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Enhancement of the Behavioral Effects of 2,5-Dimethoxy-4-Methyl-Amphetamine (DOM) by Pretreatment with *p*-Chlorophenylalanine

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COMMISSARIS, R. L., W. H. LYNESS, K. E. MOORE AND R. H. RECH. *Enhancement of the behavioral effects of 2,5-dimethoxy-4-methyl-amphetamine (DOM) by pretreatment with* p-*chlorophenylalanine*. PHARMAC. BIOCHEM. BEHAV. **13**(4) 605–608, 1980.—Seven food deprived male rats were trained to press a bar on a fixed ratio-40 (FR-40) schedule of food reinforcement. Administration of 0.5 mg/kg 2,5-dimethoxy-4-methyl amphetamine (DOM) immediately before the start of the session resulted in cessation of responding for some portion of the 40-min test session. Three successive days of *p*-chlorophenylalanine (PCPA) administration (100 mg/kg) 30 min after each session reduced 5-hydroxytryptamine (5-HT) to 15–26% of control concentrations in various brain regions but did not alter control rates of responding under the FR-40 schedule. Administration of 0.5 mg/kg DOM following this PCPA pretreatment resulted in a greater amount of non-responding than observed earlier. These data suggest that the effects of the phenethylamine hallucinogen DOM are enhanced by disruption of 5-HT neuronal activity.

5-Hydroxytryptamine DOM Hallucinogens

THE behavioral effects of hallucinogens have been suggested to result from interactions of these agents with 5-hydroxytryptamine (5-HT) neurons in the brain. Results of electrophysiological [1,9] and drug discrimination experiments [11,13] with hallucinogens of both the phenethylamine and indoleamine classes have supported this hypothesis. Moreover, it has been reported that perturbations of 5-HT neuronal balance induced by the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) or the 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) enhance the effects of the indolealkylamine hallucinogen LSD and the phenethylamine hallucinogen mescaline on fixed ratio (FR) operant responding in rats [2, 3, 10]. The FR disruptive effects of amphetamine, however, are not altered by these treatments [2]. We now report that the behavioral effects of the amphetamine analogue 2,5-dimethoxy-4-methylamphetamine (DOM), another phenethylamine hallucinogen, are enhanced following treatment with the 5-HT synthesis inhibitor PCPA.

METHOD

Animals

Seven male Sprague-Dawley rats (Spartan Farms, Haslett, MI) weighing 300–350 g at the time of the experiment were used. All animals were maintained at 70% of their free-feeding weights (relative to free-feeding controls of the same age) and were housed individually in a room with 12-hour day-night light cycle (lights on from 0700 to 1900 hr). Although all subjects had been exposed to hallucinogens, a two-week drug-free period was imposed prior to the start of this experiment.

Apparatus

Behavioral testing was conducted between 1200 and 1400 hr in one of four standard (LVE No. 143-20-215) operant chambers equipped with food pellet dispensers; these chambers were located in sound-attenuating boxes. Each chamber contained a single lever which required a force of 10–15 g to activate. All experimental events were controlled by electromechanical programming circuits and responses were recorded on electromagnetic counters and cumulative recorders. Two parameters were monitored in the experimental sessions: (1) the number of reinforcers obtained, a reflection of the average response rate, and (2) the period of non-responding, or "pausing". To quantify the period of non-responsing during operant sessions, a 10-sec interval counter [5] was incorporated into the program as described below.

Each response by the subject reset a 10-sec timer. If the animal responded before 10 sec elapsed, the timer reset and the program continued. If the animal failed to respond during this 10-sec interval, a count was registered and the timer automatically reset. Therefore, the number of counts registered by this interval timer was an index of the extent of non-responding in terms of cumulated 10-sec pause intervals.

Procedure

The animals were first trained to respond on a continuous

reinforcement (CRF) schedule for food (45 mg Noyes pellets). Daily sessions were 40 min in duration. Each animal was run at the same time of day and in the same cage seven days a week. After rats were responding on the CRF schedule (approximately 2–4 days) a fixed ratio (FR) schedule was introduced and gradually (2–3 weeks) increased to FR-40. After an additional 2–3 weeks of control FR-40 sessions, these animals were exposed to hallucinogens at various doses, the results of which will be reported elsewhere. Following a two-week drug-free period, the present study was begun. Days 1–3 of the present study were control FR-40 sessions. On Day 4 all subjects were given an IP injection of 0.5 mg/kg DOM immediately prior to the start of the FR-40 session. The 0.5 mg/kg dose was chosen because pilot studies had indicated that this dose of DOM produced a significant, yet submaximal disruption of FR-40 responding. Days 5–7 were control sessions. Again on Day 8, 0.5 mg/kg DOM was given before the start of the session. No drugs were given before operant sessions on Days 9–11. However, shortly (approximately 30 min) after each of these sessions all subjects were administered 100 mg/kg PCPA (50 mg/ml suspended in 0.5% methylcellulose). On Day 12, after 3 days of 100 mg/kg PCPA per day, 0.5 mg/kg DOM was administered prior to the start of the FR-40 session. Approximately six hours after the last DOM test session (Day 12) the animals were sacrificed by decapitation, their brains were removed and the concentrations of 5-HT, dopamine (DA) and norepinephrine (NE) were determined in various brain regions by fluorometric methods [4,8].

Statistical Analysis

The effects of DOM alone were analyzed by Student's *t*-test for paired data, as each animal served as its own control. For these comparisons, the baseline for each rat, defined as the average of the three non-drug days immediately prior to the test day, was compared to the test day for that animal. The effects of repeated PCPA administration (Days 10,11) alone on FR-40 performance were evaluated using Student's *t*-tests for paired data and repeated measures. The effects of DOM administration following the PCPA regimen were compared to the two "control" DOM test days using analysis of variance in a block design. In all statistical evaluations $p<0.05$ was used as the criterion for significance.

Drugs

p-Chlorophenylalanine was obtained from Aldrich Chemical Company. DOM hydrochloride was obtained from N.I.D.A.; dose refers to the salt dissolved in saline.

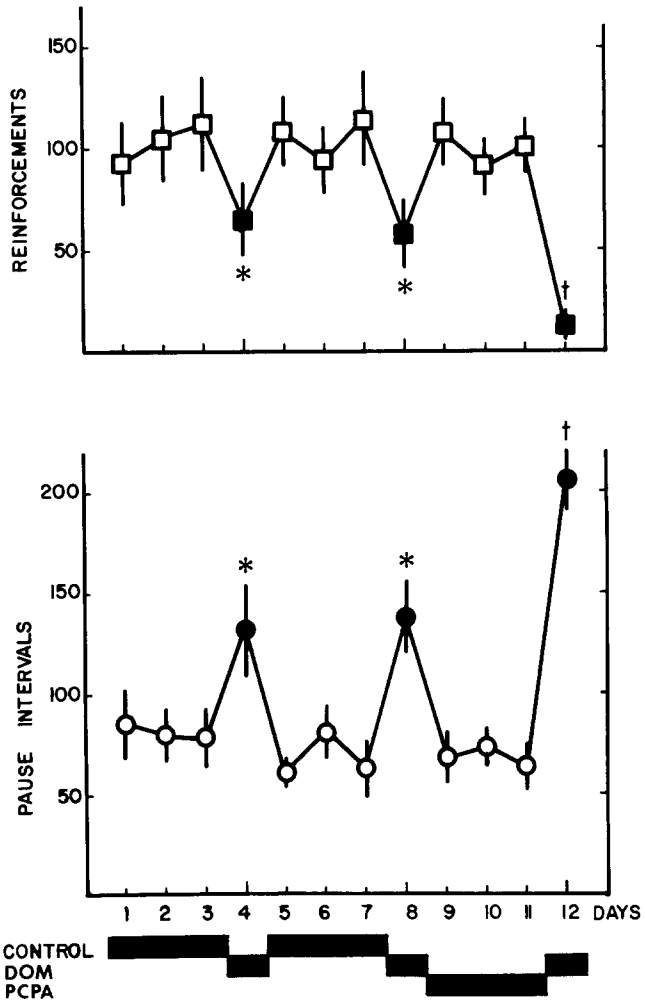

FIG. 1. The effects of PCPA treatment on the FR-40 response to DOM. *Ordinate:* The number of reinforcements received (top panel) and pause intervals produced (bottom panel) by various treatments. One pause interval represents a 10-sec interval without a response. Each point represents the mean ± SEM for seven rats. *Abscissa:* Days of the study and various treatments. Days 1–3 and 5–7 and 9–11 were control days (no injection before session). DOM, 0.5 mg/kg was administered IP on Days 4, 8 and 12 immediately before the start of the 40-min FR-40 session. PCPA, 100 mg/kg, was administered IP 30 min *after* the FR-40 session on Days 9–11. On Day 12 the effects of 0.5 mg/kg DOM were determined 24 hr following the last PCPA injection. *Significantly different ($p<0.05$) from control. †DOM response on Day 12 (after PCPA) significantly different from DOM responses on Day 4 and 8.

RESULTS

The behavioral results are shown in Fig. 1. The number of pause intervals produced during control FR-40 sessions (Days 1–3, 5–7 and 9) did not change over the course of the experiment. Daily administration of 100 mg/kg PCPA failed to alter the number of pause intervals produced in subsequent control sessions (Days 10–11). Administration of a low dose (0.5 mg/kg) of DOM produced a slight but significant increase in the number of pause intervals (Days 4,8).

After 3 days of PCPA administration, however, this same dose of DOM (Day 12) resulted in a significantly greater "pause" than produced by DOM previously, $F(2,12)=6.66$, $p<0.05$. Cumulative recordings obtained from four subjects illustrating the above-mentioned results are shown in Fig. 2.

Table 1 summarizes the biochemical data obtained from these animals. 5-HT concentrations were significantly decreased in all brain regions following PCPA treatment. A modest, though significant, decrease in the concentration of NE in the hypothalamus was observed following this treatment. Hippocampal NE and striatal DA concentrations were not significantly different from control values.

DISCUSSION

The results presented here demonstrate that the behavioral effects of the phenethylamine hallucinogen, DOM, can be enhanced by pretreatment with PCPA for three days. Al-

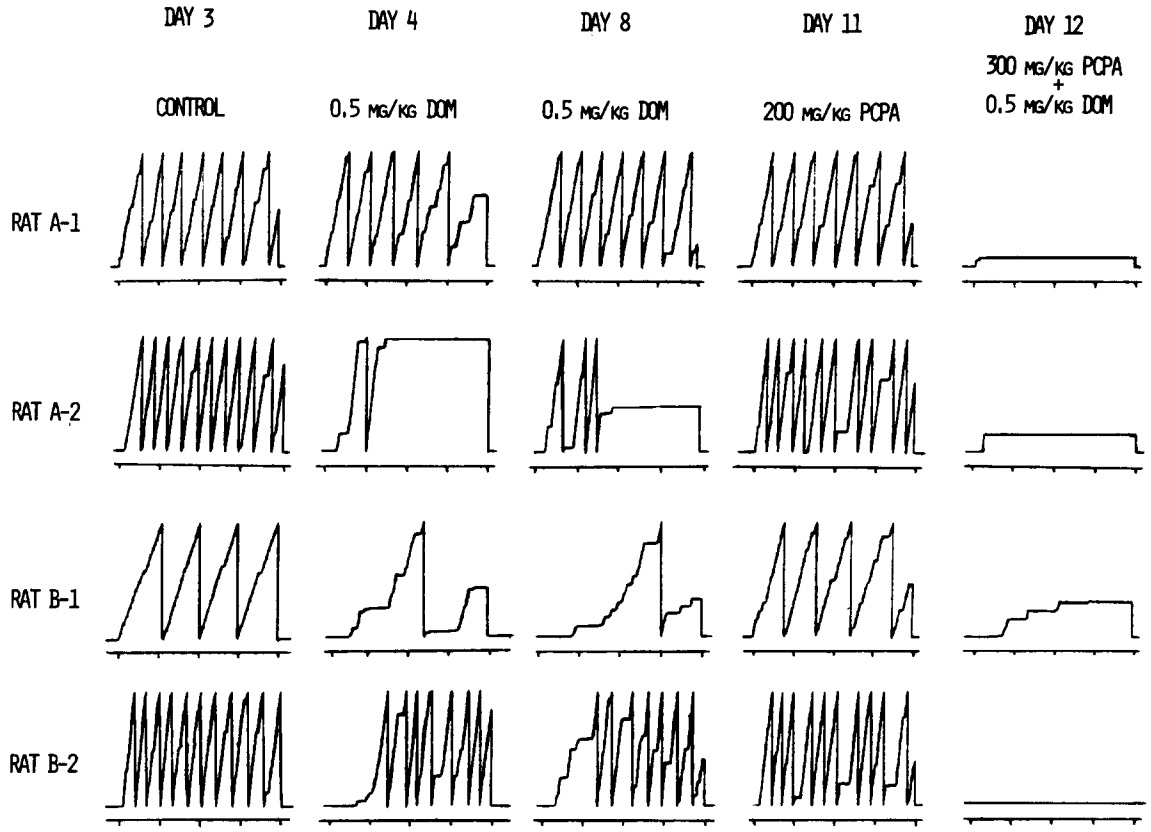

FIG. 2. Cumulative recordings illustrating the response patterns of four rats (B-1, B-2, A-1, A-2) receiving the schedule of drug treatments as indicated in Fig. 1. Treatment and day are indicated by column headings. See legend for Fig. 1 for further information. Hash marks on the abscissa indicate 10-min intervals.

TABLE 1

THE CONCENTRATIONS OF 5-HT, NE AND DA IN VARIOUS BRAIN REGIONS FOLLOWING ADMINISTRATION OF PCPA (100 mg/kg/day) FOR 3 DAYS

	Cortex		Hippocampus		Hypothalmus		Striatum	
	Control	PCPA	Control	PCPA	Control	PCPA	Control	PCPA
5-HT	417 ± 25	110 ± 18* (26%)	468 ± 41	97 ± 27* (21%)	1270 ± 75	187 ± 60* (15%)	472 ± 30	117 ± 32* (25%)
NE	N.D.	N.D.	442 ± 24	385 ± 35 (87%)	2542 ± 161	1859 ± 197* (73%)	N.D.	N.D.
DA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	9470 ± 850	8210 ± 1240 (87%)

Values represent concentration of amines (ng/g wet weight); each value represents mean ± SEM for 7 rats. Animals were sacrificed approximately 30 hours after the last PCPA injection. Numbers in parentheses indicate percent of control.

*$p<0.05$ compared to control values.

N.D. - Not Determined.

though PCPA treatment significantly decreased the concentration of norepinephrine (NE) in the hypothalamus, it is unlikely that this moderate decrease in NE is responsible for the potentiation of the effects of DOM observed, since we have found that decreasing the concentration of NE in this area to less than 10 percent of control with the neurotoxin 6-hydroxydopamine did not alter the response to DOM (Commissaris *et al.*, paper in preparation).

It seems likely that the observed potentiation of the effects of DOM by PCPA are due to the effects of PCPA on 5-HT neurons. Reports by other investigators have indicated that the effects of the indolealkylamine hallucinogen LSD

and the phenethylamine hallucinogen mescaline on FR operant responding are enhanced by disruption of 5-HT neuronal activity as produced by PCPA or the neurotoxin 5,7-DHT [2, 3, 10]. Moreover, Appel *et al.* [2] have shown that the effects of *d*-amphetamine on FR operant responding are not altered by PCPA treatment. These data indicate that in the FR-40 operant paradigm, the effects of the amphetamine analogue DOM are different from those of *d*-amphetamine, yet similar to the hallucinogens LSD and mescaline.

These studies suggest that the 5-HT hypothesis for the behavioral effects hallucinogens holds for both the indoleamine and phenethylamine classes of hallucinogens. However, a comparison of the *magnitude* of the PCPA-induced enhancement of DOM in our study to the magnitude of the enhancement of LSD and mescaline in other studies is not possible, because of differences in behavioral design and other variables. Future experiments should be conducted in which the effects of both classes of hallucinogens are tested in animals with the same behavioral design and similar disruption of 5-HT neuronal activity.

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