CYPROTERONE ACETATE DIMINISHES SEXUAL ACTIVITY IN MALE RABBITS

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Summary. Cyproterone acetate (CA) was injected daily in eleven rabbits for 3 weeks at a dose of 20 mg/day, and for a further week at a dose of 40 mg/day. After 3 weeks of treatment, the ejaculation frequency was reduced but other measures of sexual behaviour were not significantly changed. There was no reduction in the fructose concentration of the semen, but the volume of the ejaculates decreased. The vesicular glands from the experimental animals showed histological changes typical of those occurring after castration. It was concluded that CA reduced the activity of at least one of the accessory sex glands as well as sexual behaviour. This lends support to the current hypothesis that the endocrine regulation of rabbit sexual behaviour differs from that of the rat.

INTRODUCTION

Cyproterone acetate (1,2α-methylene-6-chloropregnan-4,6-dien-17β-ol-3,20-dione-17 acetate, CA), an antiandrogen, has been shown to compete with testosterone for androgenic receptors in the cell nucleus in rat ventral prostate (Walsh & Korenman, 1970). Because of its progestational activity, CA also exerts a negative feedback at the hypothalamic level, thereby decreasing gonadotrophin release (Neumann, Elger & Steinbeck, 1968). These effects result in a reduction in the size and secretory function of the accessory sex glands in rats and mice (Beach & Westbrook, 1968; Whalen & Edwards, 1969; Edwards, 1970). Cyproterone acetate has also been found to reduce sexual activity in men and dogs (Ott & Hoffet, 1968; Schmidtke & Schmidtke, 1968). Nevertheless, many studies have failed to reveal any effects of CA on sexual behaviour in rodents (Zucker, 1966; Beach & Westbrook, 1968; Davidson & Bloch, 1969; Whalen & Edwards, 1969). These discrepancies may result from species differences and/or differences in the methods used. In order to study these problems further, we investigated the effects of CA on sexual behaviour in intact rabbits, and studied the functional state of the accessory sex glands. The formation and secretion of fructose is highly dependent on circulating androgens (Mann, 1964), and the fructose concentration can be used as an index of androgenic activity in the accessory sex glands. In this way, the relationship between central and peripheral actions of CA can be studied.
Since the effect of CA on sexual behaviour might be modified by the degree of experience of the animals, both those with considerable experience and completely inexperienced animals were used in this study.

MATERIALS AND METHODS

Animals
Rabbits of mixed strains, 8 to 12 months old and weighing 3.2±0.15 kg, were obtained from a breeder who guaranteed that the animals had had no heterosexual experience. They were housed in individual stainless steel cages in a room with constant temperature (17 to 18°C) and a 12 hr light/12 hr dark cycle. They were fed a diet consisting of hay and, three times a week, 0.1 litre of concentrated food (a mixture of oats and pellets).

The females to be used as copulation partners were ovariectomized, and injected subcutaneously with oestradiol benzoate (60 µg in 0.25 ml arachis oil) 74 and 48 hr before being used. This treatment brought the females into a highly receptive state.

Procedure
The males were allotted to two groups consisting of five and six animals respectively. Three animals in each group were given extensive copulatory experience during the 4 weeks immediately preceding the present experiment.

From Day 0 onwards, the rabbits in one of the groups were injected subcutaneously with 20 mg CA/animal in 0.5 ml arachis oil/benzyl benzoate, 1/1, and those in the other group were given the oil vehicle only. After twenty-one daily injections, the CA dose was increased to 40 mg. This dose was injected for 7 days. The treatment was then reversed, so that the group formerly given vehicle was now given 20 mg CA and the CA group was now given the oil vehicle only. This treatment lasted 21 days, after which time the dose was increased as previously described. From Day 0 until the end of the experiment, the animals were subjected to a weekly mating test lasting 10 min. An oestrous female was put in the male's cage, and the behaviour of the male towards the female was carefully observed. The following parameters were registered: the ejaculation latency (time from introduction of female until ejaculation), the post-ejaculatory interval (time from one ejaculation to the next), and the number of ejaculations.

During the last few years, this method has been extensively used in our laboratory for quantifying the sexual behaviour of rabbits. It has been found to give highly reliable results (Agmo & Kihlström, 1974).

On the day before each mating test, semen was obtained from all animals using an artificial vagina and an oestrous female as a stimulus. The semen was collected in graduated micro test-tubes, the volume was determined and the concentration of fructose was estimated by the method described by Degerman & Kihlström (1970).

Two of the animals given CA were killed by a blow on the head 24 hr after the last injection, and the penis, testes and vesicular glands were carefully dissected out and immediately weighed. The penis and vesicular gland were
then fixed in Clarke's solution and embedded in paraffin wax. The 5-μm sections were stained with eosin and haematoxylin. The penis and vesicular gland from intact and castrated animals were treated in the same way. The castrated animals were not used for 2 months after the operation, and had not been used in tests for sexual behaviour. The thickness of the vesicular epithelium was measured as described by Bern (1949).

Statistical analysis was performed by the Friedman two-way analysis of variance or the Mann-Whitney U-test for the behavioural data, and the F-test followed by the t test for the physiological data.

RESULTS

Effects of cyproterone acetate on sexual behaviour

No difference was observed between experienced and inexperienced animals with regard to the response to the CA treatment, and these data were pooled.

The animals given the oil vehicle greatly increased their number of ejaculations/test between Day 0 and Day 7 (Text-fig. 1a). A much smaller increase was seen in the rabbits treated with CA. The increase in ejaculatory performance was due to the sexually inexperienced animals, all of which showed very little sexual activity on Day 0. The rabbits receiving CA continued to show a low level of sexual activity during the treatment, but their sexual activity 4 weeks after the end of treatment was the same as that of the animals given the oil vehicle during the first part of the experiment. The control animals for the first part of the experiment, however, showed a sharp decline in sexual activity during the second part of the experiment, i.e. when they were injected with CA (P<0.05, Friedman two-way analysis of variance).

No consistent changes were observed in the ejaculation latencies (Text-fig. 1b). The post-ejaculatory intervals decreased during the first tests (Text-fig. 1c). This decrease was of the same magnitude for inexperienced and experienced animals. A tendency to lengthening of the post-ejaculatory interval was observed for both groups at the last tests during CA treatment. Due to the low number of animals showing sexual activity at the end of CA treatment, this tendency did not reach statistical significance.

Effects of cyproterone acetate on seminal fructose, ejaculate volume and histology of the vesicular gland

The mean amount of fructose in the semen of rabbits treated with CA for 28 days was not significantly different from that of control rabbits (Table 1). No significant changes were observed during the course of treatment. The mean ejaculate volume±S.E.M. decreased during CA treatment from 0.48±0.02 ml on Day 0 to 0.35±0.04 ml on Day 27 (P<0.02, F-test) and was significantly lower than that of control rabbits on Day 27 (Table 1). No changes in the ejaculate volume were observed when oil was injected (0.51±0.3 ml on Day 0 versus 0.47±0.02 ml on Day 27).

The weights of the penis and vesicular glands were not affected by the CA treatment. Both these organs were very much reduced in size in the castrated animals (Table 1). Testis weight was not changed by the CA treatment (Table
The effect of cyproterone acetate (CA) on the mating behaviour of male rabbits. Tests were performed once a week. (a) Mean no. of ejaculations; (b) ejaculation latencies; (c) post-ejaculatory interval. O, Six rabbits injected with CA from Day 0 to Day 28, and from Day 29 onwards with oil; Δ, five rabbits injected with the oil vehicle from Day 0 to Day 28, and with CA from Day 29 onwards. Results marked with an asterisk are significantly different (P < 0.05, Mann-Whitney U-test).

1). Noticeable differences were found between the vesicular glands from intact and castrated animals. In the former, the lumen was wide and filled with secretions, and the epithelial cells appeared to be actively secretory (Pl. 1, Fig. 1). In the latter, the vesicular glands were generally atrophied, as indicated by a decrease in total parenchyma and an increase in fibromuscular tissue (Pl. 1, Fig. 2). Vesicular glands from the rabbits receiving CA presented an intermediate appearance: a reduction in epithelial thickness (Table 1) was evident, as were slight increases in the amount of fibromuscular tissue. The lumen was not as wide as in the glands from the intact animals, and did not contain such abundant secretions (Pl. 1, Fig. 3).
Fig. 1. Vesicular gland from an intact rabbit, 12 months old. × 30.
Fig. 2. Vesicular gland from a rabbit which had been castrated 2 months before death. × 30.
Fig. 3. Vesicular gland from a rabbit which had been injected with cyproterone acetate. × 30.
Table 1. Effects of injections of cyproterone acetate on reproductive organs, seminal fructose and ejaculate volume in male rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fructose conc. (mg/ml)</th>
<th>Vol. of semen (ml)</th>
<th>Wt of penis (mg/kg body wt)</th>
<th>Wt of vesicular gland (mg/kg body wt)</th>
<th>Wt of testis (g/kg body wt)</th>
<th>Thickness of vesicular gland epithelium (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyproterone acetate</td>
<td>0.82 ± 0.08 (10)</td>
<td>0.35 ± 0.04 (10)*</td>
<td>106 (2)</td>
<td>78 (2)</td>
<td>2.06 (4)</td>
<td>18 ± 2 (10)*</td>
</tr>
<tr>
<td>Oil</td>
<td>0.95 ± 0.12 (11)</td>
<td>0.47 ± 0.02 (11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>88 (2)</td>
<td>72 (2)</td>
<td>1.99 (4)</td>
<td></td>
<td></td>
<td>28 ± 3 (10)</td>
</tr>
<tr>
<td>Castration</td>
<td>31 (2)</td>
<td>40 (2)</td>
<td></td>
<td></td>
<td></td>
<td>5 ± 1 (10)</td>
</tr>
</tbody>
</table>

The values shown (Mean ± S.E.M.) were obtained on Day 27 of treatment or immediately after the end of treatment. The numbers in parentheses indicate the number of observations.

* Different from control (P < 0.05, t test).
DISCUSSION

In contrast to the many studies on rodents in which CA has been found to be without effect, we obtained a clear-cut effect of CA on rabbit sexual behaviour. The weight and secretory function (as expressed by seminal fructose) of the vesicular gland were unaltered by CA, although it was possible to detect a decrease in the ejaculate volume. From these data, it is evident that administration of CA does not completely imitate the effects of castration, since seminal fructose is greatly reduced within 2 weeks after castration (Mann & Parsons, 1947) but not after CA treatment.

The histological changes observed in the vesicular gland resembled those observed by Bern (1949) in his 'low androgen' animals. These were castrated rabbits injected with 0.25 mg testosterone propionate daily, a dose well below that necessary to prevent the effects of castration. Thus, it seems reasonable to suppose that CA, at least partly, antagonizes the actions of endogenous testosterone in the vesicular gland. The relative weight of the testes was unchanged by the CA treatment, and any reduction in circulating gonadotrophins was probably too small to affect testicular function. Any reduction in circulating gonadotrophins, brought about, for example, by the administration of small doses of testosterone, within a short period of time causes a marked decrease in testicular weight (Rubinstein & Kurland, 1941).

A combination of the behavioural and histological data suggests that CA, in the doses used in this study, antagonizes the actions of endogenous testosterone on sexual behaviour and the vesicular gland. The CA doses used by Beach & Westbrook (1968) and Whalen & Edwards (1969) in rodents were, on a weight basis, several times higher than the doses used here (10 to 20 mg/rat versus 20 to 40 mg/rabbit, i.e. about 20 to 40 mg/kg rat versus 7 to 13 mg/kg rabbit). This difference probably accounts for the relatively slight effect of CA on vesicular gland histology and the lack of effect on fructose concentration.

It has recently been found that oestradiol benzoate is quite effective in activating sexual behaviour in castrated male rats (Larsson, Södersten & Beyer, 1973; Södersten, 1973; Feder, Naftolin & Ryan, 1974), and a combination of oestradiol benzoate and dihydrotestosterone (DHT) results in completely normal male sexual behaviour in such rats (Baum & Vreeburg, 1973; Larsson et al., 1973; Feder et al., 1974). Treatment with DHT alone is without effect on rat sexual behaviour (McDonald & co-authors, 1970). This has led to the suggestion that, in the rat, oestradiol is the centrally acting metabolite of testosterone, whereas DHT acts peripherally. These data accord well with the established failure of CA to affect rat sexual behaviour. In the rabbit, oestradiol benzoate, alone or in combination with DHT, is ineffective in activating the sexual behaviour to normal levels (A. Ågmo and P. Södersten, unpublished work). This suggests that testosterone itself is the centrally acting hormone in this species, and further support for this hypothesis is provided by the results of this study. Interestingly, Wilson & Gloyna (1970) found that the rabbit prostate lacks testosterone-5α-reductase, and it is therefore possible that testosterone itself is the active molecule, not only in the brain, but also in the acces-
sory sex glands. In a recent report (Ågmo, 1974), it was demonstrated that the concentration of fructose in semen and the intensity of the sexual behaviour vary independently in intact rabbits, and respond differently to exogenous testosterone in castrated animals. This suggests that the sensitivity to androgenic stimulation in the brain and in the accessory sex glands may vary independently, but does not exclude the possibility that they respond to the same hormone.

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REFERENCES