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Sexual Behavior in Castrated Male Rats Treated with Monoamine Synthesis Inhibitors and Testosterone

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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(3) 319–327, 1976. – Castrated male rats treated daily with the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA, 20 mg/kg) started to display mounts, intromissions and ejaculations more rapidly in response to daily treatment with testosterone propionate (TP, 0.15 mg/kg) than NaCl-treated rats. Daily treatment with the catecholamine (CA) synthesis inhibitor α-methyl-p-tyrosine (α-MT, 20 mg/kg) had no effect on the behavioral response to subsequent TP treatment. The acceleration of TP-induced sexual behavior by PCPA pretreatment was inhibited by pretreatment with DL-5-HTP (20 mg/kg) but not with L-DOPA (12.5 mg/kg). Analyses of brain monoamines showed that the PCPA treatment reduced brain 5-HT levels and produced a marked inhibition of the 5-HT synthesis. The 5-HTP treatment restored brain 5-HT levels to normal. Daily treatment with PCPA also reduced brain CA levels and inhibited the CA synthesis but these biochemical effects were not related to the effects of PCPA on sexual behavior. Daily treatment with PCPA (40 mg/kg for 12 days) or treatment with 126 mg/kg PCPA for 3 days induced the complete pattern of sexual behavior in 5 of 9 and 19 of 30 castrated rats respectively without concurrent TP treatment. It is suggested that 5-HT exerts a modulating influence on sexual behavior in male rats.

Testosterone α-methyl-p-tyrosine PCPA 5-HT synthesis Sexual behavior

TESTOSTERONE (T) is the main activator of sexual behavior in male rats (see [18,51]). Castration of sexually experienced males is followed by a progressive decline of sexual behavior [16] and exogenous treatment with T propionate (TP) gradually restores the sexual behavior of castrated males (see [51]). The mechanisms whereby T, either directly or after conversion to its metabolites (see [41,43]) exerts this control are unknown. However, it has been shown that in the presence of submaximal doses of TP manipulation of brain monoamine neurotransmission affects the display of sexual behavior by castrated male rats. Thus, treatment with p-chlorophenylalanine (PCPA), a relatively specific inhibitor of the synthesis of 5-hydroxytryptamine (5-HT) [32], of castrated TP-treated male rats enhanced the display of sexual behavior [23, 36, 37] and administration of 5-hydroxytryptophan (5-HTP), the precursor to 5-HT, was found to inhibit the sexual behavior of castrated TP-treated males [36]. These findings led to the hypothesis that 5-HT exerts an inhibitory effect on sexual behavior in male rats [22,36]. If T normally controls the sexual behavior of male rats by affecting central monoamine neurotransmission, then treatment with drugs which alter central monoamine metabolism would be expected to have similar effects as T on the sexual behavior of castrated male rats. In the following series of experiments we have tested this prediction.

GENERAL METHOD

Animals

Male Wistar rats (Möllegård Breeding Laboratories, Ejby, Denmark) were used in all experiments. The rats were maintained with continuous access to food and water in an air conditioned temperature-controlled colony room in which the lights were out between 1100–2300. Prepuberally castrated males were castrated at 30 days of age. Postpuberally castrated males were castrated at 55–60 days of age. All postpuberally castrated males were given three preliminary screening tests for sexual behavior

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according to the method described below, to ensure that they would not show any sexual response in the absence of hormones.

Behavioral Tests

The rats were allowed to adapt in a circular (50 cm dia.) Plexiglas mating arena for 2–5 min. Thereafter, a stimulus female made sexually receptive by combined treatment with estradiol benzoate (20 μg/rat 48 hr before testing) and progesterone (0.5 mg/rat 6 hr before testing) was introduced. The following behavioral parameters were recorded: *Mount*: mount with pelvic thrusting but without intromission. *Intromission*: mount with intromission. *Ejaculation*: mount with a final deep intromission, slow dismounting and genital grooming. The latency to the first intromission, *intromission latency*, the interval from the first intromission to ejaculation, *ejaculation latency*, and the time from ejaculation to the following intromission, *Postejaculatory interval*, were recorded. Tests were ended if the intromission latency was > 15 min, the ejaculation latency was > 30 min, or if the postejaculatory interval was > 15 min. Tests for sexual behavior started at least two hr after lights off.

Injected Materials

The following drugs and hormones were used: p-chlorophenylalanine methyl ester HCl (PCPA, H 69/17, Hässle, Mölndal, Sweden), α-methyl-p-tyrosine methyl ester HCl (α-MT, H 44/68, Hässle, Mölndal, Sweden), benserazid HCl (Ro 4-4602, Hoffman La-Roch, Basel, Switzerland), L-3,4-dihydroxyphenylalanine methyl ester HCl (L-DOPA, H 19/61, Hässle, Mölndal, Sweden), DL-5-hydroxytryptophan (5-HTP, Ajinomoto, Tokyo, Japan), 3-hydroxybenzyl hydrazine HCl (NSD 1015, synthesized by Dr. P. Martinsson, Department of Pharmacology, University of Göteborg, Sweden) and testosterone propionate (TP, Schering, Berlin, Germany). The doses refer to the forms indicated above. All drugs were dissolved in 0.9% NaCl and injected IP in a volume of 2.0 ml/kg. TP was dissolved in peanut oil and injected SC in a volume of 0.5 ml/kg.

Biochemical Methods

The rats were killed by decapitation and the whole brains (olfactory lobes excluded) were dissected out and placed in ice-cold perchloric acid. The brains were homogenized in 10 ml 0.4 N perchloric acid containing 5 mg $Na_2S_2O_5$ and 20 mg EDTA. The extracts were purified on a strong cation exchange column (Dowex 50) [31]. The following spectrofluorimetric analysis were performed: tyrosine [49], DOPA [31], dopamine (DA) [1], noradrenaline (NA) [7], tryptophan [6] and 5-HT [2]. The *in vivo* rate of brain catecholamine (CA) and 5-HT synthesis was estimated by measuring the accumulation of DOPA and 5-HTP respectively after inhibition of central aromatic amino acid decarboxylase by an injection of NSD 1015, 100 mg/kg (−30 min) [11].

Statistical Methods

Overall group differences in the number of tests in which the various behavior patterns were observed were analysed by analysis of variance and subsequent group comparisons were made with *t* test. Analyses of behavior patterns were made with Kruskal-Wallis one-way analysis of variance followed by Mann-Whitney U test. Group differences in brain momoamine levels, rate of monoamine synthesis and weight of the accessory sexual organs were evaluated by analysis of variance followed by *t* test.

EXPERIMENT 1

Induction of Sexual Behavior by PCPA in Combination with TP

This was a preliminary experiment in which we tried to induce sexual behavior in prepuberally castrated male rats by daily treatment with low doses of the 5-HT synthesis inhibitor PCPA [32] and the CA synthesis inhibitor α-MT [47].

Method

Eleven approximately 90 days old prepuberally castrated male rats were randomly divided into 3 groups and injected daily at 1000 hr with PCPA (n = 4, 20 mg/kg), α-MT (n = 4, 20 mg/kg) or NaCl (n = 3). The rats were tested for sexual behavior on Days 2, 4, 6 and 8 of drug treatment. No sexual behavior was observed in these tests. Thereafter 0.15 mg/kg of TP, a dose that previously was found to be submaximal in activating the sexual behavior of prepuberally castrated male rats [44] was given together with either PCPA or α-MT and behavioral testing was continued every second day until Day 20 of treatment. After completion of the behavior tests the rats were killed and their brains were analysed for monoamines.

Results

Behavior. Figure 1 shows that PCPA or α-MT alone failed to stimulate the sexual behavior of the rats.

However, when TP was added to the PCPA treatment there was a rapid onset of mounting and intromitting (Fig. 1). All PCPA-treated males mounted within 3 days after the initiation of the TP treatment and all these rats displayed intromissions in the subsequent tests. However, ejaculations were not observed in these rats until in the final two tests despite the fact that the rats showed 49.0 ± 7.0 mounts and 14.0 ± 3.8 intromissions (means ± SEM) in the tests prior to which ejaculations occurred. No effect was observed after α-MT treatment (Fig. 1). Group comparisons showed that PCPA + TP-treated rats displayed mounts and intromissions, but not ejaculations, in more tests than NaCl + TP- or α-MT + TP-treated rats ($p<0.05$).

Biochemistry. Table 1 shows that PCPA treatment reduced brain levels of 5-HT and CA. α-MT reduced brain levels of CA but not 5-HT.

EXPERIMENT 2

Importance of 5-HT for the Induction of Sexual Behavior by PCPA and TP

In Experiment 1 treatment with PCPA was found to reduce brain levels not only of 5-HT but also of CA. In fact, PCPA was equally effective in lowering the content of CA in the brain as the CA synthesis inhibitor α-MT. α-MT, however, had no significant effect on the brain levels of 5-HT and did not affect the behavioral response to TP significantly. The rapid onset of mounting and intromitting after TP treatment of the PCPA-treated rats in Experiment 1 was, therefore, most likely due to the effects of PCPA on

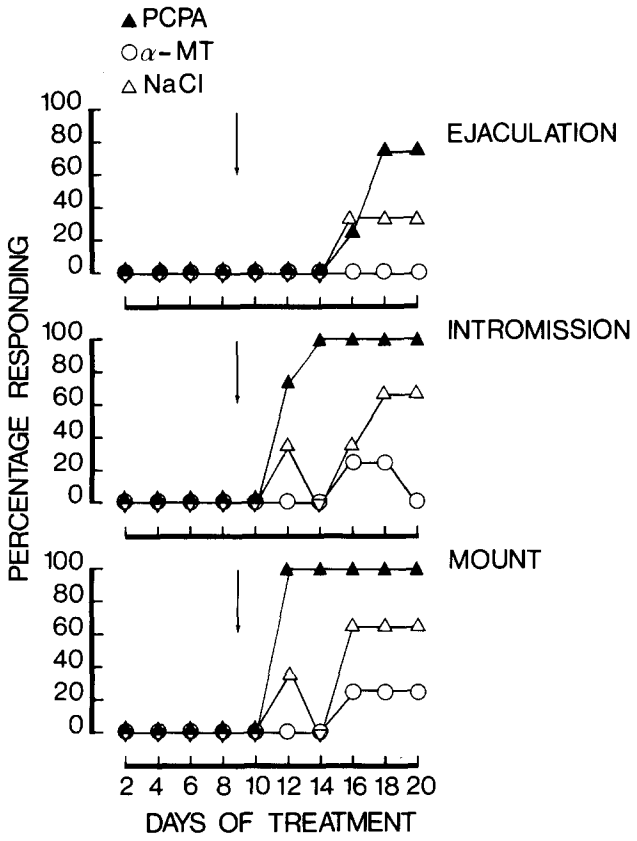

FIG. 1. Percentage of prepuberally castrated male rats showing mounts, intromissions and ejaculation after daily treatment with PCPA (20 mg/kg), α-MT (20 mg/kg) or NaCl. Daily TP (0.15 mg/kg) injections were added on Day 9 of treatment (indicated by arrows).

TABLE 1

MEAN± S.E.M. BRAIN TISSUE LEVELS (μg/g) OF NORADRENALINE (NA), DOPAMINE (DA) AND 5-HYDROXYTRYPTAMINE (5-HT) OF PREPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH P-CHLOROPHENYLALANINE (PCPA, 20 mg/kg), α-METHYL-P-TYROSINE (α-MT, 20 mg/kg) OR NaCl FOR 20 DAYS. TESTOSTERONE PROPIONATE (TP, 0.15 mg/kg) WAS ADDED DURING THE LAST 12 DAYS OF TREATMENT

Treatment	Brain tissue levels of NA	DA	5-HT	No. of rats
PCPA	0.23 ± 0.01*	0.44 ± 0.01*	0.04 ± 0.01*	4
α-MT	0.19 ± 0.01*	0.46 ± 0.02*	0.28 ± 0.01	4
NaCl	0.30 ± 0.01	0.64 ± 0.01	0.27 ± 0.01	3

* $p<0.01$ compared to NaCl.

5-HT neurotransmission. In this experiment we tested this hypothesis by studying the effects of treatment with 5-HTP, the precursor to 5-HT, or L-DOPA, the precursor to CA, on the induction of sexual behavior in castrated male rats by combined PCPA + TP-treatment. Since in Experiment 1 ejaculations did not occur in the majority of the PCPA + TP-treated prepuberally castrated rats until in the final tests postpuberally castrated males were used in the next experiment to increase the probability of ejaculations.

Method

Fifty approximately 90 days old postpuberally castrated male rats that did not show mounts or intromissions in 3 preliminary screening tests were randomly divided into the following treatment groups:

Group	No. of rats	Treatment
1	8	PCPA + benserazid + L-DOPA + TP
2	8	PCPA + benserazid + 5-HTP + TP
3	8	PCPA + benserazid + NaCl + TP
4	12	PCPA + NaCl + Oil
5	8	NaCl + NaCl + TP
6	6	NaCl + NaCl + Oil

The rats received 20 mg/kg PCPA or NaCl at 1000 hr daily for 18 days. Commencing on Day 7 of drug treatment 0.15 mg/kg of TP or oil was added at the same time. Tests for sexual behavior were given every second day. After initiation of TP treatment, L-DOPA or 5-HTP in combination with the inhibitor of peripheral aromatic amino acid decarboxylase, benserazid, were administered prior to behavioral testing as outlined in Table 2.

L-DOPA and 5-HTP treatment was omitted prior to the test on treatment Day 16. On treatment Day 18 the rats were killed and their brains were analysed for momoamines. Additionally, the adrenal glands, ventral and dorsal prostates and seminal vesicles (coagulating gland included, seminal fluid expressed) from 4 rats in treatment groups 4, 5 and 6 were dissected out, blotted dry on filter paper and weighed.

Thirteen other approximately 90 days old postpuberally castrated males were treated with 0.6 mg/kg TP for 23 days and tested for sexual behavior on treatment Days 17, 19, 21 and 23. All rats ejaculated in these tests. Thereafter, the daily TP treatment continued and the rats were randomly divided into 2 groups and treated with beserazid in combination with 5-HTP (n = 7) or NaCl (n = 6) prior to behavioral testing as outlined in Table 2. The rats were tested for sexual behavior 6 times every second day after combined TP + 5-HTP treatment.

Results

Behavior. Figure 2 shows that very little sexual behavior was observed prior to TP treatment.

After the initiation of TP treatment the PCPA-treated rats rapidly started to mount and intromit and the majority of these rats ejaculated in the tests on treatment Days 12 and 14 (Fig. 2). On the other hand, rats receiving either PCPA, TP or NaCl alone showed mounts, intromissions and ejaculations in significantly fewer tests than the PCPA + TP-treated rats ($p<0.05$, Fig. 2). Clearly, PCPA treatment facilitates the induction of all aspects of sexual behavior in postpuberally castrated male rats treated with a low dose of TP. This facilitatory effect of PCPA was unaffected by the administration of L-DOPA prior to testing (Fig. 2). 5-HTP treatment, by contrast, prevented PCPA from exerting its facilitatory effect of the display of sexual behavior (Fig. 2). Thus, rats treated with PCPA + 5-HTP + TP intromitted and ejaculated in fewer tests that PCPA + TP-treated rats ($p<0.05$).

The inhibitory effect of 5-HTP treatment on the induction of sexual behavior by PCPA and TP was probably

TABLE 2

DRUG INJECTION SCHEDULE IN RELATION TO TESTS FOR SEXUAL BEHAVIOR OR DECAPITATION IN BIOCHEMICAL EXPERIMENTS

Group	Treatment −90 min	−60 min	0 min
1	25 mg/kg benserazid	12.5 mg/kg L-DOPA	sex behavior test or decapitation
2	25 mg/kg benserazid	20.0 mg/kg 5-HTP	sex behavior test or decapitation
3	25 mg/kg benserazid	NaCl	sex behavior test or decapitation
4		NaCl	sex behavior test or decapitation
5		NaCl	sex behavior test or decapitation
6		NaCl	sex behavior test or decapitation

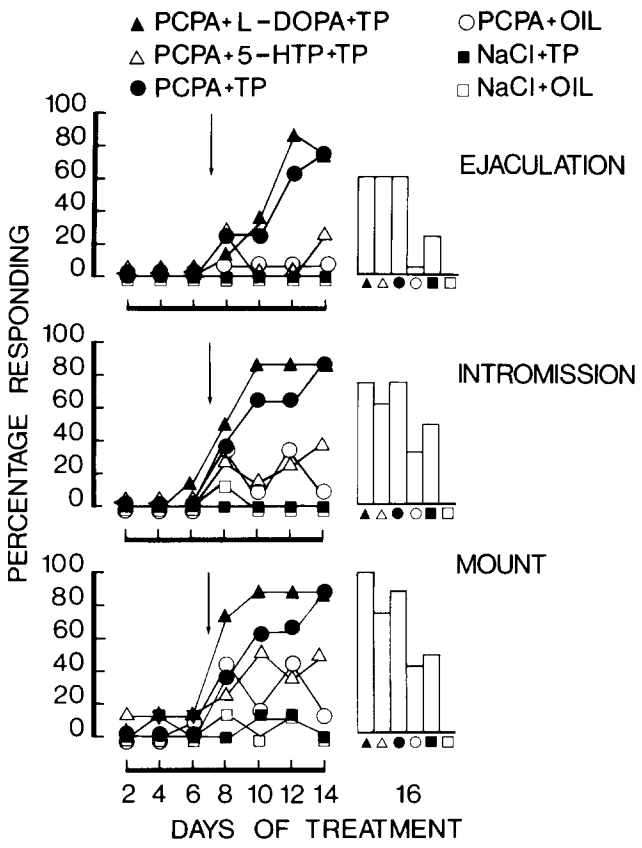

FIG. 2. Percentage of postpuberally castrated male rats showing mounts, intromissions and ejaculation after daily treatment with PCPA (20 mg/kg) and TP (0.15 mg/kg added on Day 7 of treatment, indicated by arrows). L-DOPA (12.5 mg/kg) or 5-HTP (20 mg/kg) was given prior to behavioral testing on treatment Days 8, 10, 12 and 14, but not 16, as outlined in Table 2.

not due to a general debilitating effect since the 5-HTP treatment had no comparable inhibitory effect on the sexual behavior of a group of castrated males in which the behavior had been activated by a higher dose of TP (Table 3).

Figure 2 also shows that on treatment Day 16, when the 5-HTP and L-DOPA treatments were omitted, there were no behavioral differences between the three PCPA + TP-treated groups.

It should be noted that 6 of the 12 rats treated with PCPA alone displayed mounts, that 5 rats showed intromissions and that 2 of these rats ejaculated in at least one test.

A detailed analysis of the parameters of sexual behavior displayed by the ejaculating animals in the various treatment groups on treatment Days 8, 10, 12 and 14 is shown in Table 4. There were no statistically significant group differences with the exception of the above mentioned differences in the number of tests in which the various behavior patterns were displayed.

Biochemistry. Table 5 shows that the whole brain levels of NA and 5-HT, but not DA, were significantly lowered by the administration of PCPA alone or in combination with TP. The injection of L-DOPA to animals given PCPA resulted in an increase in brain NA and DA levels. The injection of 5-HTP completely restored brain 5-HT to the NaCl + oil-treated control level. In addition, there was a statistically significantly reduction of brain NA, but not DA or 5-HT following TP treatment.

Table 6 shows that there were no changes in the rate of CA or 5-HT synthesis after TP treatment. However, there was a significant reduction in brain tyrosine levels by TP treatment. There was a significant reduction in brain CA and, in particular, 5-HT synthesis after PCPA treatment. This reduction in monoamine synthesis was not affected by simultaneous TP treatment. Brain tyrosine and tryptophan were significantly lowered by treatment with PCPA alone and in combination with TP.

Organ weights at autopsy. PCPA treatment did not stimulate the growth of the accessory sex glands whereas TP treatment did. No effect of PCPA or TP was observed on the weight of the adrenal glands (Table 7).

EXPERIMENT 3

Induction of Sexual Behavior by PCPA Alone

In Experiment 2 we found that treatment with PCPA alone stimulated the display of mounts in 6, intromissions in 5 and ejaculations in 2 of 12 rats. This suggests that PCPA treatment can induce sexual behavior in castrated male rats of our strain without concurrent TP treatment. In the next experiment we examined this possibility in detail.

Method

Forty-six approximately 90 days old postpuberally castrated male rats that did not show mounts or intromissions in 3 preliminary screening tests were randomly divided into the following treatment groups:

Group	No. of rats	Treatment
1	8	PCPA + benserazid + L-DOPA
2	9	PCPA + benserazid + 5-HTP
3	9	PCPA + benserazid + NaCl
4	7	NaCl + benserazid + L-DOPA
5	6	NaCl + TP
6	7	NaCl + NaCl

The rats received 40 mg/kg PCPA or NaCl at 1000 hr for 12 days. The rats in Group 5 were injected with 0.03 mg/kg TP. All rats were tested for sexual behavior on treatment Days 2, 4, 6, 8, 10 and 12 and they were treated with L-DOPA or 5-HTP in combination with benserazid as outlined in Table 2 prior to each test. Four rats in treatment groups 3 and 6 were killed after completion of behavioral testing and their brains were analyzed for monoamines. Additionally, 4 rats from treatment groups 3, 5 and 6 were killed and the weights of their accessory sex glands were examined as in the previous experiment.

Forty-two other approximately 90 days old postpuberally castrated male rats that, like the rats above, did not show mounts or intromissions in 3 preliminary screening tests were randomly divided into two groups and injected with 126 mg/kg PCPA at 1000 hr for 3 days (n = 30) or with NaCl (n = 12). Twenty-four hours after the last injection the rats were tested for sexual behavior.

Results

Five of 9 rats treated with 40 mg/kg of PCPA ejaculated in 2.2 ± 0.6 (mean ± SEM) of the 6 tests whereas rats treated with NaCl did not show any sexual responses ($p<0.05$). Three of the 8 PCPA + L-DOPA-treated rats ejaculated in 3.0 ± 0.6 tests. The rats in the other groups did not show ejaculations or intromissions and only infrequently displayed mounts.

Nineteen out of 30 rats that received 126 mg/kg of PCPA for 3 days displayed the complete pattern of sexual behavior when tested 24 hr after the last injection. None of the 12 NaCl-treated rats showed any sexual responses.

A detailed analysis of the pattern of sexual behavior displayed by the rats in the different PCPA treatment groups is presented in Table 8. When the behavior of the PCPA-treated groups was compared with the behavior of the TP-treated rats in Experiment 2, it was found that the rats treated daily with PCPA showed more mounts prior to ejaculation and had longer response latencies. Rats treated with 126 mg/kg of PCPA for 3 days displayed longer intromission latencies and longer postejaculatory intervals than TP-treated rats.

Biochemistry. Daily treatment with 40 mg/kg of PCPA reduced brain levels of NA (0.18 ± 0.01 μg/g (mean ± SEM) *vs* 0.29 ± 0.02 for NaCl-treated controls, $p<0.01$), DA (0.42 ± 0.02 *vs* 0.51 ± 0.02, $p<0.01$) and 5-HT (0.04 ± 0.01 *vs* 0.39 ± 0.01, $p<0.01$).

TABLE 3

SEXUAL BEHAVIOR DISPLAYED BY POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH TESTOSTERONE PROPIONATE (TP, 0.6 mg/kg) FOR 35 DAYS. THE VALUES ARE MEANS ± S.E.M. OF THE TESTS ON TREATMENT DAYS 25, 27, 29, 31, 33 AND 35. THE RATS WERE TREATED WITH 5-HTP (20 mg/kg) OR NaCl PRIOR TO TESTING AS OUTLINED IN TABLE 2

Treatment	Mounts	Intromissions	Behavior pattern Intromission latency (min)	Ejaculation latency (min)	Postejaculatory interval (min)	No. of rats	% ejac.
PCPA+5-HTP+TP	7.2 ± 2.5	14.0 ± 0.8	0.8 ± 0.4	7.6 ± 0.9	5.9 ± 0.2	6	100
PCPA+NaCl+TP	7.0 ± 0.9	12.2 ± 1.3	0.4 ± 0.2	6.2 ± 0.6	5.5 ± 0.2	7	100

TABLE 4

SEXUAL BEHAVIOR DISPLAYED BY POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH P-CHLOROPHENYLALANINE (PCPA, 20 mg/kg FOR 14 DAYS) AND TESTOSTERONE PROPIONATE (TP, 0.15 mg/kg FOR 8 DAYS COMMENCING ON DAY 7 OF PCPA TREATMENT) IN COMBINATION WITH L-DOPA (12.5 mg/kg) OR 5-HTP (20.0 mg/kg) AS OUTLINED IN TABLE 2. THE VALUES ARE MEANS ± S.E.M. OF 4 TESTS GIVEN ON TREATMENT DAYS 8, 10, 12 AND 14. ONLY TESTS WITH EJACULATIONS ARE INCLUDED

Treatment	Mounts	Intromissions	Behavior pattern Intromission latency (min)	Ejaculation latency (min)	Postejaculatory interval (min)	No. of rats	% ejac
PCPA+L-DOPA+TP	19.1 ± 3.7	19.9 ± 3.1	1.2 ± 0.5	19.9 ± 1.8	7.7 ± 0.4	8	87.5
PCPA+5-HTP+TP	15.0 ± 3.9	14.0 ± 2.0	0.9 ± 0.6	20.3 ± 3.5	6.6 ± 0.9	8	50
PCPA+NaCl+TP	15.7 ± 5.3	16.1 ± 2.7	3.1 ± 1.6	16.0 ± 2.3	8.1 ± 0.7	8	75
PCPA+NaCl+Oil	21.3 ± 5.3	16.2 ± 1.9	1.7 ± 1.3	17.6 ± 2.5	8.1 ± 0.1	12	16.5

TABLE 5

MEAN ± S.E.M. BRAIN TISSUE LEVELS (μg/kg) OF NORADRENALINE (NA), DOPAMINE (DA) AND 5-HYDROXYTRYPTAMINE (5-HT) OF POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH P- CHLOROPHENYLALANINE (PCPA, 20 mg/kg) OR NaCl FOR 18 DAYS. TESTOSTERONE PROPIONATE (TP, 0.15 mg/kg) OR OIL WAS ADDED DURING THE LAST 11 DAYS OF TREATMENT. THE ANIMALS WERE FURTHER TREATED WITH L-DOPA (12.5 mg/kg), 5-HTP (20.0 mg/kg) OR NaCl EVERY SECOND DAY THROUGH DAYS 8-18 AS OUTLINE IN TABLE 2

7	Brain tissue levels of			
Treatment	NA	DA	5-HT	No. of rats
PCPA+L-DOPA+TP	0.29 ± 0.01*	0.85 ± 0.08*†	0.05 ± 0.01†	8
PCPA+5-HTP+TP	0.24 ± 0.02†	0.64 ± 0.06	0.33 ± 0.02*	8
PCPA+NaCl+TP	0.23 ± 0.01†	0.56 ± 0.06	0.05 ± 0.01†	8
PCPA+NaCl+Oil	0.21 ± 0.01†	0.42 ± 0.05	0.05 ± 0.01†	8
NaCl+NaCl+TP	0.26 ± 0.01†	0.58 ± 0.02	0.32 ± 0.01	8
NaCl+NaCl+Oil	0.35 ± 0.03	0.62 ± 0.09	0.34 ± 0.03	3

*$p<0.05$ compared to PCPA+NaCl+TP.
†$p<0.01$ compared to NaCl+NaCl+Oil.

TABLE 6

MEAN ± S.E.M. BRAIN TISSUE LEVELS (μg/g) OF DOPA, TYROSINE, 5-HTP AND TRYPTOPHAN OF POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH P-CHLOROPHENYLALANINE (PCPA, 20 mg/kg) OR NaCl FOR 18 DAYS. TESTOSTERONE PROPIONATE (TP, 0.15 mg/kg) OR OIL WAS ADDED DURING THE LAST 11 DAYS OF TREATMENT

	Brain tissue levels of				
Treatment	DOPA	Tyrosine	5-HTP	Tryptophan	No. of rats
PCPA+TP	0.11 ± 0.01*	16.35 ± 0.72‡	0.00 ± 0.00‡	2.78 ± 0.22†	3
PCPA+Oil	0.10 ± 0.01†	16.13 ± 0.48‡	0.00 ± 0.00‡	2.87 ± 0.10†	4
NaCl+TP	0.15 ± 0.01	18.82 ± 0.74†	0.14 ± 0.01	3.28 ± 0.08	4
NaCl+Oil	0.15 ± 0.01	22.06 ± 0.05	0.14 ± 0.01	3.62 ± 0.13	4

*$p<0.05$ compared to NaCl+Oil.
†$p<0.01$ compared to NaCl+Oil.
‡$p<0.001$ compared to NaCl+Oil.

TABLE 7

MEAN ± S.E.M. BODY WEIGHTS AND WEIGHTS OF THE ACCESSORY SEX GLANDS AND ADRENAL GLANDS OF POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH P-CHLOROPHENYLALANINE (PCPA, 20 mg/kg) OR NaCl FOR 21 DAYS OR WITH TESTOSTERONE PROPIONATE (TP, 0.15 mg/kg) FOR 15 DAYS

		Organ				
Treatment	Body (g)	Ventral prostate (mg)	Dorsal prostate (mg)	Seminal vesicles (mg)	Two adrenals (mg)	No. of rats
PCPA	333.5 ± 4.1	11.5 ± 1.7	24.7 ± 7.5	40.2 ± 2.5	87.5 ± 7.9	4
NaCl	316.3 ± 14.5	14.8 ± 3.4	22.0 ± 4.9	37.1 ± 1.4	94.0 ± 5.4	4
TP	318.8 ± 6.9	94.3 ± 7.3*	98.8 ± 3.6*	155.3 ± 3.1*	83.5 ± 4.3	4

*$p<0.01$ compared to NaCl.

Organ weights at autopsy. Table 9 shows that treatment with TP stimulated the growth of the accessory sex glands but that treatment with PCPA exerted no comparable effect. There was no effect of either TP or PCPA on the weight of the adrenal glands. PCPA treatment reduced body weight.

DISCUSSION

The results of the present series of experiments confirm the findings of others that PCPA facilitates the display of sexual behavior in castrated male rats treated with subthreshold doses of TP [24,36]. In contrast to previous workers [24,45], however, we found that PCPA stimulated the sexual behavior of castrated male rats even in the absence of T. Either when administered in a small dose over several days or in a large dose over 3 days, PCPA stimulated part of the castrated rats to show a sexual behavior that differed only slightly from that shown by castrated TP-treated males.

The dose and treatment schedules of PCPA used in these experiments inhibited the synthesis of brain 5-HT and produced a marked depletion of brain 5-HT levels. The brain CA levels were less affected, although evidence was presented that PCPA treatment reduced brain CA levels (Experiments 1 and 3) and inhibited the CA synthesis (Experiment 2). However, it seems unlikely that these effects of PCPA on CA neurotransmission were related to the behavioral effects of PCPA for two reasons. First, the administration of the CA synthesis inhibitor α-MT reduced the brain levels of CA to an extent that was comparable to that produced by PCPA (Experiment 1) but had no effect on the sexual behavior. Second, the administration of the CA precursor L-DOPA to the PCPA + TP- or PCPA-treated rats failed to counteract the stimulatory behavioral effect of PCPA. Treatment with the 5-HT precursor 5-HTP, however, reversed the behavioral effects of PCPA and restored brain 5-HT levels to normal. This effect of 5-HTP was not the result of a general debilitating effect of the 5-HTP since the same dose and treatment schedule of 5-HTP failed to inhibit the sexual behavior of castrated male rats in which the behavior had been induced by a high TP dose. It is most likely, therefore, that the effects of PCPA on the sexual behavior which have been found in the present study were related to the effects of PCPA on 5-HT neurotransmission. This, of course, does not exclude a possible role also for CA in the control of sexual behavior.

The finding that a drug, through interference with 5-HT neurotransmission, can mimic the action of T, suggests that 5-HT is involved in those neural processes which control masculine sexual behavior and are normally activated by T. The first link in the chain of events triggered by a steroid hormone in the brain is the incorporation and retention of the hormone by neurons in specific brain areas (see [42]). The T-concentrating neurons in the rat brain are located in the medial preoptic-anterior hypothalamic area and ad-

TABLE 8

SEXUAL BEHAVIOR DISPLAYED BY POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH TESTOSTERONE PROPIONATE (TP, 0.6 mg/kg) FOR 35 DAYS AND TESTED EVERY SECOND DAY COMMENCING ON DAY 25 OF TREATMENT, DAILY WITH P-CHLOROPHENYLALANINE (PCPA, 40 mg/kg) FOR 12 DAYS AND TESTED EVERY SECOND DAY OR WITH PCPA (126 mg/kg) FOR THREE DAYS AND TESTED ONCE 24 HR AFTER THE LAST INJECTION. THE VALUES ARE MEANS ± S.E.M FOR THE GROUPS THAT WERE TESTED 6 TIMES

Treatment	Mounts	Intromissions	Behavior pattern Intromission latency (min)	Ejaculation latency (min)	Postejaculatory interval (min)	No. of rats	% ejac.
TP	7.0 ± 0.9	12.2 ± 1.3	0.4 ± 0.2	6.2 ± 0.6	5.5 ± 0.2	7	100
PCPA (40 mg/kg)	17.3 ± 3.4*	12.7 ± 1.2	2.8 ± 1.2*	17.1 ± 4.3*	–†	8	62.5
PCPA (126 mg/kg)	7.6 ± 1.7	10.8 ± 1.1	2.8 ± 0.2*	7.2 ± 1.0	12.5 ± 0.5*	30	63.3

* $p<0.05$ compared to TP.
†Only one rat in this group initiated a new copulatory series after ejaculation and did so in only one test.

TABLE 9

MEAN ± S.E.M. BODY WEIGHTS AND WEIGHTS OF THE ACCESSORY SEX GLANDS AND ADRENAL GLANDS OF POSTPUBERALLY CASTRATED MALE RATS TREATED DAILY WITH P-CHLOROPHENYLALANINE (PCPA, 40 mg/kg) NaCl OR TESTOSTERONE PROPIONATE (TP, 0.03 mg/kg) FOR 12 DAYS.

Treatment	Body (g)	Organ Ventral prostate (mg)	Dorsal prostate (mg)	Seminal vesicles (mg)	Two adrenals (mg)	No. of rats
PCPA	321.0 ± 5.8	19.0 ± 0.4	35.3 ± 7.6	67.0 ± 2.0	96.3 ± 7.1	4
NaCl	369.0 ± 10.5	19.0 ± 1.9	37.3 ± 5.4	65.5 ± 3.2	88.9 ± 9.4	4
TP	377.0 ± 8.1	31.3 ± 3.3*	65.0 ± 6.8*	93.0 ± 6.8*	83.3 ± 5.0	4

* $p< 0.01$ compared to NaCl.

jacent regions including the lateral septum, corticomedial amygdala and ventromedial hypothalamus (see [39]). This region coincides with the projection area of the ascending 5-HT nerve fibre systems whose cell bodies are located in the brain stem raphe nuclei [15]. It is possible, therefore, that the activity of the preoptic-anterior hypothalamic area can be altered by impulses from 5-HT nerve fibres. The facts that the preoptic-anterior hypothalamic area contains T-sensitive neurons, that lesions in this area abolish sexual behavior [10, 12, 24, 25, 27], and than hormonal or electrical stimulation of this region stimulates sexual behavior [17, 20, 29, 38, 48] suggest that this area is involved in the control of sexual behavior. In view of the present data, the ascending 5-HT system may thus exert an inhibitory influence on the preoptic-anterior hypothalamic region. Extensive lesions in the junction of the diencephalon and mesencephalon, presumably destroying the rostral connections of the raphe nuclei, result in a dramatic shortening of the postejaculatory interval of male rat sexual behavior [5, 14, 27], thus further supporting the existence of ascending 5-HT neural mechanisms exerting inhibitory influences upon the mating behavior of male rats. Interestingly, the discharge of the raphe 5-HT neurons in the rat is slower during the dark phase than during the light phase [40], and it has been shown that male rats are sexually most active during the night of the day–night cycle [33]. However, ascending CA fibres have also been implicated in te inhibitory control of male rat sexual behavior [14].

Since the adrenal glands produce appreciable amounts of sex steroids (see [46]) and since monoamines are involved in the neural control of pituitary ACTH secretion (see [21]), it is possible that the behavioral effects of PCPA which we observed in this study were mediated by an ACTH induced secretion of adrenal androgens. Malmnäs [36] found that ACTH treatment of castrated male rats stimulated the display of sexual behavior. However, since it takes less androgen to stimulate the growth of the accessory sex glands than to stimulate sexual behavior ([19] and Experiment 2 and 3) one would expect an increase in the accessory sex organ weights associated with the display of sexual behavior by the PCPA-treated males, if PCPA exerted its effect on sexual behavior by stimulating the secretion of adrenal androgens. However, in the present study none of the PCPA injection schedules used stimulated the growth of the accessory sex glands. Moreover, androstenedione, the major androgen produced by the adrenals (see [46]), has only weak behavioral effects [8]. It seems unlikely, therefore, that the stimulatory effect of PCPA on sexual behavior resulted from an activation of the adrenal cortex.

PCPA enhances the responsiveness to painful shock [26], and to gustatory [13] and other sensory stimuli (see [50]). 5-HT has, therefore, been implicated in the neural control of the state of arousal [30]. Accordingly, the action of PCPA on sexual behavior may have been due to an increase of arousal rather than to an action on the mechanisms involved in the control of copulatory behavior. Stimulation by handling [34] or electrical shock applied to the tail [3,9], presumably having arousing effects, were found to increase sexual activity in male rats. However, Barfield and Sachs [4] found that stimulation with tail shocks delayed, but did not prevent, the decline of copulatory behavior after castration. Furthermore, in unpublished experiments, we were unable to activate the sexual behavior of castrated male rats with amphetamine, a classical central stimulating drug. Moreover, the fact that the PCPA-treated rats in the present study displayed prolonged response latencies argues against the possibility that they were generally aroused.

The possibility that PCPA and T influence the sexual behavior of male rats via a common mechanism should be considered. Work to clarify this problem is currently underway in this laboratory.

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