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Effect of Cyproterone Acetate on Steroid-Induced Sexual Behavior in Adult Ewes[1]

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FABRE-NYS, C. AND J. P. SIGNORET. *Effect of cyproterone acetate on steroid-induced sexual behavior in adult ewes.* PHARMAC. BIOCHEM. BEHAV. 12(3) 359–363, 1980.—The inhibitory effect of cyproterone acetate on sexual behavior has been investigated in adult ovariectomized ewes treated with either testosterone propionate (10 mg/day) or estradiol benzoate (200 μg/day) to induce male activity and female receptivity simultaneously. Intra-muscular injections of 100 mg cyproterone acetate/day resulted in a rapid decrease in some of the male sexual responses after both testosterone and estradiol treatments, whereas the female reactions were eliminated by cyproterone acetate only when they had been induced by testosterone.

Cyproterone acetate Testosterone Estradiol Male sexual behavior Female receptivity Ewe

IT IS well established that cyproterone acetate (6-chloro-17-hydroxy-1α, 2α-methylenepregna-4,6-dione-3,20-dione acetate has strong antiandrogenic effects on male accessory sex glands. It has been shown to antagonize the effects of androgens (mainly dihydrotestosterone and testosterone) by competition for androgen receptors [8,41] and to have very few effects on the metabolism of androgens [8,11].

However, its action on the central nervous system remains controversial. Gonadotrophin release may be decreased [32,40], unchanged [30], or increased [12]. Similarly, male sexual behavior is reported to be inhibited in dogs and men [14, 25, 30, 36] and unchanged in many rodents [7, 24, 39, 41, 43].

Cyproterone acetate may act on the central nervous system through several possible mechanisms. It may act by competitive antagonism with testosterone (or its active metabolite, dihydrotestosterone) for androgenic receptors [3,35]. According to this hypothesis, cyproterone acetate may inhibit sexual behavior in species in which testosterone and dihydrotestosterone act directly to induce male behavior but may be ineffective in species in which the aromatization of androgen to estrogen is necessary for the effects on sexual responses. The results in the rabbit [1] and rat [7,43] agree with this hypothesis but those in the mouse differ [24]. In this species, DHT induces male sexual behavior but cyproterone acetate has no inhibitory effect.

Cyproterone acetate is also a potent progestagen [21,40] and progesterone has been shown to have an inhibiting effect on the hypothalamohypophysial axis [20], on female sexual behavior [29, 38, 44] and even on male sexual behavior [15, 16, 17]. Thus, the effects of cyproterone acetate could be a consequence of its action as a progestagen.

Cyproterone acetate can also act on the uterus as an anti-estrogen [11] and inhibit female sexual behavior. However, Luttge *et al.* [26] could not find competitive inhibition between estradiol and cyproterone acetate with the doses generally used in such experiments.

Furthermore, a possible indirect effect of cyproterone acetate on sexual behavior could result from its action on the genital tract in altering peripheral sensory mechanisms. Sensitivity of these peripheral organs, often androgen-dependent, may play an important role in sexual behavior [2,6]. However, most experiments with cyproterone acetate have not demonstrated any clear effects on sensitive structures like penile spines [7, 10, 27].

The present study was designed to investigate the effects of cyproterone acetate on the sexual behavior of ewes which, when ovariectomized as adults and treated with repeated injections of steroids (estradiol or testosterone) show both constant receptivity and male-like patterns of sexual behavior, which the normal female lacks [18].

In such a model, the most important action of steroids is on the central nervous system as the genital tract of the animal remains female. Thus it has been possible to study the effect of cyproterone acetate without interference from its action on the copulatory organs.

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METHOD

Animals and Treatments

Adult Ile-de-France ewes, ovariectomized as adults, were used in all the experiments. All treatments were given by daily intramuscular injections. Steroid hormones were dissolved in olive oil and cyproterone acetate (generously supplied by Schering Ag., Berlin) was dissolved in olive oil + 10% benzyl alcohol.

Experiment 1

Ten ewes were given daily intramuscular injections of testosterone propionate (Steraloids, Inc., Wilton, NH; 10 mg/day in olive oil) for 32 days to make them reach a high and constant level of male and female sexual activity. They were then divided into two groups of five animals each, the level of male sexual activity (measured by the mean number of nudgings) being similar between the two groups at the time of division. The treatment was maintained in one group for 28 additional days to act as a control. The other group received during this period a supplementary injection of cyproterone acetate (100 mg/day) immediately after the daily injection of testosterone propionate.

Experiment 2

Male and female sexual behavior in this experiment was induced by daily injections of estradiol benzoate (Steraloids, Inc., Wilton, NH: 100 or 300 μg/day for 64 days). The ten females were divided at random into two groups. The control group was treated for 20 additional days with 200 μg/day estradiol benzoate, the other group received the same treatment plus 100 mg/day cyproterone acetate just after the steroid injection.

Measurement of sexual behavior. The level of male sexual activity was measured by presenting the experimental animals to four ovariectomized ewes for 10 min at intervals of 3–4 days, two of the latter being treated with progesterone and estrogen to be in estrus on the test day [34]. These experimental conditions have been shown to be sufficiently standardized to collect meaningful data and not stressful in the sense that male sexual behavior can be shown by the ewes [18]. The frequency of the male sexual patterns of nosing, flehmen, nudging and mounting [4] was recorded.

The female receptivity of the experimental animals was assessed by presentation to a sexually active male, on each test day (as above) and they were considered to be receptive if they accepted mounting once or more.

Statistics. Since there was always a limited number of data (less than 20), drawn from an unknown distribution, non parametric tests were used [37]. The Mann-Whitney test was used to compare numerical data (male sexual behavior) and the Fisher test was used to compare qualitative data (receptivity). All were one-tailed tests.

RESULTS

Experiment 1

Male sexual behavior. Treatment with cyproterone acetate resulted in a decrease in the male sexual activity induced by daily injections of testosterone propionate (Fig. 1). This effect appeared as soon as the first treatment test for mounting and nudging was given and the level of statistical

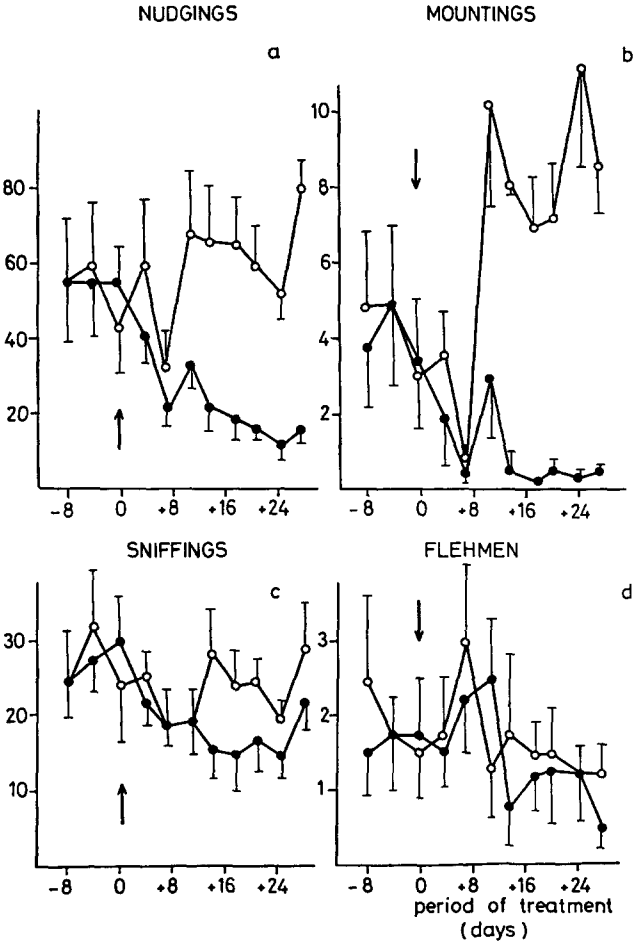

FIG. 1. Effect of cyproterone acetate on the male sexual behavior induced by testosterone propionate in the ovariectomized ewe: a, nudgings; b, mountings; c, sniffings; d, flehmen. Values are means ± SEM of the activity during a 10 min test with 5 ewes each time. ○– – –○: control ewes given testosterone propionate alone (10 mg/day). ●– – –●: ewes given testosterone propionate (10 mg/day) plus cyproterone acetate (100 mg/day). The arrows indicate the onset of cyproterone acetate treatment on Day 0.

significance was reached at test 3 and 4 respectively (Day 11 and 14 of treatment, $p \leq 0.05$ and $p \leq 0.01$).

The anogenital nosing test gave less clear results (Fig. 1c) and no effect could be seen with the flehmen test (Fig. 1d).

Female sexual behavior. Female sexual behavior induced by testosterone propionate was inhibited by treatment with cyproterone acetate (Fig. 3a). This effect was statistically significant from Day 16 of treatment ($p \leq 0.025$).

Experiment 2

Male sexual behavior. Male behavior induced by a long-lasting treatment with estradiol benzoate was dramatically inhibited by cyproterone acetate when tested by nudging and mounting (Fig. 2a, b). The level of statistical significance was reached as soon as the first test (Day 4 of treatment, $p \leq 0.05$ and $p \leq 0.01$ respectively). Later, the difference in the mounting test was no longer significant.

The number of nosing and flehmen reactions was unaffected by the treatment (Fig. 2c, d).

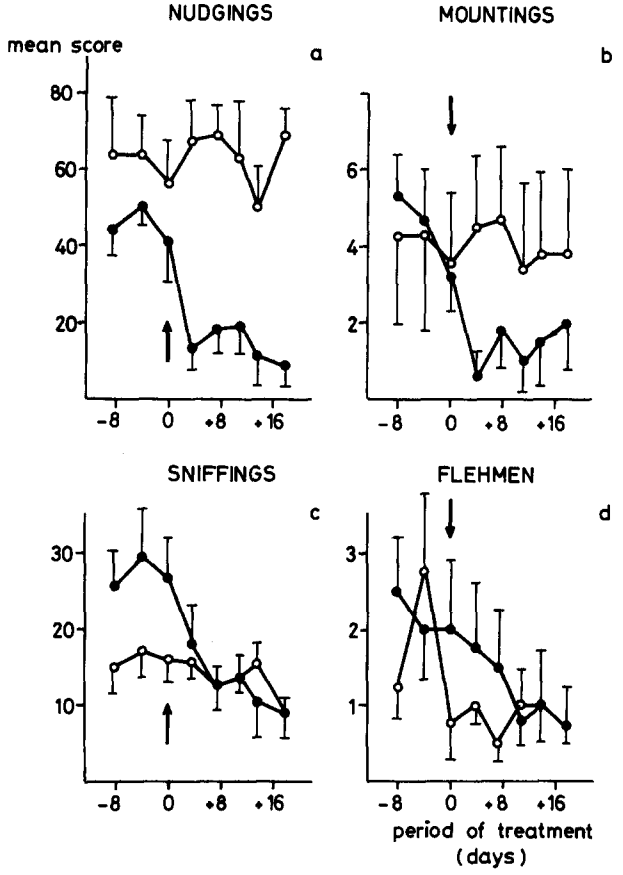

FIG. 2. Effect of cyproterone acetate on the male sexual behavior induced by estradiol benzoate in the ovariectomized ewe: a, nudgings; b, mountings; c, sniffings; d, flehmen. Values are means ± SEM of the activity during a 10 min test with 5 ewes each time. ○-----○: control ewes given estradiol benzoate alone (200 μg/day). ●-----●: ewes given estradiol benzoate (200 μg/day) plus cyproterone acetate (100 mg/day). The arrows indicate the onset of cyproterone acetate treatment on Day 0.

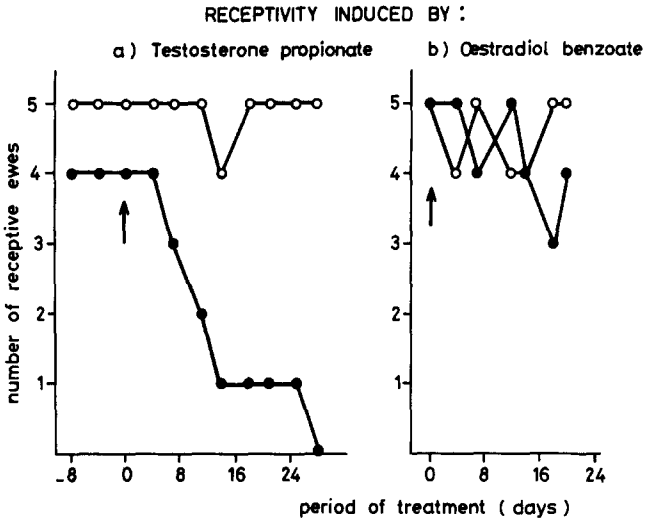

FIG. 3. Effect of cyproterone acetate on the receptivity of ovariectomized ewes treated with steroid hormones: a, experiment 1 ewes given testosterone propionate (10 mg/day); b, experiment 2 ewes given estradiol benzoate (200 μg/day). ○-----○: control ewes given steroid hormone alone. ●-----●: ewes given steroid hormone plus cyproterone acetate (100 mg/day). The arrows indicate the onset of cyproterone acetate treatment.

Female sexual behavior. In this experiment, in contrast with Experiment 1, treatment with cyproterone acetate had no noticeable effect on receptivity (Fig. 3b).

DISCUSSION

Our results show that in ovariectomized ewes treated with testosterone propionate, cyproterone acetate has some obvious inhibitory effects on sexual behavior. However, the plasma concentrations of testosterone (radioimmunoassay using the technique of Garnier, Cotta and Terqui [22] with the help of Garnier) were unaffected by the treatment, the mean value in the treated group being 2.9 ng/ml (SE=0.2, n=34, range of values 1.3–5.5) and in the control group 3.3 ng/ml (SE=0.2, n=40, range of values 1.2–7.7). These results agree with observations in man [25], rabbit [1] and dog [36].

TABLE 1

EFFECT OF CYPROTERONE ACETATE ON THE RECEPTIVITY OF OVARIECTOMIZED EWES TREATED WITH TESTOSTERONE PROPIONATE

Days from the beginning of CA treatment	Number of receptive ewes −8	−4	0	4	7	11	14	18	21	25	28
Control group* (5)‡	5	5	5	5	5	5	4	5	5	5	5
CA treated group† (5)‡	4	4	4	4	3	2	1	1§	1§	1§	0¶

*Ewes given testosterone propionate alone (10 mg/day).
†Ewes given testosterone propionate (10 mg/day) plus cyproterone acetate (100 mg/day).
‡Total number of ewes in the group.
§$p<0.025$.
¶$p<0.005$.

The inhibitory effect could be due to competitive antagonism by cyproterone acetate for those androgenic receptors implicated in sexual behavior as is the case with the androgenic receptors in the accessory sex glands. This supposes however, that in ewes, as in the rabbit, testosterone causes directly (or after transformation to dihydrotestosterone) some sexual behavior without aromatization. The second experiment, in agreement with previous results [19], shows that estradiol induces male sexual activity at a dose of about 50 times less than of testosterone, indicating a direct effect of estradiol.

Three hypotheses could account for the inhibitory effects of cyproterone acetate.

The first is that cyproterone acetate prevents the transformation of testosterone to estradiol or another metabolite which acts on behavior. Several studies have shown that cyproterone acetate has no effect on the metabolism of testosterone [8,31]. However, such studies were dealing only with 5α reduced androgens and not with the low ratio of aromatized metabolites. Such an action, if it exists, does not seem to be sufficient to inhibit sexual behavior in the rat, a species in which inhibitors of aromatization have been shown to decrease sexual activity of testosterone-treated, castrated males [9, 1].

The second hypothesis, which does not exclude the first, is that cyproterone acetate may act not only as an anti-androgen but also as an anti-estrogen. In our experiment, cyproterone acetate had a severe inhibitory effect on the male behavior induced by estradiol benzoate. These results are in agreement with this second hypothesis and with the results of Luttge, *et al.* [27] on the female sexual behavior in the rat. The mechanism of this action is not well known but these authors considered that it does not act by competitive inhibition [26].

It should also be pointed out that cyproterone acetate is, in addition, a potent progestagen. Progesterone is only a weak anti-androgen due to its limited binding with specific androgen receptors [42] and its slight interference with androgen metabolism [33]. On the other hand, its inhibiting action on estrogen-induced female sexual receptivity is well-established. Although the mechanism of this action is not completely understood, it does not seem to act by competitive inhibition with specific estrogen receptors [28]. Thus, the anti-estrogen action of cyproterone acetate could be due to its progestagenic properties.

The various male-like patterns of sexual activity do not undergo the same evolution. Without invoking a true hierarchical organization of these patterns, as described by some authors [5,23], it may be seen that the effects of cyproterone acetate on the nosing phenomenon were less clear and occurred later than the nudging and mounting behaviors. Nosing is also the last pattern to undergo a decrease after castration of resumption of androgen treatment [4]. These results support previous observations [18].

Finally, despite the similarities in male and female behavior patterns induced by estrogens or androgens, the inhibitory action of cyproterone acetate is of variable effectiveness according to the steroid used and the behavior pattern considered. The male sexual patterns are inhibited more than is female receptivity. This could be due to a difference in sensitivity as smaller doses of steroids (androgen or estrogen) are necessary to induce female sexual behavior than those required to induce male sexual behavior. Cyproterone acetate seems to be less active when sexual behavior is induced by an homologous hormone (male behavior caused by testosterone) than when it is induced by an heterologous hormone (male behavior caused by estradiol or female behavior induced by testosterone).

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