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# The Effect of Cyproterone Acetate on Shock Elicited Aggression in Rats

VINETTA PRASAD AND MICHAEL H. SHEARD

*Yale University School of Medicine, and Connecticut Mental Health Center*
*34 Park Street, New Haven, CT 06508*

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PRASAD, V. AND M. H. SHEARD. *The effect of cyproterone acetate on shock elicited aggression in rats*. PHARMAC. BIOCHEM. BEHAV. **15**(5) 691–694, 1981.—The aggressive behavior of male rats treated chronically with cyproterone acetate was measured following electric footshock. Shock elicited fighting behavior and body weight was recorded once every week for 4 weeks. The level of plasma testosterone, brain 5-HT and 5-HIAA and weight of testes were measured 24 hours after the last injection. No significant change was observed in the level of testosterone or brain 5-HT and 5-HIAA. A trend towards an increase in plasma testosterone and shock elicited fighting in rats treated with a higher dose of CA (10 mg/kg) may be indicative of some androgenic property of CA. On the other hand a slower gain in the body weight and a significant reduction in the testes weight of CA treated rats corroborate the well known antiandrogen property of this steroid. The balance of these androgenic and antiandrogenic properties of CA may account for the absence of significant changes in behavioral and biochemical measures.

Aggression Cyproterone acetate 5-HT 5-HIAA Testosterone

CYPROTERONE acetate (CA) has been shown to have antiandrogenic, antigonadotropic, progestogenic [4, 18, 19] and some androgenic [8] properties. It has been used frequently in animal studies investigating the mechanism by which testosterone acts on the central nervous elements involved in the control of androgen dependent behavior. In humans, it has been used for the treatment of hypersexuality [16], sexual precocity [24] and hirsutism [11,12]. Still, the mode of action of CA remains controversial, particularly its role on sex-related aggressive behavior. Studies investigating its effect on intermale aggressive behavior have yielded contradictory results [17]. A reduction in the fighting behavior has been reported in socially isolated male mice treated with CA [14,20], whereas other studies [4, 5, 22] failed to confirm a decrease. However, agreement does exist about a reduction in the weights of peripheral androgen target tissues [1, 4, 14, 15, 29] which is suggestive of an antiandrogenic effect.

In order to investigate this problem further, we conducted a study into the effect of CA on fighting behavior produced by electric foot shock in rats. The shock induced fighting in rats has been shown to be a defensive behavior unrelated to sex-related aggressive behavior [3]. However, a decrease in shock elicited aggression was observed following castration [7], presumably as a result of lowered testosterone, and testosterone treated animals have been shown to have higher brain 5-HT levels [2]. Since 5-HT also plays a role in the modulation of shock-elicited fighting (SEF) [26, 27, 31] measures of plasma testosterone and brain serotonin were obtained.

## METHOD

### *Animals*

The rats used were male albino Sprague-Dawley (Charles River Co.) weighing between 210–240 g. They were housed three to a cage in a room that was maintained on a 12:12 light-dark schedule (light on at 7 a.m. and off at 7 p.m.). Food and water were continuously available. Rats were paired on the basis of similar weights and the fighting pairs of animals were housed separately.

### *Apparatus*

SEF was determined by procedure described earlier [26] in a 39×28×24 cm Plexiglas cage housed in a Lehigh Valley sound attenuating enclosure. Rats received a sequence of 30 shocks at an intensity of 1.5 mA for a duration of 0.5 sec/shock and the intershock interval was 7 sec.

### *Procedure*

Prior to drug treatment all pairs of rats were pretested for initial levels of shock-elicited fighting (SEF). Fights were defined as a directed movement towards the opponent resulting in contact plus one of the following: biting, sparring, upright attack posture, or supine submissive posture adopted by the attacked rat. Based on the total number of fights for each pair during the pretest, the pairs were matched into three groups which had similar mean levels of SEF.

Cyproterone acetate (Schering A. G. West Berlin, Germany) dissolved in peanut oil was injected subcutaneously.

TABLE 1
EFFECT OF CYPROTERONE ACETATE ON THE BODY WEIGHT OF RATS

| Treatments | Body Weight (Mean ± SEM in g) Weeks 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Oil | 222.5 ± 3.5 | 264.2 ± 4.7 | 285.0 ± 9.1 | 319.7 ± 5.3 | 338.3 ± 5.59 |
| CA (2.5 mg/kg) | 225.4 ± 3.9 | 251.4 ± 4.7 | 262.5 ± 4.8† | 278.2 ± 5.7‡ | 291.6 ± 6.19‡ |
| CA (10 mg/kg) | 227.6 ± 2.1 | 241.4 ± 3.5* | 258.7 ± 5.3† | 270.5 ± 6.6‡ | 279.0 ± 6.61‡ |

Independent $t$-tests were carried out in comparison with oil treated controls: *$p<0.001$; †$p<0.025$; ‡$p<0.005$.

TABLE 2
EFFECT OF CYPROTERONE ACETATE ON TESTES WEIGHT, TESTOSTERONE, BRAIN 5HT AND 5HIAA

| Treatments | Testes wt, (g) | Testosterone (ng/ml) | 5HT (ng/g) | 5HIAA (ng/g) |
|---|---|---|---|---|
| Oil | 3.01 ± 0.05 | 6.90 ± 1.95 | 361.86 ± 13.48 | 293.43 ± 10.15 |
| CA (2.5 mg/kg) | 2.76 ± 0.07* | 5.42 ± 1.27 | 318.12 ± 24.80 | 275.12 ± 17.10 |
| CA (10.0 mg/kg) | 2.79 ± 0.05* | 8.82 ± 2.13 | 324.75 ± 16.20 | 271.25 ± 9.06 |

Independent $t$-test in comparison to that of oil controls: *$p<0.01$.

Care was taken to administer the drug at a different site every day between 1500 and 1700 hours. Group 1 rats served as controls and received only oil vehicle. The rats in Group 2 each received a daily dose of 2.5 mg CA per kg body weight and those in Group 3 received 10 mg CA per kg body weight daily for 4 weeks. Rats were tested once every week for SEF between 1400 and 1700 hour. On these days CA or oil vehicle was always administered after testing. In a separate experiment rats were pretested and divided into two groups. One group of rats received saline (1 ml/kg body weight) and the other group received oil vehicle once every day for 4 weeks. These rats were processed exactly as CA treated rats. All rats were killed 24 hours after last injection by decapitation. Trunk blood collected in heparinized tubes was centrifuged and plasma was stored at −20°C until assay. Forebrain was collected for the assay of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). Testes were removed and wet weight was taken.

*Testosterone Assay*

Testosterone concentrations were measured by the radioimmunoassay procedure using T/DHT-Kit (Amersham). Duplicated plasma samples (200 μl) were extracted twice with ether (3 ml) in glass centrifuge tubes (75×12 cm) by shaking (2–4 min) on shaker at full speed. The ether layer was then removed by freezing the aqueous layer in an acetone/dry ice bath and pouring the ether into clean tubes. The combined ether extracts were evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in tris buffer pH 8.0. An aliquot (200 μl) of the final extracts in buffer was used and remainder of the assay was as described in the kit. The recoveries of $^3$H-testosterone ranged from 80–90% in different assays. The water blank values were always less than 5 pg. The inter and intra assay coefficients of variation were 9% and 5% respectively.

*Assay of 5-HT and 5-HIAA*

Brain 5-HT and 5-HIAA was assayed by a spectrofluorometric technique [10].

## RESULTS

*Body Weight*

Rats were weighed regularly and an increase in the body weight of all rats was observed. The body weight of rats receiving saline or oil vehicle was very close throughout the period of four weeks of treatment. A two way analysis of variance revealed significant effects of Dose $F(2,21)=16.94$, $p<0.01$, Days $F(3,63)=193.6$, $p<0.01$, and Dose × Days interaction $F(6,63)=12.41$, $p<0.01$. Further analysis showed that CA treated rats gained weight more slowly than controls, lower doses of CA (2.5 mg/kg) produced a significant reduction ($p<0.005$) in body weight after two weeks of treatment and the higher dose of CA (10 mg/kg) showed a significant reduction ($p<0.001$) even after one week's treatment (Table 1).

*Testes Weight*

Wet weights of the testes were recorded at the end of the experiment. The weight of the testes were very close in saline (3.09±0.07) or oil (3.13±0.08) treated rats. A significant decrease ($p<0.01$) in the weight of testes was observed in CA treated rats (Table 2).

*Shock-Elicited Fighting*

SEF after one week of treatment decreased slightly in oil

TABLE 3
EFFECT OF CYPROTERONE ACETATE ON SHOCK ELICITED FIGHTING

| | % Fighting | | | | |
|---|---|---|---|---|---|
| | | Weeks | | | |
| Treatments | Pretest | 1 | 2 | 3 | 4 |
| Oil | 44 | 27.5 | 40.1 | 45.5 | 47.7 |
| CA (2.5 mg/kg) | 44 | 44.1 | 17.5* | 36.6 | 43.3 |
| CA (10.0 mg/kg) | 44 | 48.3 | 48.3 | 50.0 | 60.0 |

Independent $t$-test in comparison to oil treated control: *$p<0.025$.

treated rats and remained unchanged in CA treated rats (Table 3). On second week the SEF returned back to pretest level in oil injected rats, decreased in CA (2.5 mg/kg) treated rats and remained same in CA (10 mg/kg) treated group. In third and fourth week of treatment, the level of fighting in all the groups remained very close to pretest level. A trend towards an increasing fighting level was observed in rats treated with higher dose of CA (10 mg/kg). A two way analysis of variance with repeated measures revealed no significant effects of Dose, $F(2,9)=2.98$, $p>0.05$, Days $F(3,27)=1.59$, $p>0.05$, and Dose × Days interaction $F(6,27)=1.02$, $p>0.05$. Further analysis showed a decrease (not statistically significant) in fighting in oil treated rats (after 1st week) and a significant decrease, $t(5)=2.89$, $p<0.025$, in CA treated rats (2.5 mg/kg) (after second week). This can be explained on the basis that one pair of rats in each group did not fight at all during this particular testing session. SEF in saline and oil treated rats remained close to pretest level throughout the four weeks of treatment.

*Testosterone Levels*

Plasma testosterone levels showed a wide variation in each group. This wide variation in the level of testosterone within the rat has been shown earlier [23]. The mean level of testosterone in saline (5.89±0.80) or oil (6.22±1.04) treated rats was very close. No significant difference in the level of testosterone was observed in CA treated rats in comparison to that of controls (Table 2).

*5-HT and 5-HIAA Levels*

No significant difference was observed in the level of brain 5-HT and 5-HIAA of CA treated rats when compared to the controls although the CA treated rats showed lower levels of 5-HT and 5-HIAA. There was also no difference between the saline and oil treated rats' level of 5-HT or 5-HIAA (Table 2).

## DISCUSSION

No significant change in the intermale fighting behavior, plasma testosterone or brain serotonin was observed as a result of administration of two doses of CA to rats for a period of four weeks.

The loss of body weight observed in CA treated rats could be due to other anitanabolic effects and is in agreement with the literature [4, 13, 15, 17, 29, 30]. A significant reduction observed in the wet weight of testes of CA treated rats indicated an antiandrogenic property of this steroid. Similar observations have been reported by Matte and Fabian [17] and Jones [13]. A decreasing trend in the level of brain 5-HT and 5-HIAA and the tendency of testosterone level to go up in CA treated rats may have a counterbalancing effect on the level of fighting and help to account for the lack of significant difference from controls. The interaction between the effects of hormones and behavioral consequences on neurotransmitter levels is a problem and needs further investigation.

Finally the balance of androgenic properties as indicated by such findings as those of Early and Leonard [8] and antiandrogenic properties of CA may account for the absence of a significant effect on aggressive behavior as well as for the previously reported controversial findings.

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