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The Effects of p-Chlorophenylalanine, Fenfluramine and α-Methyltyrosine on Marking Responses in the Male Mongolian Gerbil *(Meriones unguiculatus)*

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BATTY, J. AND B. J. MEYERSON. *The effects of p-chlorophenylalanine, fenfluramine and α-methyltryosine on marking responses in the male Mongolian gerbil* (Meriones unguiculatus). PHARMAC. BIOCHEM. BEHAV. 12(2) 181–184, 1980.—Marking behavior was maintained by testosterone propionate treatment in castrated Mongolian gerbils. p-Chlorophenylalanine (PCPA) enhanced and α-methyltyrosine (α-MT) inhibited responses. No effect was seen by PCPA in non-TP-treated subjects. Fenfluramine inhibited the response in intact gerbils. The data suggest that serotonergic inhibitory mechanisms exist which are implicated in testosterone dependent marking behavior in the Mongolian gerbil.

Marking behavior Gerbil Testosterone p-Chlorophenylalanine α-Methyltyrosine Fenfluramine

SEXUAL responses in the male rat are dependent to a large extent on the amount of circulating androgens [1]. Castration results in a progressive decline in sexual behavior [2], and treatment with testosterone propionate (TP) restores the behavior to precastration levels [3].

There is now much data showing that the levels of monoamines in the brain also have effects on sexual responses. For example the serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) [6] can increase sexual responses in intact [14] or castrated testosterone treated male rats [7]. This stimulatory effect of PCPA is not mediated by changes in endogenous testosterone production, since the drug is also effective in castrate adrenalectomized subjects injected with low doses of TP [8]. Administration of the serotonin precursor DL-5-hydroxytryptophan results in a decreased sexual response in castrated testosterone treated male rats [8], further indicating serotonergic mechanisms to be implicated in mounting behavior. In contrast to PCPA, the catecholamine synthesis inhibitor α-methyltyrosine (α-MT) decreases sexual responses in castrate male rats, and treatment with catecholamine precursors such as L-dihydroxyphenylalanine increases sexual responses [8].

These results (and many others not cited, see review by Meyerson and Malmnäs [10]) suggest that monoamines are involved in the mechanisms which control the display of testosterone dependent measures of sexual behavior in the male rat.

This paper examines another testosterone dependent behavior—ventral marking in the male Mongolian gerbil *(Meriones unguiculatus)*. Gerbils have a ventral sebaceous gland which is used to "mark" objects on the ground in their environment [15]. The behavior is easily observed in the laboratory and consists of an approach to an object, some sniffing, followed by a mount and rubbing of the gland over the object's surface with one forward movement. Marking responses in the male decline after castration, but can be restored with injections of TP [15]. This paper investigates whether drugs which influence monoaminergic transmission have effects on the display of marking responses in the male Mongolian gerbil. Experiments 1 and 2 examine the effects of altered serotonin function and Experiment 3 examines the effects of altered catecholamine function.

METHOD

Housing

Animals were weaned at 25 days and housed in single sex groups until 10–11 weeks. At this age they were isolated into single cages (Macrolon) and used in the experiment at 12–13 weeks. Lighting was on a reverse 12/12 cycle (light off 8:30 a.m.) and room temperature was maintained at 22°C. Standard laboratory chow and water were available ad lib.

Behavior Observation

Marking responses were observed in a 50×50 cm open field, with 15 cm walls and 4 raised pegs equidistantly placed on the floor which was marked off into squares (Fig. 1). Each test session was 5 min, and the number of discrete ventral rubs and the number of lines crossed was recorded. Tests were conducted between 9 a.m. and 4 p.m. under dimmed illumination. After each test the floor area was thoroughly cleaned with 70% alcohol.

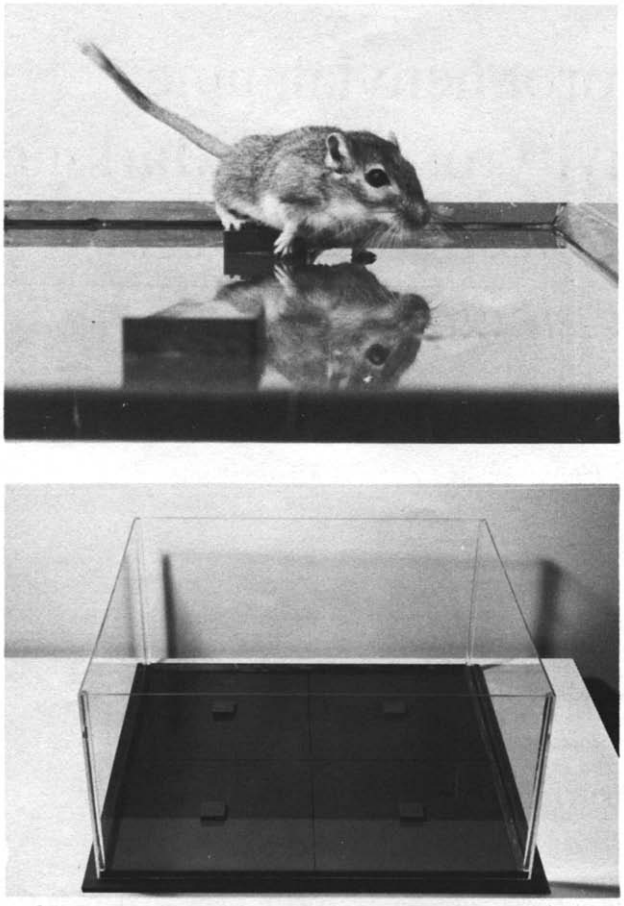

FIG. 1. The marking response in the Mongolian gerbil and the observation arena.

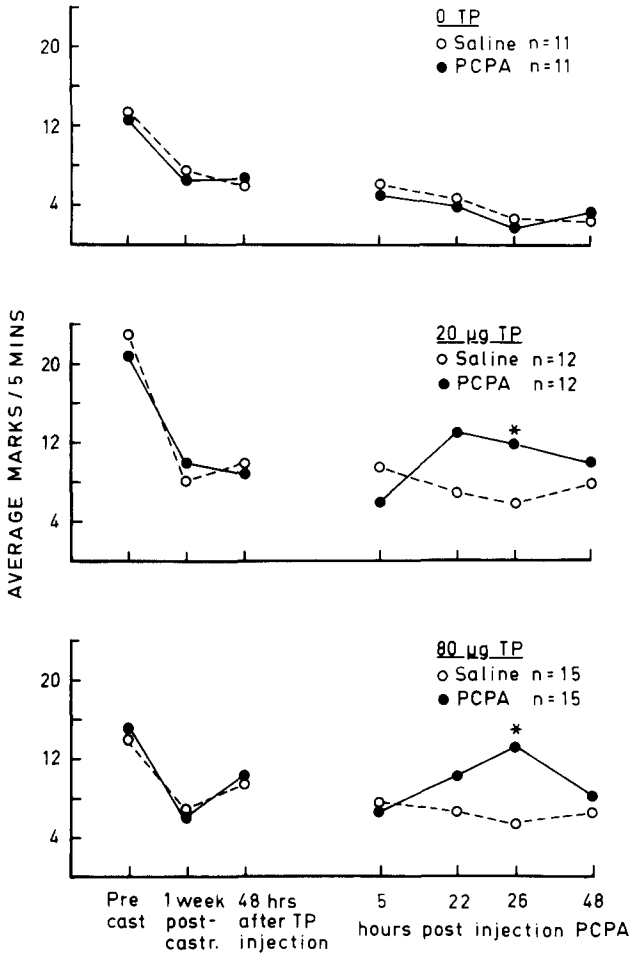

FIG. 2. The effect of p-chlorophenylalanine (PCPA) on marking responses in testosterone propionate (TP) treated castrated Mongolian gerbils.

Hormones and Drugs

Testosterone propionate (TP, Organon) was dissolved in olive oil and DL-para-chlorophenylalanine methylester HCl (PCPA, Labkemi, Stockholm), α-methyl-p-tyrosine HCl (α-MT, Labkemi, Stockholm) and fenfluramine HCl (Benson, Copenhagen) were dissolved in saline. Injections were made subcutaneously in the "scruff" of the neck. The doses refer to the salt.

Castration

Animals were castrated under ether anesthesia via a midscrotal incision.

EXPERIMENT 1

Effects of PCPA on Marking Responses

This experiment examined the effects of the serotonin synthesis inhibitor PCPA on marking responses in castrated testosterone treated male gerbils.

METHOD

Marking responses of intact male gerbils were observed on two consecutive days and subjects were then castrated and retested for marking responses after one week. Only those animals which showed more than 10 marks in the precastration test session, and which after castration showed at least a 40% reduction in marking were used (approximately 65% of animals tested fulfilled this criterion). This was because the experiment was designed to investigate the effects of PCPA on testosterone dependent aspects of marking behavior. Subjects were then injected immediately after the postcastration test with either 0 µg TP (n=22), 20 µg TP (n=24) or 80 µg TP (n=30) in a volume of 0.05 ml oil. Forty-eight hours later subjects were retested for marking responses and half from each hormone dose group were injected with saline (0.1 ml) and half with PCPA (200 mg/kg in 0.1 ml saline). Behavior was observed after 5, 22, 26 and 48 hr.

RESULTS

The marking responses for the three experimental groups are shown in Fig. 2.

After castration and injections of TP, the three groups showed different levels of marking responses. Two days after injection the controls and experimental subjects of the 0 µg TP group displayed on the average 48% of their precastration levels an the 20 µg TP and 80 µg TP group displayed

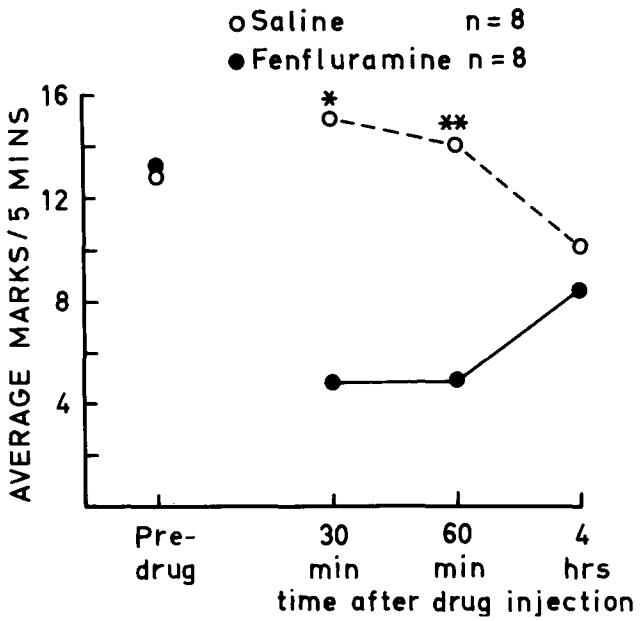

FIG. 3. The effect of fenfluramine on marking responses in intact Mongolian gerbils.

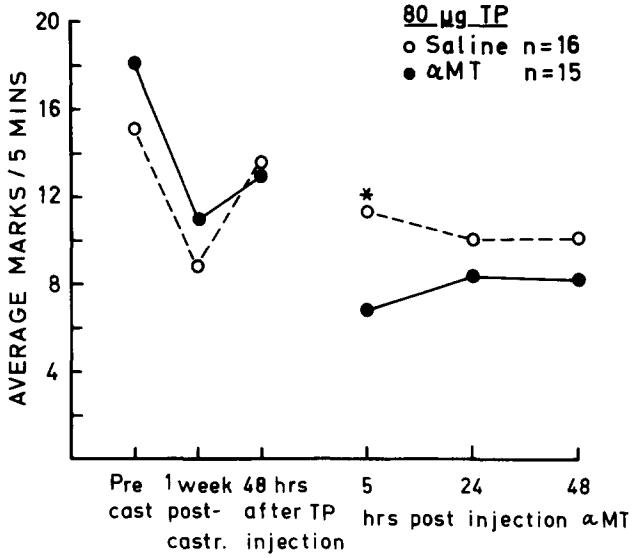

FIG. 4. The effect of alpha-methyl-p-tyrosine (α-MT) on marking responses in testosterone propionate (TP) treated castrated Mongolian gerbils.

53% and 68% respectively. Four days after the injection of TP the control subjects of the 0 µg TP group displayed 20% of precastration marking levels, and the 20 µg TP and 80 µg TP groups displayed 32% and 50%, respectively. The effects of PCPA were studied during a time when the effects of the TP treatment were declining.

In the 0 µg TP group, there were no significant differences in the marking scores between the saline and PCPA treated animals in any of the tests. In the 20 µg and 80 µg TP groups there was an increased marking frequency in the PCPA treated animals in 22, 26 and 48 hr, which was significant for both groups at 26 hr (Mann-Whitney U test, 20 µg TP $U=33$, $p<0.03$; 80 µg TP $U=62$, $p<0.05$).

There were no significant differences between saline and PCPA treated animals in the number of lines crossed in any of the experimental groups, and the PCPA treated groups showed no disturbance in overt behavior at any time. However, the subcutaneous injection of PCPA did cause considerable skin irritation and the subjects scratched the injection site.

EXPERIMENT 2

The Effects of Fenfluramine on Marking Behavior

The previous experiment has shown that the serotonin synthesis inhibitior PCPA can increase the display of marking responses in the castrate, testosterone treated male gerbil. This experiment examined whether fenfluramine, an amphetamine derivative with a stimulatory effect on serotonergic activity [5,11] also has effects on marking response.

METHOD

Pilot experiments using castrate testosterone injected males suggested that fenfluramine had effects on marking responses less than an hour after drug injection, and that these effects had disappeared after 4 hr. Since the drug effect had such a rapid onset it was decided to use intact males, rather than castrates, since a change in endogenous testosterone levels is unlikely to affect marking behavior in so short a time period.

Marking responses in intact male gerbils were observed on 2 consecutive days and subjects showing an average of more than 10 marks per test were used as experimental subjects. The following day subjects were retested (Pre-drug Fig. 3) and immediately following the test were injected with fenfluramine (6 mg/kg, n=8; or saline only, n=8). Behavior was observed 0.5, 1 and 4 hr after injection.

RESULTS

The marking responses for experimental and control groups are shown in Fig. 3.

Prior to drug treatment there were no significant differences in marking responses between the two groups, but at 30 and 60 min post injection, the fenfluramine treated group showed a significant decrease in marking frequency (Mann-Whitney U-test; $U=6$, $p<0.004$, $U=9.5$, $p<0.02$). Four hours after injection there was no significant differences between the drug treated and saline treated animals. There were no significant differences in the number of lines crossed between saline and fenfluramine treated animals in any of the tests and the drug treated animals showed no disturbance in overt behavior.

EXPERIMENT 3

The Effect of α-Methyltyrosine on Marking Behavior

This experiment examines the effects of decreased catecholamine activity on marking responses, α-methyltyrosine inhibits catecholamine synthesis at the tyrosine hydroxylase level [2].

METHOD

Marking response of intact male gerbils were observed on two consecutive days, and subjects were then castrated and

retested for marking responses after one week. Only animals which showed a postcastration decline in marking responses of at least 30% were used in the experiment. Subjects were then injected with 80 μg TP in 0.05 ml oil and were retested for marking responses 48 hours later. After this test animals were injected with either 100 mg α-MT (n=15), or with saline only (n=16). Behavior was observed after 5, 24 and 48 hr.

RESULTS

The marking response of drug and saline treated groups are shown in Fig. 4.

Prior to drug treatment there were no significant differences in marking responses between the two groups. Five hours after injection, the α-MT treated groups showed a significantly lower level of marking compared to controls (Mann-Whitney U-test, U=62, $p<0.03$). There were no significant differences between the groups 24 and 48 hr after injection. There were no significant differences in the number of lines crossed between the saline and α-MT treated groups, and the drug treated animals showed no disturbance in overt behavior.

DISCUSSION

Our results show that after castration the marking behavior of the male gerbil gradually decreases over a period of about 2 weeks. During this period it is possible to maintain or restore the behavior to a submaximal response level by injections of TP. The response measured 48 hr after TP injection was greater than the one seen after 70–74 hr. During this time of declining TP-induced response, PCPA treatment resulted in a significant increase of the number of markings made 26 hr after PCPA injection. The extent of this facilitatory effect was such that the response became equivalent to the level of the 48 hr TP-induced response. This might mean that the PCPA treatment maintained the 48 hr response. The biochemical effects of PCPA in the gerbil are almost unknown. However, in the laboratory rat it is well established that systemic administration of PCPA produces relatively long term decrease of brain serotonin [6] by suppressing tryptophan hydroxylase, the rate limiting enzyme in serotonin production. There is also some evidence that PCPA, in addition to its inhibition of serotonin synthesis, releases serotonin from storage sites with a subsequent depletion and long-term impaired serotonergic activity [9]. Initially, however, the biosynthesis of catecholamines is influenced as well [6] and a decrease in the catecholamine synthesis might have influenced the results. The delay of the facilitatory effect may have been due to a decreased catecholamine synthesis as a very clearcut decrease of the marking response resulted after administration of the catecholamine synthesis inhibitor α-methyltyrosine in analogous experiments.

The major functional effect of fenfluramine is an increase of the serotonergic activity by a release of serotonin from storage sites with a subsequent increase of activity at postsynaptic receptors [11,15]. The clearcut inhibitory effect of fenflurmaine on the marking behavior gives further support to the hypothesis that the stimulatory effect of PCPA is related to serotonin depletion and that the marking response is inhibited by increased a facilitated by decreased serotonergic activity.

As no effect was seen by PCPA in non-TP treated subjects, the present data suggest that the serotonergic and testosterone dependent neuronal mechanisms are related.

So far the marking response seems to be analogous to the mounting behavior in the rat, in that the behavior is facilitated by PCPA and decreased by α-MT.

In conclusion there is evidence that there exist serotonergic inhibitor mechanisms which are implicated in the control of testosterone induced marking behavior in the Mongolian gerbil and that a diminished serotonergic activity removes this inhibition.

ACKNOWLEDGEMENTS

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REFERENCES

1. Davidson, J. M. In: *Hormones and Reproductive Behavior*, edited by S. Levine. New York: Academic Press, 1972, pp. 63–103.
2. Davidson, J. M. Characteristics of sex behavior in male rats following castration. *Anim. Behav.* **14:** 266–272, 1966.
3. Beach, F. A. and A. M. Holz-Tucker. Effects of different concentrations of androgen upon sexual behavior in castrated male rats. *J. comp. physiol. Psychol.* **42:** 433–453, 1949.
4. Mitler, M. M., B. Morden, S. Levine and D. Dement. The effects of parachlorophenylalanine on the mating behavior of male rats. *Physiol. Behav.* **8:** 1147–1150, 1972.
5. Costa, E., A. Groppetti and A. Revuelta. Action of fenfluramine on monoamine stores of rat tissues. *Br. J. Pharmac.* **41:** 57–64, 1971.
6. Koe, B. K. and A. Weissman. p-Chlorophenylalanine in a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
7. Malmnäs, C. O. and B. J. Meyerson. p-Chlorophenylalanine and copulatory behavior in the male rat. *Nature* **232:** 398–400, 1971.
8. Malmnäs, C. O. Monoaminergic influence on testosterone-activated copulatory behavior in the castrated male rat. *Acta physiol. scand.* Suppl. 395, pp. 1–128, 1973.
9. Meyerson, B. J. and T. Lewander. Serotonin synthesis inhibition and estrous behaviour in female rats. *Life Sci.* **9:** 661–671, 1970.
10. Meyerson, B. J. and C. O. Malmnäs. Brain monoamines and sexual behaviour. In: *Biological Determinants of Sexual Behaviour*, edited by J. B. Hutchinson. Chichester: Wileye, 1978, pp. 521–554.
11. Michanek, A. and B. J. Meyerson. A comparative study of different amphetamines on copulatory behavior and stereotype activity in the female rat. *Psychopharmacology* **53:** 175–183, 1977.
12. Spector, S., A. Sjoerdsma and S. Udenfriend. Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.* **147:** 86–95, 1965.
13. Södersten, P., K. Larsson, S. Ahlenius and J. Engel. Sexual behavior in castrated male rats treated with monoamine synthesis inhibitors and testosterone. *Pharmac. Biochem. Behav.* **5:** 319–327, 1976.
14. Tagliamonte, A., W. Fratta, G. Mercuro and G. L. Gessa. Failure of p-chlorophenylalanine (PCPA) to restore copulatory behaviour in male castrated rats. *Riv. Farm. Terap.* **III:** 399–403, 1972.
15. Thiessen, D. D., H. C. Friend and G. Lindzey. Androgen control of territorial marking in the Mongolian gerbil. *Science* **160:** 26–30, 1968.