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Effects of Neonatal Cyproterone Acetate Administration on Isolation-Induced Fighting Behavior and Mounting Behavior in Male and Female TO Strain Albino Mice

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The effects of a single dose (1 mg) of cyproterone acetate administered on either day 1 or day 20 of life on the adult behaviors of male and female TO strain albino mice were studied. The mice were tested both in a "standard opponent"-type situation and in a similar test using a hormonally primed receptive female, after being gonadectomized and maintained with testosterone propionate as adults. Neonatal treatment with this compound had little effect on subsequent fighting behavior in either sex, but clear evidence was produced that this treatment masculinized the sexual behavioral potentialities of the females, an effect which was apparent in animals which had been injected on either day 1 or day 20 of life. Indications were obtained that females treated neonatally with cyproterone acetate were capable of differentiating between the male and female "opponents" in a manner similar to the male. The effects of this treatment on fighting behavior consequently appear to be dissimilar to the effects of neonatal castration in this species. However, the effects on mounting behavior in the females, evidenced in adulthood, seem likely to be a consequence of the weak androgenic properties of the antiandrogen. The administration of cyproterone acetate neonatally appears to have a more dramatic effect on the adult weights of endocrine organs in females than in males.

Key words: aggression, anti-androgen, mounting, neonatal, mice

INTRODUCTION

Neonatal hormone administration can lead to marked changes in both endocrine and behavioral potentialities in adulthood (see review by Brain, 1971). For instance, it is said that neonatal treatment of female albino mice with androgens or estrogens can result in individuals which are more likely to show male-type fighting behavior as adults following ovariectomy and androgen treatment (Edwards, 1968, 1969) than are untreated controls. There has been some recent indication that under appropriate conditions of androgen treatment, female mice

who have not been "androgenized" can be induced to show fighting behavior (Svare et al., 1974; Brain and Evans, 1975). It has been reported that neonatal castration of male mice results, if androgen replacement is not given early enough, in individuals who show little fighting behavior in response to adult androgen treatment. Meyerson (1968) also indicated that rats castrated 1–5 days after birth were more capable of showing lordotic-type behavior in response to appropriate treatment with estrogen and progesterone than were individuals castrated in later life. The basic conclusions to be drawn from these and related studies appear to be that the presence of sex steroids in early life may result in "masculinization" of behavioral potentialities in rodents and that the absence of these factors leads to feminine potentialities. Such changes may not be absolute but seem to be related to the relative ease with which certain behaviors can be elicited.

Neumann and Elger (1965b) found that perinatal treatment of rats with the antiandrogenic steroid cyproterone acetate* led to genetic males with a female type of hypothalamic function in adulthood. Treatment of a variety of adult rodents with this compound results in marked suppression of a number of indices of androgen action, especially the weights of various sex accessory glands (e.g., Neumann and Elger, 1965a; Whalen and Edwards, 1969; Edwards, 1970; Brain et al., 1974). However, this steroid is apparently without influence on adult aggressive motivation in male mice (Edwards, 1970; Brain et al., 1974), although such treatment has an indirect effect on fighting behavior in that it suppresses the amount of fighting directed toward a treated animal, presumably by suppressing "pheromone" production (Nowell and Wouters, 1973).

As neonatal treatment appears to have produced effects on endocrine functioning and fighting behavior in adulthood in mice, an attempt was made to determine whether early treatment with cyproterone acetate had any lasting action on physiology and behavior in male and female mice. In an initial study the effects of early treatment with this compound on the relative weights of various adult endocrine glands were assessed. In a second study, the effects of treatment on the behaviors of adult gonadectomized mice which had been treated with testosterone propionate (TP) were determined.

EXPERIMENT 1

Materials and Methods

Animals. Mice utilized in this study were litters from TO strain albino animals obtained from A. Tuck and Sons Ltd., Rayleigh, Essex. The litters were produced as a result of postpartum mating in pairs of these mice, and the litters were reduced to 8 on the day of birth. All mice were housed in opaque, plastic cages measuring 30 x 12 x 11 cm, and the animals were supplied with food and water ad libitum. The animals were kept in a quiet room which housed only this strain

*6-chloro-17-hydroxy-1$^{\alpha}$, 2$^{\alpha}$, methylenepregna-4, 6-diene-3, 20-dione acetate.

of mouse, and temperature and lighting conditions were controlled (a reversed lighting schedule was imposed).

Injections of Cyproterone Acetate. Cyproterone acetate (A. G. Schering, Berlin, West Germany) was suspended in a vehicle consisting of 20% ethyl oleate and 80% arachis oil. Mice received i.p. injections of 0.05 ml vehicle or a similar volume containing, respectively, 1 or 2 mg of cyproterone acetate. Only a single injection was given, and the injections were given on the day of birth (day 1) or on the 10th day of life.

Subsequent Housing. On weaning at 18–22 days of age, the mice were sexed and were then housed in groups approximating to 6 animals of the same sex and treatment group.

Organ Weight Data. At 122 days of age, mice of both sexes were killed by cervicle dislocation. In the male the weights of the body, right preputial gland (minus sebum), right testis, right seminal vesicle (plus coagulating gland but minus contents of both), and ventral prostate were obtained. In the female the weights of the right hemiuterus and ovary were obtained in addition to the weight of the animal. Glands were rapidly excised and cleaned on saline-moistened filter paper before being weighed on a torsion balance.

Results

Mean organ weight data ± standard errors for the male is presented in Table I and comparable data for the female in Table II. Significant comparisons on the Student t test are also indicated between treatment and placebo categories. No consistent effects of cyproterone acetate treatment on body weight were discernable. Whilst early injection of cyproterone had no consistent effect on relative testis weight in the treatment categories of males employed (although the category treated with 1 mg of the steroid administered on the 1st day of life did show a significant increase in the relative weight of this organ over the placebo-injected animals), treatment on both days 1 and 10 in the female resulted in a significant decline in relative ovarian weight, a response which appeared to be dose-dependent and more marked in animals treated on day 10 than on day 1. These results were largely supported by the relative weights of sex accessory structures, as there appeared to be no consistent differences between mean relative weights of the preputial gland, the seminal vesicle, and the ventral prostate

EXPERIMENT 2

Materials and Methods

Similar methods were employed in this experiment with respect to treatment and housing. The categories in this experiment consisted of animals treated with a single injection of vehicle or of a suspension containing 1 mg of cyproterone

TABLE I. The Effect of Neonatal Injection of Cyproterone Acetate on Endocrine Function in Male Albino Mice

Treatment category	Mean body weight (gm)	Mean relative right testis weight (mg/100 gm)	Mean relative preputial weight (mg/100 gm)	Mean relative seminal vesicle weight (mg/100 gm)	Mean relative ventral prostate weight (mg/100 gm)
Placebo, day 1	29.1 ±0.7	392.4 ±13.3	102.4 ±7.7	196.0 ±23.8	39.5 ±2.5
1 mg cyproterone acetate, day 1	25.0* ±0.6	528.0* ±11.6	113.9 ±12.3	164.8 ±11.3	48.1 ±4.1
2 mg cyproterone acetate, day 1	28.0 ±0.9	512.4 ±7.4	90.9 ±12.5	170.3 ±10.0	48.0 ±10.0
1 mg cyproterone acetate, day 10	28.7 ±0.9	395.6 ±15.7	117.2 ±6.9	165.9 ±9.6	43.0 ±5.4
2 mg cyproterone acetate, day 10	28.3 ±1.8	394.3 ±23.2	102.3 ±10.1	192.3 ±18.2	47.8 ±9.6

*$P < 0.001$ different from placebo (Student t test).

TABLE II. The Effect of Neonatal Injection of Cyproterone Acetate on Endocrine Function in Female Albino Mice

Treatment Category	Mean body weight (gm)	Mean relative right ovary weight (mg/100 gm)	Mean relative right hemiuterus weight (mg/100 gm)
Placebo, day 1	26.7 ±1.8	41.3 ±6.9	197.2 ±49.5
1 mg cyproterone acetate, day 1	23.4 ±1.4	32.7 ±3.7	178.9 ±36.6
2 mg cyproterone acetate, day 1	26.4 ±0.7	23.4* ±2.3	155.4 ±37.3
Placebo, day 10	29.7 ±0.9	50.9 ±3.4	204.8 ±15.0
1 mg cyproterone acetate, day 10	22.9 ±0.4	24.1*** ±1.2	115.6** ±16.6
2 mg cyproterone acetate, day 10	27.5 ±1.1	20.9 ±3.3	105.6*** ±18.5

*P < 0.05.
**P < 0.01.
***P < 0.001 (different from appropriate placebo on Student t test).

acetate on either the 1st or the 20th day of life. Solutions were identical in composition and volume to those used in Experiment 1. The interval between the first and second categories was extended from that used in the first study as it was thought this would demonstrate more clearly the presence of a "sensitive period" effect of injection with cyproterone acetate.

At about 100 days of age, the mice were bilaterally gonadectomized under ether anaesthesia and housed individually for 18 days. Throughout this period, and for the duration of behavioral tests, each mouse received daily i.m. injections in the different treatment categories of the male, whilst early cyproterone treatment caused a decline in adulthood of mean relative hemiuterus weight (which is utilized as an index of estrogen secretion) in the female. This latter effect reached significance at both dose levels of antiandrogen when the treatment was applied on day 10 ($p < 0.02$ and $p < 0.01$ for 1 and 2 mg, respectively).

of 250 μg of testosterone propionate (TP) (Evans Medical Ltd.) in 0.1 ml of vehicle (20% ethyl oleate and 80% arachis oil).

After 18 days isolation the mice in each of the 8 experimental catagories (N= 5–7) were given a 7 min "standard opponent" aggression test (described fully by Brain and Poole, 1974) against a 30–40-day-old group-housed male mouse of the same strain. The tests were carried out in the home cage of the test animal and the mice received a total of 3 such tests over 3 consecutive days. All tests were conducted under red lighting conditions.

The behavioral measures recorded included the mean latency of attack (the mean interval between the introduction of the opponent into the cage of the test animal and the first overt biting attack by the latter animal), the mean accumulated attacking time (the mean duration of time spent by the test animal in biting the opponent), and the mean number of biting attacks directed by that test animal toward the standard opponent. These measures have been shown to be good indices of aggressiveness in this strain of mouse (Brain and Poole, 1974). Any mounting behavior that occurred was recorded, and the percentage of tests in which attack or mounting behavior was apparent were recorded separately.

After this series of tests, each mouse received three 7 min tests (at 4-day intervals) in the home cage of an hormonally primed, receptive female mouse. These females had been ovariectomized and were injected with 0.1 ml of vehicle containing 2 μg of estradiol benzoate on two consecutive days, followed by a single injection of 100 μg of progesterone (both steroids obtained from Evans Medical Ltd.). The behavioral encounters took place 2 days after the last injection of estradiol benzoate and 6 hr after the injection of progesterone. Although 7 min is a relatively short period for a reproductive behavioral test, it was selected because of the problem of possible circadian variations in behavior in mice and in order to be able to relate the amount of fighting in this and the previous set of tests. The measures recorded included the latency to the first mount and the mean number of mounts by the test animal. The percentage of tests in which mounting or attack behavior occurred were recorded separately as in the standard opponent test.

Results

The mean behavioral test data (± standard error) obtained over the three "standard opponent" tests are presented in Table III and corresponding data, obtained using primed female animals, in Table IV.

The results of this experiment suggest that in the case of the behavioral test employing a male standard opponent, there is a sex difference in the aggressiveness of the experimental animals. For example, male mice treated with placebo on day 1 of life showed a significantly greater ($p < 0.009$) number of attacks and an increase in the accumulated attacking time ($p < 0.007$) directed toward the

standard opponent after castration and TP injection than did similarly treated females also subjected to adult gonadectomy and injection with TP. There was also some indication that the "stress" of injection on the 1st day of life increased adult aggressiveness of both the males and females following gonadectomy and androgen treatment in this test compared with mice of the same sex receiving similar injections on the 20th day of life. Although this effect did not reach significance for the females, males treated with placebo in the earlier-injection category evidenced shorter latencies, extended accumulated attacking times, and increased numbers of attacks (all $p < 0.05$), compared with mice of the same sex which had received injection on the 20th day of life. There was little evidence, using this particular test, that neonatal treatment with cyproterone acetate could alter the levels of aggressiveness generated by TP treatment of isolated, gonadectomized animals compared with similarly treated categories receiving early placebo injections.

The behavioral data obtained using primed females as opponents clearly demonstrated that the major effect of neonatal cyproterone acetate injection is to masculinize reproductive behavioral potentiality in the female mice of this strain. Female mice treated with the antiandrogen on day 1 of life exhibited shortened mount latencies ($p < 0.01$) and increased number of mounts ($p < 0.01$) when gonadectomized and treated with TP, compared with corresponding early placebo-treated animals. Similar effects were also apparent when the treatments were given on the 20th day of life. In this case, the significance values were $p < 0.005$ and $p < 0.007$, respectively. Neonatal treatment with cyproterone acetate in the male resulted in some evidence of reduced fighting and increased mounting behavior, but comparisons with the respective placebo-injected categories did not reach significance.

DISCUSSION

Whilst early injection of cyproterone acetate in Experiment 1 had no consistent effects on any of the organ weights recorded for the male (although the category treated with 1 mg of the steroid on day 1 did show a significant increase in relative testis weight over the placebo-injected animals), treatment on both day 1 and day 10 in the females resulted in a significant decline in relative ovarian weight and also caused a decline in mean relative hemiuterus weight (utilized as an index of estrogen secretion). In this respect, the effects of this compound appear to be opposite to those of neonatal administration to the mouse of androgens or estrogens (Bronson and Desjardins, 1968). Early injection of these sex steroids normally results in persistent cornification and hyperplasia of the uterus in the mature mouse (Boris and Trmal, 1967).

The results of Experiment 2, in general, show that the major behavioral effects

TABLE III. Behavioral Measures Obtained in a 7 Min "Standard Opponent" Test for Male and Female Albino Mice Treated with 1 mg Cyproterone Acetate or Placebo on Day 1 or Day 20 of Life

Treatment	Fight (%)	Mean latency of attack (secs)	Mean no. of attacks	Mean accumulated attacking time	Mount (%)
Male, day 1, placebo	66	322	4.58	14.08	8
Male, day 1, cyproterone acetate	44	345	1.27	5.16	27
Male, day 20, placebo	38	346	2.47	10.71	57
Male, day 20, cyproterone acetate	33	331	3.76	17.90	23
Female, day 1 placebo	25	316	1.08	2.54	16
Female, day 1, cyproterone acetate	22	345	1.50	3.27	27
Female, day 20, placebo	0	420	0	0	20
Female, day 20, cyproterone acetate	6	393	0.30	1.13	60

TABLE IV. Behavioral Measures Obtained in a 7 Min Test with a Primed Female for Male and Female Albino Mice Treated with 1 mg Cyproterone Acetate or Placebo on Day 1 or Day 20 of Life

Treatment	Mount (%)	Mean no. of mounts	Mean latency of 1st mount	Fight (%)
Male, day 1, placebo	0	0	420	0
Male, day 1, cyproterone acetate	11	0.5	398	0
Male, day 20, placebo	22	0.55	394	0
Male, day 20, cyproterone acetate	28	0.76	397	4
Female, day 1, placebo	8	0.25	408	29
Female, day 1, cyproterone acetate	38	0.72	377	5
Female, day 20, placebo	7	0.14	417	21
Female, day 20, cyproterone acetate	53	1.00	327	6

of cyproterone acetate are on mounting behavior in tests employing hormonally primed females. The results obtained in such tests indicate that treatment of the females with this antiandrogen in early life induces effects that seem similar to those of neonatal testosterone injection in female mice (Bronson and Desjardins, 1968) with respect to increased male behavioral potentialities. Cyproterone acetate treatment had little effect on the sexual behavior of the males, however, a result that seems rather different from the effect of this compound in male rats (Nadler, 1968). It is possible that the low level of mounting by the males in this situation could have been largely a consequence of the short duration of the test, but this was implicit in the design.

The fact that neonatal administration of this compound had little effect on fighting behavior in the standard opponent test could be a consequence of the stimulus animal's failing to stimulate high levels of fighting (presumably due to

the low production of "pheromones" as a consequence of it's relatively low androgen secretion) in control categories, thus masking any differential effects of the treatment.

It is interesting to note that the males used in this study appeared capable of differentiating between male and female stimulus animals, a very low level of aggressiveness being directed toward primed females. However, a relatively high incidence of homosexual mounting behavior was recorded for some categories of males. The reason for this is not clear, although this too could be attributed to the low pheromone production on the part of the stimulus animal, resulting in conflicting motivations. Had mature male opponents been utilized, higher levels of aggressiveness might have resulted but in such a situation it is more difficult to quantify the relative contributions of the test and the stimulus animals.

There was evidence of a similar discrimination by females that had been treated neonatally with cyproterone acetate and had been ovariectomized as adults and had been given injections of TP, but similarly treated females which had received placebo injections showed relatively high levels of fighting behavior directed toward the primed females. This could have interfered with the manifestation of mounting behavior and tentatively indicates that animals, which have not been subjected to sex steroids in early life, although capable of showing fighting behavior following ovariectomy and TP treatment, are not as likely to differentiate between different types of opponent as are animals which have been "androgenized."

The fact that persistent effects of early cyproterone acetate treatment were evident in Experiment 1 in females, tentatively indicates that the treatment may influence sexual differentiation in this sex. If this is the case, the behavioral data reported in Experiment 2 are at variance with the data reported by Edwards (1968) and Bronson and Desjardins (1968). Such differences could be a consequence of strain or mode of behavioral testing. Taken together, the two experiments also supply some support for Davidson's (1972) view that different brain structures may be involved as androgen receptors for the regulation of gonadotrophin secretion, sex behavior, and aggressive behavior.

The results in general suggest that, far from having an antiandrogenic effect with respect to effects on the hypothalamus, cyproterone acetate administration appears to have its major effect when administered to females, causing weak androgenization resulting in masculine behaviors, an effect which may be evidenced by a disruption in the normal cyclicity of females but which seems to differ from the normally reported effects of neonatal sex-steroid treatment on ovarian functioning. It perhaps should be noted that the previously reported effects of perinatal cyproterone acetate administration are known to be related to dose, duration of treatment, and the age of the animal (Davidson and Bloch, 1969).

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