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Maintenance of Sexual Behavior in Castrate Male SW Mice Using the Anti-androgen, Cyproterone Acetate[1]

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HALL, N. R. AND W. G. LUTTGE. *Maintenance of sexual behavior in castrate male SW mice using the anti-androgen, cyproterone acetate.* PHARMAC. BIOCHEM. BEHAV. 3(4) 551–555, 1975. – The present study was designed to test the hypothesis that cyproterone acetate (C) might selectively block the actions of dihydrotestosterone (D) and via this action, function as an anti-androgen in male sexual behavior. Sexually experienced male SW mice, a strain previously shown to respond to D following castration, were divided randomly into six groups. Beginning on the day after castration, animals received SC injections for 21 days of either testosterone (T), (D), (C), (T+C), (D+C) or vehicle (V). C was found to significantly reduce seminal vesicle and body weights in all androgen treated groups. There was no evidence to support the contention that C selectively blocks the action of D. To the contrary, in sex tests C maintained palpations, thrust mounts, mounts with intromissions and mounts with ejaculations. Indeed, only animals receiving C alone or in combination with T or D exhibited ejaculations throughout the testing. These results suggest that in the SW mouse, C can work like an androgen in the maintenance of male sexual behavior.

Cyproterone acetate Dihydrotestosterone Copulation Sexual behavior Anti-androgen

CYPROTERONE ACETATE has been shown to exert marked anti-androgenic effects upon androgen target organs in male rodents [22]. Despite the potency of this synthetic steroid in blocking androgenic effects in the periphery, it has no diminishing effects upon androgen dependent male sexual behavior in rats [3, 9, 31, 32, 38] or in guinea pigs [38]. Although some of the evidence is difficult to interpret, cyproterone acetate has been reported to decrease libido in human males [22].

One possible explanation for this peripheral vs. behavioral dichotomy is that cyproterone acetate fails to cross the blood-brain barrier in rodents and is thus unable to modulate central regions associated with androgen induced sexual behavior [31,35]. This is unlikely, however, since cyproterone acetate has been shown to block the uptake of ^{3}H-testosterone in the hypothalamus, septum and hippocampus as well as in the anterior pituitary [24]. This anti-androgen has further been shown to increase plasma gonadotropin levels presumably via hypothalamic regions involved in LH and FSH release [4, 8, 12]. Another possibility is that cyproterone acetate functions as an anti-androgen in the periphery and in feedback control of gonadotropin release by blocking the action of dihydrotestosterone. This 5α reduced androgen has been shown to be more potent than testosterone in the negative feedback inhibition of gonadotropin secretion [5, 18, 21, 28, 37] as well as in the stimulation of the prostate, penis and seminal vesicles [2, 16, 17, 18, 25, 26, 33].

Dihydrotestosterone has also been shown to stimulate male sexual behavior in SW mice [16,18], hamsters [30], rabbits [7] and rhesus monkeys [23]. If human primates are similar to rhesus monkeys in that dihydrotestosterone is

[1] Testosterone (4-androsten-17β-ol-3-one) and dihydrotestosterone (5α-androstan-17β-ol-3-one) were purchased from Steraloids, Inc., Pawling, N.Y. The synthetic steroid, cyproterone acetate (6-chloro-17-hydroxy-1α,2α-methylenepregna-4,6-dione-3,20-dione acetate) was generously provided by Schering A. G., West Germany. Additional supplies of cyproterone acetate were supplied by Drs. Richard E. Whalen and Julian M. Davidson. Progesterone (Prolutin) and estradiol benzoate (progynon) were provided by Schering Corporation, Bloomfield, N.J.

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capable of stimulating male sexual behavior, then cyproterone acetate could reduce libido in humans by selectively blocking this androgen metabolite of testosterone. Animals such as rats, which do not respond to cyproterone acetate treatment, also do not display sexual behavior when given exogenous dihydrotestosterone following castration [6, 14, 17, 19, 33, 34]. This raises the possibility that cyproterone acetate selectively blocks the effects of dihydrotestosterone and by doing so blocks copulation in those species that respond to dihydrotestosterone therapy following castration.

To test this possibility, we examined the effects of cyproterone acetate on the maintenance of male sexual behavior in a strain of mice that has previously been shown to respond behaviorally to dihydrotestosterone [16,18].

METHOD

Animals

Male SW mice, purchased from Marland Farms, Wayne, N.J., were housed in 8.5 X 27.5 X 11.5 cm clear plastic isolation cages with food and water available ad lib during baseline tests and throughout the experiment. Lights remained on from 2400 to 1200 hr with injections scheduled between 0800 and 0900 hr and all behavioral tests between 1400 and 1700 hr. Data from two animals that became sick during the course of the experiment were excluded.

Procedure

All experimental animals were preselected on the basis of sexuality. Only those animals demonstrating full mounts with intromissions on a minimum of three successive tests prior to the onset of the experiment were used. Animals were bilaterally orchidectomized using ether anesthesia with daily hormone treatment commencing on the following day. The first behavioral test under exogenous hormone was conducted 3 days after Day 1 of injection onset and subsequent tests at 3 day intervals for the next 18 days. Animals were randomly assigned to treatment groups receiving either testosterone (T), dihydrotestosterone (D), cyproterone acetate (C), T+C, D+C or oil: benzyl benzoate vehicle (V) (50:50, v:v). SC doses of 400 μg/day of T and D were dissolved in 0.05 cc of vehicle while C was delivered in a separate SC injection at a concentration of 3 mg/day in 0.05 cc of vehicle. To prevent saturation induced precipitation all solutions were stored at 38° C.

All behavioral tests were conducted in the animal colony under dim illumination. Males were allowed to explore the 22 cm dia., 14 cm high clear glass circular testing chambers for 20 min prior to the introduction of a stimulus female. Each chamber had a 1 to 2 cm layer of clean SAN-I-CEL bedding material covering the floor. During 20 min tests, the following behaviors were recorded on an Esterline Angus 10 pen event recorder: palpations, thrust mounts, mounts with intromissions and mounts with ejaculations. These behaviors have been described previously [20]. Within hours after the final test, the experimental males were sacrificed and weighed. The seminal vesicles were excised, extraneous adipose tissue removed, expressed of fluid and weighed on a Mettler H-20 analytical balance. Using a replicate design, the experiment was divided into two parts. Eleven weeks after the first half of the animals had been tested, the exact same design was again used in testing the remaining animals. Data from the two replicates were combined for statistical analyses.

Stimulus females were brought into sexual receptivity with a single SC injection of estradiol benzoate (10 μg in oil) 48 hr and a single SC injection of progesterone (500 μg in oil) 6 hr before testing [13]. Receptivity was verified prior to testing by using stud males. Throughout the test, the same stimulus female was used unless the experimental male failed to show an intromission within the first 10 min. When this situation arose, the original female was replaced with a fresh stimulus animal for the second half of the test.

RESULTS

Figure 1 illustrates the percentage of animals in each group displaying mounts with intromissions and mounts with ejaculations. No significant differences were observed between any of the treatment groups on the last test (Fisher Exact Probability Test), however, each of the groups was found to differ from the control group with respect to palpations, thrust mounts, and mounts with intromissions ($p<0.005$: 1 tail). With the exception of a single animal in the T group who ejaculated once during the first test, only animals receiving C alone or in combination with D or T were observed to exhibit ejaculations. Grouping animals into C-vs. non-C groups did not reveal a significant difference on this measure, however.

Based upon a two factor analysis of variance, intromission frequencies were found to vary significantly between the groups $F(5,47) = 6.79$; $p<0.01$. Newman-Keuls *a posteriori* tests of all possible comparisons [36] indicated that the following individual differences were significant: T>C ($p<0.01$) and (T,TC,D, & DC)>V ($p<0.05$). The same analysis revealed an overall difference based upon intromission duration, $F(5,47) = 5.54$; $p<0.01$, with Newman-Keuls tests showing that T>C ($p<0.01$) and (T,TC,D, & DC)>V ($p<0.05$). Although the C group was not found to vary significantly from the vehicle control group on the frequency and duration measures using the Newman-Keuls tests, a paired t test showed that those animals receiving C did exhibit a higher rate of responding when compared to the control animals on Test 7, $t(16) = 2.74$; $p<0.01$.

Body weights (Table 1) were also found to vary significantly using a single factor analysis of variance, $F(5,47) = 7.01$; $p<0.01$. Newman-Keuls tests indicated that the following individual differences were significant: (V & D)>(DC,C & TC) and T>TC ($p<0.05$). Seminal vesicle weights were also found to vary, $F(5,47) = 35.28$; $p<0.01$, with the following significant individual differences: (D & T)>(C & V) ($p<0.01$).

DISCUSSION

The present results indicate that cyproterone acetate is capable of maintaining sexual behavior in castrated, experienced male SW mice. Furthermore, only animals receiving cyproterone acetate either alone or in combination with testosterone or dihydrotestosterone were found to show ejaculatory behavior throughout the testing. This finding is in close agreement with data of Bloch and Davidson [9] who demonstrated that cyproterone acetate treatment retarded the loss of ejaculatory behavior in rats following castration.

In contrast to the apparent facilitory action of cyproterone acetate upon sexual behavior, the synthetic steroid

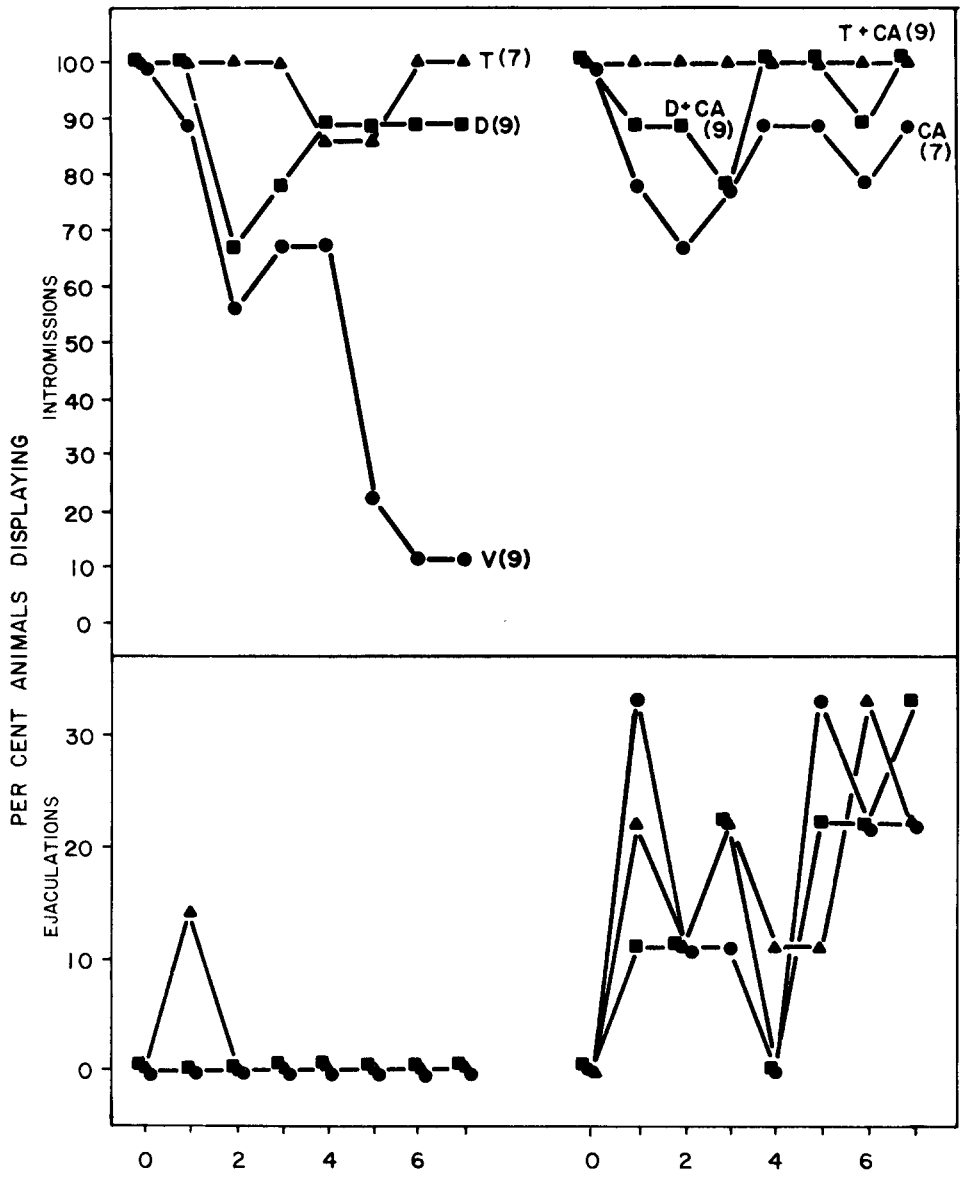

FIG. 1. Percentage of castrated male SW mice in each group displaying mounts with intromissions and mounts with ejaculations. Test 0 represents precastration and pre-hormone treatment data. Test 1 was conducted 3 days following initiation of injections and subsequent tests were run at 3 day intervals for next 18 days. Numbers in parentheses represent the N for each group.

was found to exert an anti-androgenic influence upon the seminal vesicles. Thus, the action of cyproterone acetate upon peripheral target tissues in the SW mouse appears to be the same as that in rats [3, 9, 22, 31, 32, 38] and guinea pigs [38]. Also in agreement with the rat data was the finding that cyproterone acetate significantly reduced body weight [10, 27, 29].

Because limited evidence suggests that humans may respond with decreased libido to cyproterone acetate treatment [22], and because it has been shown that rhesus monkeys are capable of responding behaviorally to dihydrotestosterone therapy following castration [23], it was hypothesized that cyproterone acetate may inhibit human sexuality by acting as an anti-dihydrotestosterone. If this were the case, then, it would be expected that cyproterone acetate might block dihydrotestosterone induced sexual behavior in those animals capable of responding to this 5α reduced androgen. The present results do not support this contention. To the contrary, the data suggests that groups receiving cyproterone acetate concurrently with dihydrotestosterone showed a slightly higher frequency of palpations, thrust mounts and mounts with intromissions than did those animals receiving dihydrotestosterone alone. This stimulatory effect of cyproterone acetate was even more

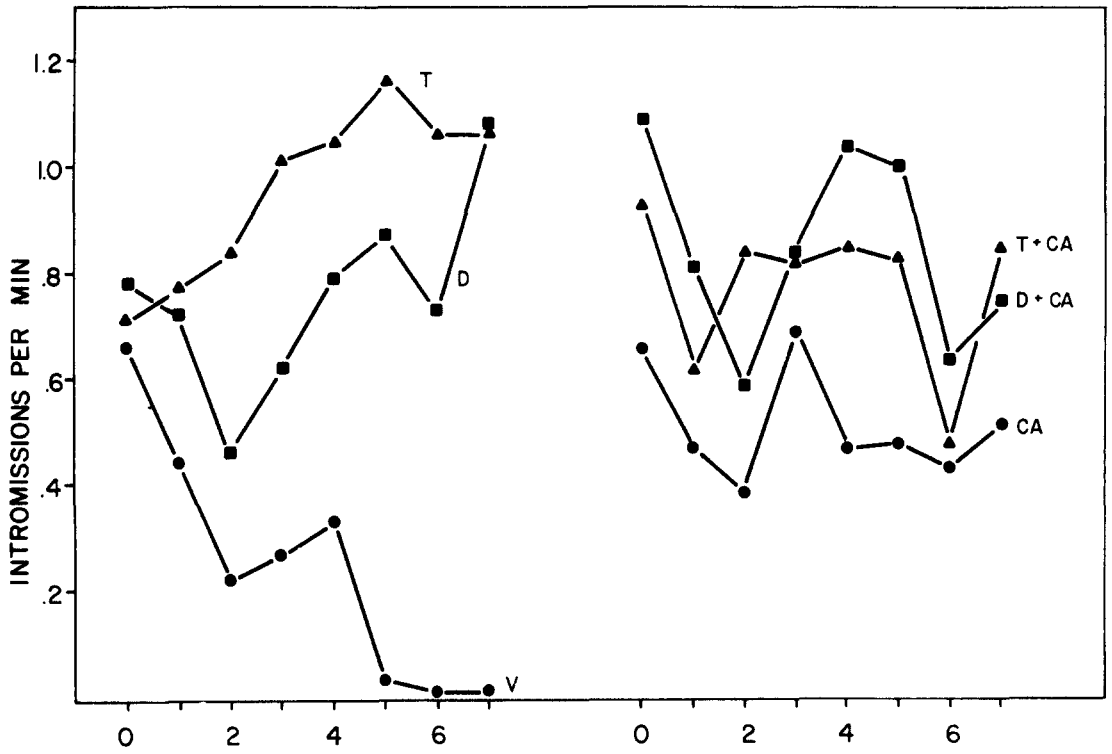

FIG. 2. Mean intromission frequency displayed by control and treatment groups prior to castration and hormone treatment (Test 0) and after castration and hormone treatment (Tests 1–7).

TABLE 1

MEAN (± SEM) BODY WEIGHTS AND SEMINAL VESICLE WEIGHTS FOR EACH GROUP

Group	Body Weight (g)	Seminal Vesicle Weight (mg)
T	40.1 ± 0.7	87.8 ± 4.9
D	41.9 ± 1.1	96.1 ± 7.3
TC	34.4 ± 1.2	56.7 ± 5.1
DC	36.8 ± 1.3	54.3 ± 3.8
C	36.7 ± 1.1	33.0 ± 3.1
V	42.8 ± 1.7	26.5 ± 3.4

apparent with respect to mounts with ejaculations, however, none of these differences attained significance.

Following the completion of this experiment, it was learned that dihydrotestosterone can restore sexual behavior in the prepuberally castrated male guinea pig [1], a species in which it had been previously demonstrated that cyproterone acetate exerts no effect upon sexual behavior [38]. Thus, the evidence does not support the hypothesis that cyproterone acetate specifically antagonizes the action of dihydrotestosterone.

It has been suggested that the hypothalamic receptors involved in androgen dependent behavior differ from the target tissue receptors and those involved in androgen feedback control of gonadotropin secretion [9, 16, 18]. However, it has since been shown that cyproterone acetate can significantly reduce the uptake of ^{3}H-testosterone in a variety of brain sites as well as in the anterior pituitary, seminal vesicles and ventral prostate [24]. It is especially worthy of note that one of the brain sites at which ^{3}H-testosterone uptake was reduced by cyproterone acetate was the preoptic area, a region implicated in the regulation of androgen induced sexual behavior [11,15]. If receptor differences do exist, then it would appear that these differences are manifested at a more complex level than simply at the binding site.

In summary, the present data would suggest that in the SW mouse, cyproterone acetate is capable of stimulating behavioral receptors in the hypothalamus in much the same way that they are stimulated by androgens.

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