyproterone acetate, equally as potent as flutamide in interfering with androgen expression on androgen-dependent target tissues [20,21] also has powerful anti-estrogenic activity. Possible antagonism or enhancement by flutamide of estrogen-progesterone induced lordosis was therefore evaluated.

METHOD

Animals and Treatment

Sixteen ovariectomized female rats known to be lordotically responsive to EB and P were assigned to a Latin Square Design for repeated measures to each of the following treatment conditions: (1) EB + PROP; (2) EB + SCH; (3) OIL + PROP; (4) OIL + SCH. The animals were tested once per week for four weeks for the display of lordosis. Each week animals received a treatment condition different than that of the previous week according to their placement in the Latin Square. Thus each animal was tested under each treatment condition over the four week period. At the end of the four week period, another four week period of testing began using a different dosage of EB. The first four week period (Series 1) involved the administration of 0.5 μg EB or OIL given 48 and 24 hr before testing with the addition of P (500 μg) 4 hr before the start of testing. The second four week period (Series 2) separated from Series 1 by two weeks, involved the administration of a lower dose of EB (0.25 μg) 48 and 24 hr before testing with the addition of P (500 μg) 4 hr before the onset of testing.

Flutamide (5 mg) or PROP was given concurrently with EB, 48 and 24 hr before testing. The same dose of SCH was used in both series.

Statistical Analyses

Data were analyzed for each series using the analysis of variance for Latin Squares with repeated measures according to Kirk [12]. Mean comparisons among treatments were made using a Student-Newman-Keuls (SNK) test [22,33].

RESULTS

Flutamide did not interfere with estrogen-progesterone induced sexual receptivity at either dose of EB tested (Table 1). At the 0.5 μg EB dose level an overall significant effect of treatment condition, $F(3,48)=605.16$, $p<0.0001$, but not among animals or over repeated testing (time), $F(3,48)=2.437$, $p>0.05$ and $F(3,48)=0.840$, $p>0.5$, respectively, was observed. Treatment effects were wholly accounted for by differences between EB-treated and OIL-treated animals ($p>0.05$, SNK). No significant differences between EB + PROP and EB + SCH treatment conditions were found ($p>0.1$, SNK). Table 1 reveals that at either dose of EB, mean lordosis quotients for EB + PROP and EB + SCH conditions are virtually identical.

TABLE 1

MEAN LORDOSIS QUOTIENTS IN FEMALES PRIMED WITH EITHER ESTRADIOL BENZOATE (EB) OR OIL AND ADMINISTERED EITHER FLUTAMIDE (SCH) OR THE CONTROL VEHICLE, PROPYLENE GLYCOL (PROP)

Treatment*	0.5 μg EB	0.25 μg EB
EB + PROP	90.00 ± 2.42†	62.50 ± 6.61
EB + SCH	88.12 ± 3.32	76.88 ± 7.34
OIL + PROP	3.13 ± 1.20	3.40 ± 1.19
OIL + SCH	3.13 ± 1.19	3.00 ± 1.00

*N=16, all animals received all treatments in a Latin-Square repeated measures design.

†Mean ± SEM. SCH (5 mg) or PROP given concurrently with EB or OIL. All treatments administered 48 and 24 hr before test. Progesterone (500 μg) administered 4 hr before test.

At the 0.25 μg EB dose similar results were obtained with an overall treatment effect, $F(3,48)=61.654$, $p<0.0001$ observed. No significant differences between EB + PROP and EB + SCH or between OIL + PROP and OIL + SCH were observed. Treatment effects were wholly accounted for by EB vs OIL group comparisons ($p<0.01$, SNK).

The possibility that flutamide might have subtle estrogenic actions was not supported since lordosis quotients of females when treated with OIL + SCH did not significantly differ from LQ's obtained from treatment with OIL + PROP ($p>0.05$, SNK, Table 1), both levels of lordosis behavior being quite low.

EXPERIMENT 2

The results of Experiment 1 indicated that flutamide failed to exert an anti-estrogenic effect on estrogen-progesterone induced lordosis. These results are in agreement with other studies showing that the primary mode of action of flutamide is antiandrogenic. Whalen *et al.* [31] have shown TP to induce lordosis in female rats and that such lordosis may be prevented by concurrent anti-hormone treatment with the anti-estrogen CI-628, which acts upon the estrogen receptor. Since flutamide did not appear to have any action on estrogen receptors which might influence behavior (Experiment 1), any alteration by flutamide of lordosis in TP-treated animals would appear to be related to androgen-receptor activity.

The action of the aromatase inhibitor ATD upon TP-induced lordosis was compared with that of flutamide. It was hypothesized that blocking either testosterone uptake to the androgen receptor via SCH or interfering with conversion of T or E via ATD would diminish the probability of lordosis in ovariectomized female rats.

METHOD

Twenty-eight ovariectomized female rats known to be lordotically responsive to ovarian hormones were assigned (according to a Latin Square Design for repeated measures as indicated in Experiment 1) to the following four treatment conditions: (1) TP (250 μg) + PROP (propylene glycol, vehicle for ATD); (2) TP + SCH (10 mg flutamide); (3) TP + ATD (10 mg 1,4,6-androstatriene-3,17-dione); and (4) OIL (vehicle for TP) + ATD (10 mg). In this experiment, only one dose of activating hormone (250 μg TP) was used, hence there is only one 4 week series of tests. TP or OIL injections were given 48 hr prior to tests for lordosis. PROP, SCH and ATD were administered IM (in 0.2 cc volume) 48 and 24 hr before testing. All animals received P (500 μg) 4 hr before the start of behavior tests.

Under this experimental design, all animals were exposed to each treatment condition once, on a different test week; the experiment lasting four weeks.

Data, in the form of lordosis quotients, were analyzed with an analysis of variance for Latin Square Design with repeated measures according to Kirk [12]. Mean comparisons for treatment conditions were made using the Student-Newman-Keuls test indicated earlier [22,33].

RESULTS

Treatment with flutamide or ATD significantly reduced LQ in TP-treated animals ($p<0.01$, SNK). Treatment with ATD resulted in significantly lower lordosis quotients than SCH treatment ($p<0.05$, SNK). Animals exposed to OIL + ATD did not significantly differ from treatment with TP + ATD ($p>0.05$, SNK) (Fig. 1). In terms of relative lordosis responding the overall treatment condition effects can be summarized as follows: TP + PROP > TP + SCH > TP + ATD=OIL+ATD. The statistic derived from the overall analysis of variance for treatments was $F(3,96)=60.026$, $p<0.0001$.

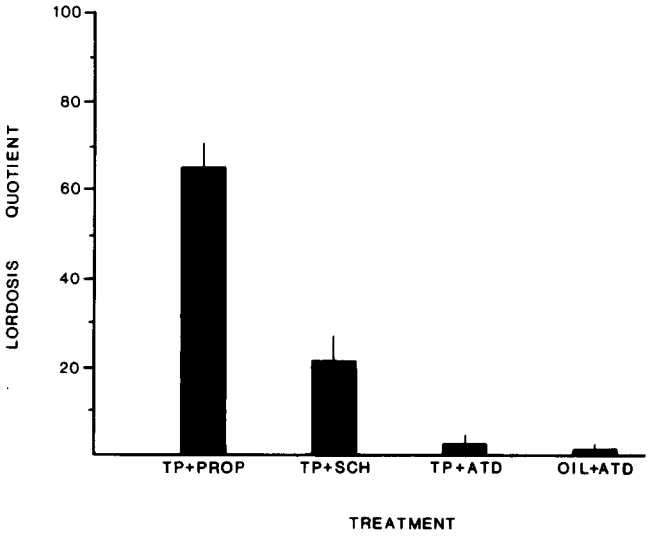

FIG. 1. Mean lordosis quotient in female rats treated with TP (250 μg) or OIL in combination with progesterone (500 μg) and given either flutamide (SCH, 10 mg), an aromatization inhibitor (ATD, 10 mg) or control vehicle (PROP). Vertical bars indicate S.E.M., n=28 for all treatment conditions. See text for details.

Further analysis revealed no overall effect on time (repeated testing) and an absence of significant residual effects of treatments, $F(3,96)=0.672$, $p>0.6$ and $F(6,96)=0.886$, $p>0.6$, respectively.

EXPERIMENT 3

While it was demonstrated in Experiment 1 that SCH had no overt facilitative or inhibitory action on estrogen-progesterone induced lordosis it was not readily apparent that this was also the case for ATD. Since ATD blocks the action of T on the induction of lordosis and, according to results obtained in Experiment 2, ATD appears to have no facilitatory effect on inducing lordosis by itself, it becomes necessary to determine whether ATD exerted its inhibitory action on T-induced lordosis by acting as an aromatase inhibitor or whether ATD was capable of interfering with the action of estrogen per se.

METHOD

Animals from Experiment 2 were used in this experiment. Two of these animals had died following completion of Experiment 2, but the remaining 26 females were in good health. They were allowed a one week interval between experiments before being assigned to the following treatment

groups: (1) EB (10 μg) + PROP and (2) EB (10 μg) + ATD (10 mg). A single injection of EB was administered 48 hr before testing, with the addition of P (500 μg) to all animals 4 hr before testing. PROP or ATD was given 48 and 24 hr before behavioral testing. Animals given a particular treatment one week were given the opposite treatment the following week. Hence, all animals were exposed to both treatments over the two-week period in a crossover repeated measures design.

Data from this experiment were analyzed using analysis of variance for treatment cross-over with repeated measures according to Gill [7].

RESULTS

Animals exposed to EB + ATD (Mean lordosis quotient = 92.31 ± 4.0 SEM) or EB + PROP (Mean LQ = 95.77 ± 1.68 SEM) did not significantly differ from each other, $F(1,24)=0.835$, $p>0.3$, (Fig. 2).

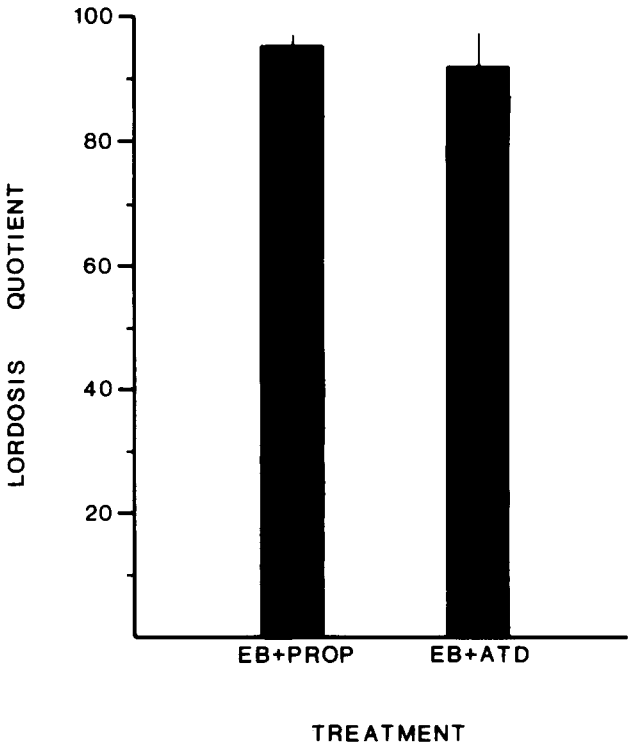

FIG. 2. Mean lordosis quotients in female rats treated with EB (10 μg) and progesterone (500 μg) and given either an aromatization inhibitor (ATD, 10 mg) or control vehicle (PROP). Vertical bars indicate S.E.M., n=26 for both treatment conditions. See text for details.

GENERAL DISCUSSION

Androgens, as well as estrogens, are capable of inducing lordotic behavior in ovariectomized female rats [1, 3, 6, 11, 24, 29, 31, 32, 34]. The present series of experiments sought to further characterize this hormonal mediation of lordosis. Specifically, ATD, a selective inhibitor of the aromatization pathway, completely blocked lordosis in female rats primed with TP and progesterone. Lordotic behavior induced by treatment with EB and progesterone, however, was not affected by ATD. Flutamide, a potent antiandrogen, significantly reduced but did not abolish lordotic behavior displayed by females receiving TP and progesterone while not altering lordosis in females primed with EB and progesterone.

It seems likely that androgen is converted to estrogen metabolites prior to activating a lordotic response since androgens yielding to this aromatic conversion have been shown to be more effective in the induction of lordosis [2, 16, 19, 30] than non-aromatizable androgens. This conclusion is supported by the finding that the antiestrogen CI-628 diminished lordotic behavior in androgen treated females, presumably by blocking estrogen receptors [31]. In the present study, results from Experiment 2 further substantiate a conversion hypothesis in that lordosis was eliminated in TP plus progesterone primed females when ATD, a steroid known to interfere with enzymatic conversion of androgens to estrogens [14,25] was administered. This inhibition was probably not the result of general debilitation or antiestrogenic action since ATD did not produce a similar alteration of lordotic behavior in females receiving EB and progesterone (Experiment 3).

The reduction in lordosis following flutamide (SCH) treatment of TP plus progesterone primed females is less easily interpreted. Testosterone induced lordosis may arise from the activation of testosterone (T) receptors. Such receptor activation by testosterone is capable of being prevented by prior treatment with the anti-androgen flutamide [13, 17, 23]. Consequently, a decrease in TP-induced lordosis may reflect the unavailability or inactivation of T-receptors in these animals. However, the possibility that T-receptors are directly involved in lordosis is unlikely. Sufficient levels of testosterone receptors should have been present in Experiment 2 in ATD treated TP-primed females to induce lordosis since ATD has no known effect upon T uptake [14]. Since ATD treated females had lower lordosis quotients than SCH-treated animals, it appears that additional steps are required to mediate T effects on lordosis beyond association with T-receptors.

It is possible then, that the enzymatic conversion of androgens to estrogens is the key element in testosterone-induced lordosis, and that such conversion may occur only after the testosterone molecule becomes bound to some receptor. Such receptor binding may be prevented by the antiandrogen flutamide. Consequently, by interfering with the hormone-receptor interaction, conversion of testosterone to its estrogen metabolite is prevented. Since the suppression of lordosis by flutamide was not as complete as that observed in ATD treated females, it is likely that this dose of flutamide (10 mg) fails to inactivate all available hormone-receptor interactions through which aromatic conversion might proceed.

Another alternative interpretation of flutamide action on TP-induced lordosis may be that flutamide acts to interfere directly with aromatization and not solely by receptor blockade per se. If flutamide is capable of competitively interfering with hormone uptake at the androgen receptor level it is also possible that flutamide may possess enough androgen-like configurational appearance to interfere with aromatic conversion of T to estradiol at one of the aromatization enzyme sites. Such enzyme receptor site interference would appear to account for the ability of flutamide to block TP-

induced lordosis. In this context, the failure of the given dose (10 mg) of flutamide to abolish lordosis, as did an equal quantity of ATD may reflect a failure of flutamide to effectively inhibit all conversion enzyme active sites as well as ATD.

Finally, flutamide does not appear to alter lordotic behavior by acting on estrogen receptors. Experiment 1 clearly demonstrated that flutamide does not exert an estrogenic or anti-estrogenic influence over estrogen-induced lordosis in females receiving small quantities of EB in combination with progesterone. This is in marked contrast to the actions of the anti-androgen, cyproterone acetate, which is known to possess estrogenic, anti-estrogenic as well as progestagenic properties [16,21]. The interpretations of flutamide actions in adults may thus rule out the likelihood that this anti-androgen directly interferes with estrogenic expression.

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