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The Effect of Testosterone and Cyproterone Acetate on the Concentration of γ-Aminobutyric Acid in Brain Areas of Aggressive and Non-Aggressive Mice

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EARLEY, C. J. AND B. E. LEONARD. *The effect of testosterone and cyproterone acetate on the concentration of γ-aminobutyric acid in brain areas of aggressive and non-aggressive mice.* PHARMAC. BIOCHEM. BEHAV. 6(4)409–413, 1977. Mice which had been housed in groups were introduced into the home cages of isolated mice and the aggressive defensive behaviour assessed. The grouped mice were chronically treated with testosterone, cyproterone acetate or arachis oil. The concentration of GABA was determined in 10 regions following the last behavioural assessment. The grouped mice which were treated with testosterone or with the anti-androgen, cyproterone acetate, were subjected to fewer aggressive attacks by the isolated mice than the grouped controls. The concentration of GABA in particular brain areas appears to depend on the degree of aggressive behaviour exhibited, the type of environment into which the mouse is introduced, the hormone treatment, and the housing conditions.

Aggression Brain Cyproterone acetate GABA Testosterone

IT IS well established that when male mice are isolated for several weeks, they become aggressive when placed in a neutral environment with another mouse [24]. This experimental situation has been used to study the effects of various centrally active drugs on behaviour [19].

The primary aim of the present investigation was to see what effects changes in the androgen status of nonaggressive mice, had on the aggression of the isolated animal. The non aggressive mice were housed in groups and were used as opponents in aggression tests with isolated mice. Changes in androgen status were achieved by injecting testosterone or the anti-androgen, cyproterone acetate, into the non-aggressive oponent mouse.

An important consideration in this experiment was the use of intact animals. Stress has been shown to reduce testosterone in man [21] and in animals [3]. It would follow that the stress induced by defeat or by introduction into a novel environment [7, 13, 14, 15, 16], would depress testosterone concentrations. Therefore in order to maintain androgen levels, testosterone was given to intact animals. In this experimental design, if androgens are reduced by environmental stress and if the responses measured are androgen dependent, then cyproterone acetate should not produce an effect in opposition to the effects established by androgen treatment. Also it would appear from the work of others that cyproterone or cyproterone acetate has androgen-like qualities which have been demonstrated in studies on sexual and aggressive behaviour [4, 10, 23]. If cyproterone acetate is a potential androgen, at least in terms of certain types of behaviour then it would be expected to stimulate responses which are in accordance with testosterone stimulated behaviour.

As an ancillary part of this study, changes in the concentrations of the inhibitory transmitter, γ-aminobutyric acid (GABA) were determined in different brain regions of mice. The possible relationship between GABA concentrations and environmental factors associated with aggression is supported by several studies. Brody et al [5], using micro-cannulae implants in the hypothalamus of cats, reported that GABA inhibited attack responses and increased the threshold for electrically induced attack. Using a synaptosomal fraction which was prepared from brains of mice which had been differentially housed, De Feudis *et al*, [6] found that the binding capacity of the particulate fraction for GABA was less in the brains of isolated mice compared with grouped mice. The possible involvement of GABA in aggressive behaviour was also suggested by the observation in this laboratory that the concentration of this amino acid was reduced in the septum of rats which showed pronounced aggression following the administration of apomorphine (Kenny, Lynch and Leonard, unpublished).

METHOD

Experiment 1

Animals. One hundred twenty albino mice, 3–4 months of age and weighing 25–30 g at the start of the experiment were used. The mice were randomly assigned to 2 housing

conditions either isolated or grouped. The 60 isolated mice were housed in individual cages (30 x 13 x 11 cm) for 6 weeks prior to testing. The grouped mice were randomly assigned to 1 of 3 cages (41 x 25 x 11 cm). Each set of cage-mates was randomly assigned to treatment groups. Each group was housed in the appropriate cages 6 weeks prior to testing.

Specification of treatment groups. Several groups were formed from the 60 mice in the isolation-housing condition. Six animals were randomly chosen as isolation housing controls (Group A). These animals were maintained under the same environmental conditions as the other isolated mice but were otherwise untreated and not subjected to the behavioural situations. Another 6 animals were selected on the observation that during the period of preliminary testing none exhibited any aggressive behaviour towards the opponent mice. These mice constituted the non-fighter group and were used only in the behavioural part of this study. The remaining 48 mice constituted the fighter group. The behaviour assessment of aggression was based solely on the recorded observations on the fighter mice. In the interim between the conclusion of the behavioural testing and the start of the biochemical procedures the animals were ranked according to the behaviour observed, and then two groups (B and C) of 6 animals each were selected from the fighter group. Group B animals exhibited the highest aggressive tendency over all treatment opponents and over all behavioural parameters. Group C showed the lowest aggressive tendency over all conditions.

All mice which were assigned to group-housing conditions were randomly assigned to treatment groups on the same day as housing assignments were made. Two groups of six mice each were formed to control for housing conditions (CH) and for injection procedures (CI). The injection controls were administered arachis oil (0.1 ml) in accordance with drug-treatment procedures. These two groups never encountered the behavioural assessment conditions. Twenty-one animals were selected as opponents to the non-fighter group, and were used to evaluate the biochemical effects of being introduced into a strange environment. This group was equally divided into drug treatment subgroups: oil environment group (OE), testosterone-environment group (TE), and cyproterone acetate-environment group (CE). The remaining 27 animals were assigned to the defeated group which was the only group that encountered members of the fighter group. They were also the only group used in the assessment of defensive behaviour. The defeated group was subdivided into drug-treatment group: oil-defeated (OD), testosterone-defeated (TD) and cyproterone acetate-defeated (CD).

Preliminary procedure. Procedures for identification of non-fighter mice were commenced 3 weeks after the housing assignments had been made, and continued for 4 daily sessions. All isolated mice, except for 6 animals which were selected as isolation-housing controls (Group A), encountered daily and at random a member from the defeated group. After the fourth session, each isolated mouse had encountered 4 grouped-mice. Six of the isolated mice were chosen on the observation that none showed any aggressive tendency towards the grouped mice which had been introduced into their cages. These mice constitute the non-fighter group.

At the conclusion of the 4th session, the bedding in the isolated mice's cages was changed. All treatment procedures for the grouped mice were begun. Treatment with testosterone (1 mg/kg sc), cyproterone acetate (1 mg/kg sc) or arachis oil (0.1 ml) were initiated 18 days prior to aggression testing. The drugs were also given daily throughout the period of behavioural observation. Injections were given at 1800 hr and testing commenced at 2100 hr.

Assessment of aggressive and defensive behaviour. Assessment followed 18 days after the 4th preliminary testing session. The remaining procedures were adapted from those of Mugford and Nowell [17]. The isolated mice (fighters) were equally divided into 2 groups and each group was tested on alternate days for 6 periods each (12 consecutive days overall). Eight grouped mice from each defeated-treatment groups (O, TD, CD) were chosen as opponents to the isolated mice. Each grouped mouse was introduced into the cage of the fighter mouse and left there for 4 min. Each fighter mouse had encountered one mouse from each treatment class by the end of the 3rd test session; it encountered two from each treatment group by the end of the 6th test session. The results were taken from the second encounter. The following indices of aggression/defense were recorded: (1) Duration of aggression - taken as the time spent by the fighter mouse chasing, biting or wrestling with drug- or oil-treated opponent. (2) Latency to attack taken as the time between the opponent mice entering the cage and the first bite. (3) Number of times the fighter bites the opponent mouse. (4) Duration of the defense upright posture by the opponent mouse.

Groups OE, TE, and CE consisted of 6 animals each. With the exception that these three groups encountered the nonfighter mice, the drug and behavioural procedure were the same as those with the Defeated Group.

RESULTS

The results from the effects of testosterone and cyproterone administered to the opponent mice (TD, CD) on the various parameters of aggression shown by the fighter mice are summarized in Fig. 1. The results were expressed as a percentage of the control values. Control values in the first three instances were the response of fighter mice to the control-opponent (OD) while in the fourth instance, Defense upright posture, control values were representative of the oil-recipients' responses.

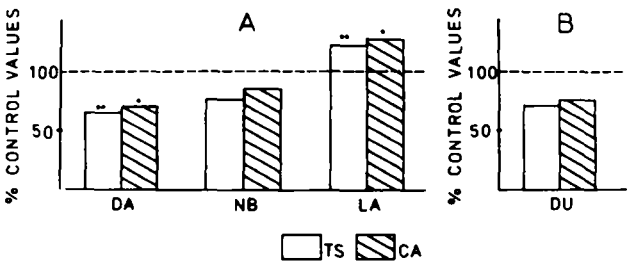

FIG. 1. The effects of testosterone (TS) and cyproterone acetate (CA) on aggressive or defensive behaviour. In Fig. 1A, the responses of the fighter mice to the hormone-treated opponents are duration of aggression (DA), number of bites (NB), and the latency to attack (LA). The fighter's mean responses to the control opponents are DA (23.5 sec), NB (8.2 bites), and CA (129.5 sec). In Fig. 1B, the control opponents mean score for the duration of upright posture (Du) is 51.3 sec. $*p<0.05$ $**p<0.01$ $\cdot p<0.005$

It can be seen that testosterone and cyproterone acetate have a qualitatively similar effect on all 4 parameters of aggression. It is interesting to note that the duration of aggression was significantly reduced in both groups following the drug treatment and that the latency to attack was significantly increased in comparison to the oil treated opponents. The number of bites and the duration of defensive upright postures were also reduced following the administration of testosterone or cyproterone but these changes did not reach the 95% level of statistical significance.

DISCUSSION

The results of this investigation contrast with the findings of other investigators who have shown that testosterone increases aggressiveness of isolated mice [2,18]. However, whereas most investigators have studied aggression in a neutral environment or in the fighter's home-cage which has fresh-bedding, the present study recorded the effects of a high-scented environment on aggression shown to the treated opponents.

Clearly, testosterone treatment of the opponent significantly modified the aggressive behaviour of the fighter mice. Thus the duration of aggression was reduced and the latency to attack was significantly increased towards testosterone recipient. One possible explanation for this was that testosterone stimulates an increase in an aggression-inhibiting pheromone [11].

The effects of cyproterone acetate were qualitatively similar to those of testosterone. These results are compatible with the effect of cyproterone on sexual behaviour [4]. An equipotent non-steroidal anti-androgen, flutamide was also ineffective in antagonizing sexual and aggressive behaviour in rodents, although its effects on peripheral tissues were indicative of its anti-androgen action [12,25].

Three points seem worthy of consideration. First, in this experiment cyproterone acetate-recipients exhibited behaviour which was similar to behaviour induced by testosterone. Second, cyproterone acetate when given alone to castrated mice, stimulated the sexual response [10], and third, previous work (to be published) using the same experimental design, has shown that progesterone administration had no significant effect on either aggressive or defensive behaviour. When progesterone was administered to intact mice there was a decrease in aggression [26]. Further work which was reported would suggest that progesterone has an anti-androgen effect on the CNS [8].

With these considerations in mind, it would appear that with respect to aggressive-defensive behaviour, cyproterone acetate acts like an androgen and shows only minimal anti-androgen qualities.

Experiment 2

Changes in brain γ-aminobutyric acid. Eleven groups of 6 mice were used. Three groups were from the isolation-housing condition (Groups A,B,C) and eight groups were from the group-housing condition (CH,CI,OE,TE,CE,OD, TD and CD). Four days after the last behavioural observations were concluded (in part 1), all animals were again subjected to similar behavioural conditions and then killed by subjecting them to microwave irradiation for 1.2 seconds. The brains were removed and dissected into 10 anatomical areas by the method of Popov *et al.* [20]. These areas were cerebellum, brain stem, mid-brain, olfactory lobe, septum, striatum, thalamus-hypothalamus, hippocampus, amygdala, and cortex. After homogenization in 0.01 N HCl (containing 10% EDTA of the disodium salt) the concentration of γ-aminobutyric acid was determined in the brain regions by the method of Uchida and O'Brien [27].

RESULTS AND DISCUSSION

The concentration of γ-aminobutyric acid in the various brain regions are shown in Table 1. The following general observations can be made: (1) Isolation control group (A) showed a decrease in GABA (specifically in striatum, hippocampus, and amygdala) when compared with the

TABLE 1

CONCENTRATION OF GABA IN DIFFERENT BRAIN AREAS

	BRAIN AREAS(Mean ± SEM in mg/g tissue)									
Isolation										
Treatment Groups	Olfact.	Sept.	Hipp.	Amygd.	Striat.	Thalam.	Cortex	Cerebl.	Brn.Stm.	Mid Brain
Housing-control ª(A)	1.67 ± .31	2.38 ± .42	0.82 ± .08	0.93 ± .13**	0.96 ± .19*	0.94 ± .14	0.80 ± .04	0.79 ± .07	0.70 ± .07	1.12 ± .13
High aggress. †(B)	1.40 ± .19*	2.43 ± .17	1.12 ± .16	0.97 ± .15	1.18 ± .12**	0.85 ± .08	0.75 ± .04	0.78 ± .08	0.70 ± .08	1.09 ± .16
Low aggres. (C)	1.88 ± .22	2.84 ± .48	1.08 ± .03	0.95 ± .03	1.78 ± .21	0.95 ± .10	0.82 ± .05	0.90 ± .06	0.83 ± .04	1.28 ± .07
Housing-control (CH)	2.01 ± .14	2.79 ± .33	0.99 ± .08	1.48 ± .06	1.51 ± .12	0.97 ± .06	0.78 ± .07	0.98 ± .05	0.83 ± .05	1.28 ± .13
Injection-control ª(CI)	1.70 ± .14	2.68 ± .26	1.12 ± .07	1.45 ± .10	1.53 ± .09	1.07 ± .05	0.88 ± .02	1.02 ± .08	0.86 ± .07	1.29 ± .06
Grouped										
Oil-environment ‡(OE)	2.09 ± .15	3.20 ± .36	1.44 ± .22	1.16 ± .07**	1.82 ± .11*	1.10 ± .19	0.83 ± .02	1.02 ± .07	1.04 ± .07**	1.45 ± .18
Testos-environ. §(TE)	1.92 ± .19	2.44 ± .45	1.05 ± .07	1.42 ± .07**	1.37 ± .10**	1.03 ± .07	0.73 ± .04	0.88 ± .08	0.78 ± .06**	1.31 ± .11
CypAcet-environ. §(CE)	1.87 ± .23	3.12 ± .25	1.22 ± .10	1.20 ± .11	1.68 ± .12	1.06 ± .08	0.76 ± .05	0.87 ± .05	0.94 ± .07	1.30 ± .10
Oil-defeat ‡(OD)	1.76 ± .18	2.51 ± .29	1.08 ± .06	1.10 ± .14**	1.36 ± .09	0.94 ± .10	0.75 ± .04	0.88 ± .08	0.91 ± .04	1.10 ± .06
Testos-defeat §(TD)	1.79 ± .20	3.35 ± .42	1.19 ± .09	1.15 ± 0.8	1.58 ± .29	1.10 ± .11	0.85 ± .03	0.94 ± .03	0.98 ± .10	1.14 ± .14
CypAcet-defeat §(CD)	1.62 ± .09	2.72 ± .39	0.92 ± .08	1.19 ± .10	1.58 ± .17	1.10 ± .13	0.79 ± .05	0.95 ± .06	0.98 ± .14	1.29 ± .14

Statistical comparison made with ªgroup-housing control, †low-aggression group, ‡injection-control, and §appropriate oil-control.
*p0.05.
**p0.01.

grouped controls (CH). (2) Differences between high and low aggressive fighters (B and C, respectively) were found in the olfactory, and striatal areas with the low-aggressive mice exhibiting higher concentrations of the amino acid. (3) Injection with oil had no significant effect on GABA concentrations, although a noted reduction in GABA was found in the olfactory lobe. (4) Group OD showed an overall reduction in GABA levels when compared with environment-controls (CE), but a comparison with injection controls (CI) showed only a significant reduction in the amygdala. (5) In the defeated animals, testosterone and cyproterone acetate increased the concentrations in the striatum and thalamus. Their effects were not compatible with their suggested differential actions but were compatible with the behavioural effects discussed in part 1. The difference between the androgen and the anti-androgen was most noticeable in the hippocampus where testosterone increased and cyproterone decreased the GABA concentration. In addition cyproterone increased the GABA concentration in the mid-brain region and testosterone increased the levels in the septum.

A finding worthy of enumeration was the effect of treatment on the drug-environment groups. Group OE animals, which were oil-recipients and encountered the non-fighter, showed increased concentrations of GABA in the brain stem, septum, striatum and hippocampus but showed a significantly reduced concentration in the amygdala. In these same brain areas, testosterone specifically opposed this change in GABA. In light of this androgenic effect, a comparison of the anti-androgen-recipients (CE) with oil-recipients (OE) showed no apparent difference, suggesting that this environmental condition played a role in altering GABA levels which were possibly reflective of an alteration in endogenous testosterone. In support of this suggestion, Jones and Nowell [13, 14, 15, 16] reported that isolated mice produced a long-acting, urinary substance which was stressful to introduced male mice. Furthermore, the study by Bliss [3] suggested that chronic noxious environments reduced testicular testosterone levels and that brain biogenic amines may control testosterone levels.

GENERAL CONCLUSIONS

In summary, there was a decrease in the aggressiveness of isolated mice towards nonisolated mice which had been chronically treated with either testosterone or cyproterone acetate.

Housing conditions alter GABA concentrations.

The tendency to exhibit certain aggressive responses was inversely related to GABA concentrations in particular brain regions (e.g. low aggressive mice showed an increase concentration of GABA relative to concentrations found in high aggressive mice.)

Defeat reduces GABA in some brain regions, while testosterone and cyproterone acetate were not found to oppose such reductions.

A novel nonaggressive environment increases GABA concentration while decreasing this amino acid in the amygdala. Testosterone was found to counteract these environmentally induced changes in GABA.

It is concluded that the inhibitory transmitter substance γ-aminobutyric acid may play a role in isolation induced aggressive behaviour. Thus one further transmitter substance must be added to the list of neurotransmitters which already include the catecholamines [28], serotonin [9] and acetylcholine [1] and which have been implicated in aggressive behaviour.

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REFERENCES

1. Allikmets, L. H. Cholinergic mechanisms in aggressive behaviour. *Med. Biol.* **52:** 19–30, 1974.
2. Beeman, E. A. The effect of male hormone on aggressive behaviour in mice. *Physiol. Zool.* **20:** 373–405, 1947.
3. Bliss, E. L., A. Frischot and L. Samuels. Brain and testicular function. *Life Sci.* **11:** 231–238, 1972.
4. Block, G. J. and J. M. Davidson. Behavioural and somatic responses to the antiandrogen cyproterone. *Horm. Behav.* **2:** 11–15, 1974.
5. Brody, J. F., P. A. De Feudis and F. V. De Feudis. Effects of micro-injections of L-glutamate into the hypothalamus on attack and flight behaviour in cats. *Nature* **224:** 1330, 1969.
6. De Feudis, F. V., P. Madtes and J. G. Camacho. Binding of glycine and γ-aminobutyric acid to synaptosomal fractions of the brains of differentially-housed mice. *Expl. Neurol.* **50:** 207–213, 1976.
7. Eleftheriou, B. E. and R. L. Church. Brain levels of serotonin and norepinephrine in mice after exposure to aggression and defeat. *Physiol. Behav.* **3:** 977–980, 1968.
8. Floody, O. R. and D. W. Pfaff. Steroid hormones and aggressive behaviour: approaches to the study of hormone sensitive brain mechanisms for behaviour. In: *Aggression*, edited by S. H. Frazier. New York: The Williams and Wilkins Company, 1974.
9. Goldberg, M. E., D. Dubnic, M. Hefner and A. I. Salama. Influence of chlorpromazine on brain serotonin turnover and body temperature in isolated aggressive mice. *Neuropharmacology* **12:** 249–260, 1973.
10. Hall, N. R. and W. G. Luttge. Maintenance of sexual behaviour in castrate male SW mice using the anti-androgen cyproterone acetate. *Pharmac. Biochem. Behav.* **3:** 551–555, 1975.
11. Harmatz, P., R. C. Boelkins and S. Kessler. Postisolation aggression and olfactory cues. *Behav. Biol.* **13:** 219–224, 1975.
12. Heilman, R. D., M. Brugmans, F. C. Greenslade and J. P. Da Vanzo. Resistance of androgen-mediated aggressive behaviour in mice to flutamide, an antiandrogen. *Psychopharmacology* **47:** 75–80, 1976.
13. Jones, R. B. and N. W. Nowell. Aversive and aggressive-promoting properties of urine from dominant and subordinate male mice. *Anim. Learn. Behav.* **1:** 207–210, 1973.
14. Jones, R. B. and N. W. Nowell. The effects of urine on the investigatory behaviour of male albino mice. *Physiol. Behav.* **11:** 35–38, 1973.
15. Jones, R. B. and N. W. Nowell. Aversive effects of the urine of a male mouse upon the investigatory behaviour of its defeated opponent. *Anim. Behav.* **21:** 707–710, 1973.
16. Jones, R. B. and N. W. Nowell. The urinary aversible pheromone of mice: species, strain and grouping effects. *Anim. Behav.* **22:** 187–191, 1974.
17. Mugford, R. A. and N. W. Nowell. The preputial glands as a source of aggressive-promoting odour in mice. *Physiol. Behav.* **6:** 247–249, 1971.
18. Mugford, R. A. and N. W. Nowell. The relationship between endocrine status of female opponents and aggressive behaviour of male mice. *Anim. Behav.* **19:** 153–155, 1971.

19. Nelson, S. R. and E. J. Wabaszek. Pharmacology of the central nervous system. *Prog. Neurol. Psychiat.* **26:** 123–149, 1971.
20. Popov, N., W. Pohle, V. Rosler and H. Matthies. Regionale Verteilung von gamma-Aminobuttersaure, glutaminsäure, Asparaginsäure, Dopamin, Noradrenalin, und Serotonin im Rattenhirn. *Acta Biol. med. germ.* **18:** 695–702, 1967.
21. Rose, R. M. Androgen response to stress. I. Psychoendocrine relationships and assessment of Androgen Activity. *Psychosom. Med.* **31:** 405–417, 1969.
22. Rose, R. M., P. G. Bourne, R. O. Poe, E. H. Mougey, D. R. Collins and J. W. Mason. Androgen response to stress II. Excretion of testosterone, epitestosterone, androsterone and etiocholanolone during basic combat training and under threat of attack. *Psychosom. Med.* **31:** 418–436, 1969.
23. Sayler, A. The effect of anti-androgen on aggressive behaviour in the gerbill. *Physiol. Behav.* **5:** 667–671, 1970.
24. Scott, J. P. and E. Frederickson. The causes of fighting in mice and rats. *Physiol. Zool.* **24:** 273–309, 1951.
25. Sodersten, P., G. Gray, D. A. Damassa, E. R. Smith and J. M. Davidson. Effects of a non-steroidal antiandrogen on sexual behaviour and pituitary-gonadal function in the male rat. *Endocrinology* **97:** 1468–1475, 1975.
26. Suchowsky, G., L. Pegrassi and A. Bonsignori. The effect of steroids on aggressive behaviour in isolated male mice. In: *Aggressive Behaviour*, edited by S. Garattini and E. B. Siggs. New York: John Wiley and Son, 1969.
27. Uchida, T. and R. O. O'Brien. The effects of hydrazine on rat brain 5-hydroxytrytamine, norepinephrine, and γ-aminobutyric acid. *Biochem. Pharmacol.* **13:** 725–730, 1964.
28. Valzelli, L. Drugs and aggressiveness. In: *Advances in Pharmacology*, edited by S. Garattini and P. A. Shore. London: Academic Press, 1967, 79–108.