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LACK OF COPULATORY BEHAVIOUR IN MALE CASTRATED RATS AFTER *p*-CHLOROPHENYLALANINE

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1 The effect of *p*-chlorophenylalanine (PCPA) on the copulatory behaviour of normal and castrated male rats with females in oestrus was studied.

2 Castration 2 months before the experiment completely prevented the increased copulatory behaviour produced by PCPA in normal rats.

3 The administration of testosterone restored the copulatory behaviour in the castrated rats indicating that testosterone is essential for this behaviour.

Introduction

p-Chlorophenylalanine (PCPA), a compound that inhibits the synthesis of 5-hydroxytryptamine (5-HT) rather selectively (Koe & Weissman, 1966), stimulates homo- and heterosexual mounting behaviour in male animals (Sheard, 1969; Tagliamonte, Tagliamonte, Gessa & Brodie, 1969; Shillito, 1970; Ferguson, Henriksen, Cohen, Mitchell, Barchas & Dement, 1970; Hoyland, Shillito & Vogt, 1970). However, we have observed that PCPA fails to cause male to male mounting behaviour in castrated rats, but this effect is restored and greatly potentiated by testosterone (Gessa, Tagliamonte, Tagliamonte & Brodie, 1970).

In agreement with our findings, Gawienowski & Hodgen (1971) reported that PCPA failed to produce homosexual mounting behaviour in sexually immature male rats, but this behaviour was produced if the animals were primed with testosterone. Moreover, Malmnäs & Meyerson (1971) found that PCPA potentiates the effect of a submaximal dose of testosterone on the copulatory behaviour of male castrated rats.

These observations and the fact that PCPA-induced sexual stimulation in intact rats is blocked by 5-hydroxytryptophan, the direct precursor of 5-HT, suggest that brain 5-HT plays an inhibitory role in male sexual behaviour and that testosterone is essential for this behaviour to occur (Gessa *et al.*, 1970). However, this theory has been challenged recently by Bond, Shillito & Vogt (1972) who reported that PCPA produces homosexual mounting behaviour in male castrated rats.

In view of these conflicting results, it was of interest to study the effect of PCPA on the copulatory behaviour of male castrated rats exposed to female rats in oestrus.

Methods

Male Wistar rats had been castrated or sham-operated 2 months before the study began. At this time, the rats were 140-150 days old. They were housed individually for at least one week before the beginning of the experimental period, under a reversed light-dark cycle (12 h light, 12 h dark with lights on at 21 h 00 min) and fed *ad libitum*. The mating tests were done in the dark phase of the cycle, from 10 h 00 min to 12 h 00 min in a red light.

Each rat underwent three mating tests with a female in oestrus at one week intervals. As a base-line level of sexual activity, we considered that shown by the rat in the third mating test. In order to show an increase in sexual activity after PCPA (Gessa, Tagliamonte & Tagliamonte, 1971), sham-operated rats with low level of sexual activity were used in the present study.

One week after the last test, intact and castrated rats were treated with DL-*p*-chlorophenylalanine methylester hydrochloride at a dose of 100 mg/kg i.p. daily for 4 days. A mating test was carried out 12 h after the last treatment.

The female rats used in this study were Wistar rats which had been ovariectomized 3 weeks before use and brought into heat by subcutaneous injections of oestradiol and progesterone in olive oil (Lindström & Meyerson, 1967). For each

mating test the female in oestrus was introduced in the male's own cage.

The effect of PCPA on brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) was studied in separate groups of male sham-operated and castrated rats of the same strain and age. 5-HT and 5-HIAA concentrations were measured fluorimetrically as previously described (Perez-Cruet, Tagliamonte, Tagliamonte & Gessa, 1971).

Results

The results are summarized in Table 1. PCPA treatment lowered the 5-HT and 5-HIAA concentrations in the brain to about 20% of the control value, in both castrated and sham-operated rats.

Forty-eight per cent of the 25 sham-operated rats exhibited mountings, intromissions and ejaculations prior to PCPA treatment. As previously reported (Tagliamonte, Tagliamonte & Gessa, 1971), the administration of PCPA to these rats increased to 80% the number of animals showing the full pattern of copulatory behaviour. On the other hand, none of the castrated rats exhibited mountings, intromissions or ejaculations either before or following the administration of PCPA at a dose schedule which greatly depleted brain 5-HT and 5-HIAA concentrations.

Ten days after the last treatment with PCPA, castrated rats were treated with testosterone propionate (0.1 mg/kg subcutaneously) daily for 4 days and retested with females in oestrus 12 h after the last treatment. As Table 1 shows, after the administration of testosterone 60% of the castrated rats exhibited mountings, intromissions and ejaculations. Thus, these rats were capable of copulating provided testosterone was administered.

Discussion

Contradictory data have been reported from different laboratories (Gessa *et al.*, 1970; Gawienowski & Hodgen, 1971; Bond *et al.*, 1972) on the stimulatory effect of PCPA on the homosexual behaviour of castrated rats.

In order to assess the role played by testosterone on the sexual stimulant effect of PCPA, the male to female copulatory behaviour of castrated rats was studied. This behaviour has been characterized by the following strict criteria 'It is a requirement that the male mounts directly from the rear, clasps its partner firmly with the forelegs, palpates the subject's sides with the forelegs and executes vigorous pelvic thrusts' (Money & Ehrhardt, 1971), and allows a more detailed and objective picture of male sexual activity.

The results of these experiments indicate the PCPA treatment *per se* cannot substitute for testosterone in eliciting the copulatory behaviour of castrated rats, thus supporting our previous hypothesis that testosterone is essential for the sexual stimulatory effect of PCPA.

A possible explanation for the discordant findings of Bond *et al.* (1972) may be the different experimental conditions used by these authors. Their observations were made on grouped animals, with a different treatment schedule and different

Table 1 Effect of *p*-chlorophenylalanine (PCPA) on brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) and on the copulatory behaviour of male sham-operated and castrated rats

Condition	Treatment	% Males exhibiting at least one*			Brain 5-HT (μg/g)	Brain 5-HIAA (μg/g)
		mounting	intromission	ejaculation		
Sham-operated	None	48	48	48	0.63 ± 0.04	0.57 ± 0.03
	PCPA	80	80	80	0.09 ± 0.07	0.07 ± 0.06
Castrated	None	0	0	0	0.65 ± 0.03	0.59 ± 0.05
	PCPA	0	0	0	0.11 ± 0.06	0.09 ± 0.04
	Testosterone	60	60	60	–	–

* Occurring within 30 min after male and female rats were paired.

Mating tests were carried out 12 h after last treatment with PCPA using 25 sham-operated and 25 castrated rats. Each biochemical value is the mean with s.e. from 6 experiments: PCPA-treated animals were killed 12 h after the last treatment.

PCPA (methylester . HCl) was given i.p. daily for 4 days at a dose of 100 mg/kg.

Ten days after last test, castrated animals were treated s.c. with testosterone propionate (0.1 mg/kg) daily for 4 days: a mating test was carried out 12 h after the last treatment with testosterone.

light-dark cycle. In addition, their very young animals (5-6 weeks old) showed, after castration, a more pronounced increase in sexual activity than rats aged between 3 and 4 months; whereas the rats used in our experiments in both homo- and heterosexual studies, were from 6 to 8 months old.

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