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Phencyclidine-Induced Dopamine-Dependent Behaviors in Chronic Haloperidol-Treated Rats

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YAMAGUCHI, K., T. NABESHIMA, M. AMANO, S. YOSHIDA, H. FURUKAWA AND T. KAMEYAMA. *Phencyclidine-induced dopamine-dependent behaviors in chronic haloperidol-treated rats.* PHARMACOL BIOCHEM BEHAV **23**(5) 803–809, 1985.—This study was designed to assess whether phencyclidine (PCP) produces dopamine (DA)-dependent behaviors such as licking, biting and gnawing at low doses after withdrawal from chronic haloperidol (HAL) treatment in rats. Low doses of PCP (2.5 and 5 mg/kg) produced licking, gnawing, biting and self-biting in rats after withdrawal from chronic HAL treatment, which were not observed in the vehicle-pretreated rats given PCP at the same dose range. These behaviors were similar to DA-dependent behaviors produced by methamphetamine and apomorphine in rats after withdrawal from chronic HAL treatment. The PCP-induced behaviors were attenuated by acute pretreatment of DA antagonist, HAL (0.25 mg/kg, IP). Furthermore, at doses of 5 or 7.5 mg/kg, PCP-induced head weaving and backpedalling, which were mediated by both DA and serotonin (5-HT) neurons, significantly increased in rats after withdrawal from chronic HAL-treatment. These results suggest that dopaminergic systems play an important role for PCP-induced behavioral responses.

Phencyclidine Dopaminergic Stereotyped behavior Methamphetamine Apomorphine Haloperidol

PHENCYCLIDINE (PCP), a general anesthetic agent developed in the late 1950's has been reported to induce psychotomimetic reaction in man [11,12]. Previous studies have demonstrated the role of dopaminergic neurons in PCP-induced behaviors; the locomotor activity and some aspects of the stereotyped behaviors produced by PCP are similar to the behavioral changes induced by *d*-amphetamine and methylphenidate [4,6]. *d*-Amphetamine and methylphenidate are believed to produce their behavioral effects mainly through presynaptic actions on dopaminergic neurons. In general, drugs which increase dopaminergic function in the brain cause a well-known pattern of behavior which includes increased locomotor and repetitive activities such as sniffing, non-specific mouth movement, licking, biting and gnawing [9,22]. Balster and Chait [2] have reported that a small dose of PCP enhances amphetamine stereotypy, which has been shown to be mediated predominantly by nigrostriatal dopamine (DA) neurons [5]. Furthermore, PCP produced ipsilateral rotation in substantia nigra-lesioned rats [7] and inhibition of DA uptake in the rat striatum [8,26]. From these results, PCP appears to have a presynaptic dopaminergic action in the striatum.

On the other hand, there have been numerous reports in which chronic blockade of synaptic transmission, by lesion of presynaptic neurons or by pharmacological antagonism of the transmitter substance at a receptor site, results in an enhancement of the observed effects associated with synaptic transmission. For example, chronic treatment with neuroleptic agents, which have dopaminergic antagonist properties, results in increased stereotyped behaviors induced by the direct-acting dopaminergic agonist, apomorphine (APO) given after withdrawal of neuroleptic agents [10, 20, 24, 25, 29]. Furthermore, Klawans and Rubovits [10] have also found that after chronic chlorpromazine treatment, rats are more sensitive to the stereotypic effect of the indirect acting dopaminergic agonist, amphetamine.

Based on the above reports and hypothesis, we investigated whether low doses of PCP produce DA-dependent behaviors (such as licking, biting and gnawing which are not observed in normal rats) in rats after withdrawal from chronic haloperidol (HAL) treatment. The behaviors induced by PCP were compared with the DA-dependent behaviors induced by methamphetamine (MAP) and APO. Furthermore, we investigated whether PCP-induced stereotyped behaviors, such as head weaving, backpedalling and turning [17, 18, 19], are potentiated in rats after withdrawal from chronic HAL treatment.

METHOD

Animals

Male Fischer 344 rats (Charles River Breeding Co. Ja-

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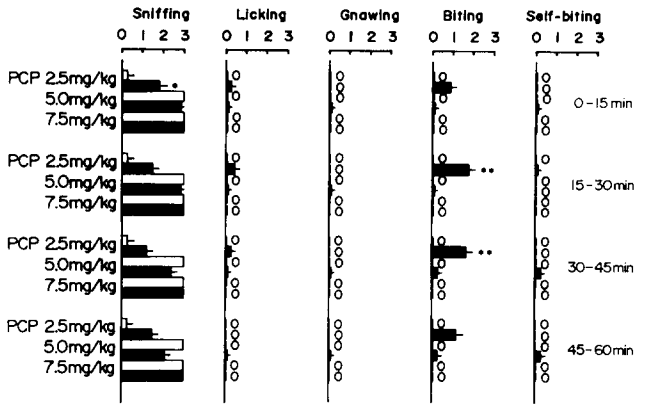

FIG. 1. Components of PCP-induced DA-dependent behaviors in HAL- and vehicle-pretreated rats. Each group of 10–20 rats received a daily injection of HAL (4 mg/kg, IP) or vehicle for 7 days. Four days after the last injection, three groups of rats were given PCP (2.5, 5 and 7.5 mg/kg, IP). DA-dependent behaviors induced by PCP were evaluated at four points during each of the four 15-min observation-periods after PCP injection by the method of all (score: 3) or none (score: 0) as described in the Method section. None of DA-dependent behaviors was induced in the control groups given vehicle (2 ml/kg, IP). Values are the mean±S.E. Open columns: Chronic vehicle + PCP; Solid columns: Chronic HAL + PCP. $*p<0.05$, $**p<0.01$ vs. Chronic vehicle + PCP (Mann-Whitney *U*-test).

pan), weighing 150–200 g, were used. The age of animals was 50–60 days at the start of study. All animals were housed with continuously available food and water on a 12:12 hr light-dark cycle (light on 8:00 a.m.). The room temperature was maintained at 23±1°C (humidity: 55±5%). All animals were kept in the room for a minimum of 1 week prior to the start of the experiment.

Drugs

Phencyclidine hydrochloride (PCP, synthesized in our laboratory) and Methamphetamine hydrochloride (MAP, Dainippon Seiyaku Co.) were dissolved in 0.9% saline. Apomorphine hydrochloride (APO, Sigma) was dissolved in 0.9% saline containing 0.1% of ascorbic acid. Haloperidol (HAL, Dainippon Seiyaku Co.) was dissolved in a minimum amount of 15% acetic acid and then diluted with water. The volume of intraperitoneal or subcutaneous injection was 0.2 ml/100 g body weight.

Chronic HAL Treatment Schedule

Rats were administered HAL chronically, following the schedule of Sayers *et al.* [24] with minor modification. Rats were given HAL (4 mg/kg, IP) once a day from 9 to 11 a.m. for 7 days. After 4 drug-free days, the rats were challenged with PCP, APO or MAP and the behavioral responses induced by these drugs were evaluated. Control rats were given the same volume of vehicle following the same schedule as the chronic HAL treatment.

Behavioral Studies

All behavioral experiments were performed in a quiet room, at a temperature of 22–24°C between 10:00 and 15:00. The animals were randomly assigned to the different drug treatment conditions. Observation of animal behavior was done in a plastic cage with dimension of 19×30×13 cm (DA-dependent behaviors) or of 30×35×17 cm (PCP-induced stereotyped behaviors). The animals were habituated to the plastic cage by placing them individually in the cage for 30 min before the experiments. Ratings of behavior were made by two of the authors, who were blind to the drug treatments.

1. Measurement of DA-dependent behaviors. DA-dependent behaviors induced by PCP, APO or MAP were assessed by the method of Watanabe *et al.* [32] with some modifications. Briefly, the DA-dependent behaviors were rated as follows; 0: inactive or asleep; 1: active but the same as saline-treated rat; 2: predominantly locomotor activity with bursts of stereotyped sniffing or rearing; 3: stereotyped sniffing or rearing over a wide area; 4: stereotyped sniffing or rearing in one location; 5: stereotyped licking of the floor or walls of the cage at least three times during the observation period; 6: gnawing the filter paper on the cage or edge of the cage at least three times during the observation period; 7: compulsive continual biting, not in one location; 8: continual biting in one location; 9: continuous biting the filter paper on the cage or edge of the cage without any interruption and maintaining this posture during the observation period; 10: biting of own tail, forepaw or hindlimb of the rat (self-biting).

Doses of PCP were set within the dose range of 2.5 and 5 mg/kg which caused DA-dependent behaviors including exploratory behavior and sniffing without licking, biting and gnawing [28] and the dose of PCP (7.5 mg/kg) was based on studies of the neurological mechanisms underlying the complex stereotyped behaviors [17, 18, 19, 28]. PCP-induced DA-dependent behaviors (sniffing, licking, gnawing, biting and self-biting) were rated as an all (score: 3) or none response (score: 0) at four points during each of the four 15-min observation-periods (0, 15, 30 and 45 min post-injection). APO (0.25 and 1 mg/kg, SC) and MAP (2.5 and 5 mg/kg)-induced DA-dependent behaviors were rated for 1-min observation-periods at 15 min intervals up to 1 and 2 hr post-injection, respectively. The maximum score of DA-dependent behaviors during observation period was used for the analysis of results.

2. Measurement of PCP-induced stereotyped behaviors. Stereotyped behaviors induced by PCP were assessed by the observational rating scales which were developed by the authors [17] as follows: head weaving (the number of times the animal made slow, side to side or lateral head movements), backpedalling (the number of times the animal moved backward), turning (the number of times the animal circled laterally to the left or right over 360° within a relatively small area). The rating of head weaving was made at four points during each of the four 3-min observation-periods (15, 30, 45 and 60 min post-injection). The ratings of backpedalling and turning were made at four points during each of the four 15-min observation-periods (0, 15, 30 and 45 min post-injection). One of the two authors recorded the behavioral scores for head weaving while the other author recorded those for backpedalling and turning.

Biochemical Studies

Determination of monoamines and their metabolites in the whole brain. On the 4th day of withdrawal from chronic HAL treatment, the animals were sacrificed by microwave irradiation (Toshiba Microwave Applicator TMW-6402A, Tokyo, Japan) for 1.5 sec at 5 kW. The brain was frozen

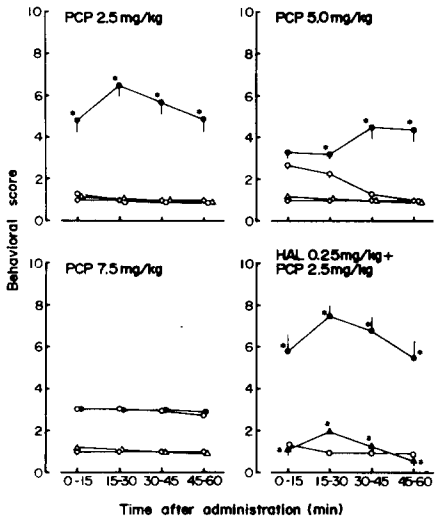

FIG. 2. Time courses of PCP-induced DA-dependent behaviors in HAL- and vehicle-pretreated rats. Each group of 10–20 rats received a daily injection of HAL (4 mg/kg, IP) or vehicle for 7 days. Four days after the last injection, four groups of rats were given PCP (0, 2.5, 5 and 7.5 mg/kg, IP). In the experiment of antagonism by acute HAL, each group of 10 rats was given vehicle or HAL (0.25 mg/kg) at 45 min before the PCP (2.5 mg/kg) injection. The DA-dependent behaviors induced by PCP were evaluated at four points during each of the four 15-min observation-periods after the PCP injection by scoring system described in the Method section. Values are mean±S.E. △—△ Chronic vehicle + Saline; ◇—◇ Chronic HAL + Saline; ○—○ Chronic vehicle + PCP; ●—● Chronic HAL + PCP; ▲—▲ Chronic HAL + Acute HAL + PCP 2.5 mg/kg. *$p<0.01$ vs. Chronic vehicle + PCP, $^{a}p<0.01$ vs. Chronic HAL + PCP (Mann-Whitney U-test). The effects of doses and times after PCP administration on the stereotyped behavior were compared by Mann-Whitney U-test for data where Kruskal-Wallis H scores were associated with a probability of less than 5%. Time effect: vehicle-pretreated groups; PCP 5 mg/kg, 0–15 min vs. 30–45 and 45–60 min, $p<0.01$, 15–30 min vs. 30–45 min, $p<0.05$, 15–30 min vs. 45–60 min, $p<0.01$ (Mann-Whitney U-test). Dose-effect: vehicle-pretreated groups; PCP 0 mg/kg vs. PCP 5 mg/kg, 0–15 and 15–30 min, $p<0.01$, PCP 0 mg/kg vs. PCP 7.5 mg/kg, all points, $p<0.01$, PCP 2.5 mg/kg vs. PCP 5 mg/kg, 0–15 and 15–30 min, $p<0.01$, PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, all points, $p<0.01$, PCP 5 mg/kg vs. PCP 7.5 mg/kg, 15–30 min, $p<0.05$, 30–45 and 45–60 min, $p<0.01$, HAL-pretreated groups; PCP 0 mg/kg vs. PCP 2.5, 5 and 7.5 mg/kg, all points, $p<0.01$, PCP 2.5 mg/kg vs. PCP 5 mg/kg, 15–30 min, $p<0.01$, PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, 15–30 min, $p<0.01$, 30–45 min, $p<0.05$ (Mann-Whitney U-test).

rapidly and stored in a deep freezer at −70°C until assayed. Extraction and determination of monoamines and their metabolites were performed according to the method of Nabeshima *et al.* [16]. Contents in vehicle-pretreated rats were as follows: DA 1027.5±35.0, 3,4-dihydroxyphenylacetic acid (DOPAC) 58.9±1.7, 3-methoxy-4-hydroxyphenylacetic acid (HVA) 72.7±2.5, serotonin (5-HT) 421.2±9.8, 5-hydroxyindoleacetic acid (5-HIAA) 321.0±17.1 (ng/g wet tissue±S.E., n=10).

Statistical Analysis

All results were expressed as the mean±S.E.. Kruskal-Wallis analysis of variance of ranks was used to analyze the effects of doses (i.e., 0, 2.5 and 5 mg/kg) and times (i.e., 30, 45 and 60 min) after drug administration on the stereotyped behavior. Statistical analysis was then carried out using pair-wise Mann-Whitney U-test for data where the Kruskal-Wallis H scores were associated with a probability of less than 5% [23]. A two factor analysis of variance (ANOVA) with repeated-measures on both factors (drug×time×subject) and a Mann-Whitney U-test were used to analyze the effects of withdrawal from chronic HAL-pretreatment on the stereotyped behavior induced by drugs [27]. A two tailed Student's t-test was used to analyze the effects of withdrawal from chronic HAL pretreatment on the contents of monoamines and their metabolites in the whole brain.

RESULTS

1. Effects of Withdrawal from Chronic HAL-Pretreatment on PCP-Induced DA-Dependent Behaviors

PCP-induced DA-dependent behaviors in rats on the 4th day of withdrawal from chronic HAL injection are shown in Fig. 1 and Fig. 2. In vehicle-pretreated rats, sniffing without any DA-dependent behaviors was induced by 2.5–7.5 mg/kg PCP (Fig. 1). At doses of 2.5 and 5 mg/kg PCP, the DA-dependent behaviors in the HAL-pretreated rats consisted mainly of sniffing and biting with occasional licking, gnawing and self-biting (Fig. 1). In the HAL-pretreated rats, significantly higher sniffing and biting scores were obtained at 0–15 and 15–45 min after the injection of 2.5 mg/kg PCP, respectively, compared with the vehicle-pretreated rats (Mann-Whitney U-test). In the HAL-pretreated rats, significantly higher DA-dependent behavioral scores were observed for the duration of 0–60 and 15–60 min after the injection of 2.5 and 5 mg/kg PCP, respectively, compared with the vehicle-treated rats (Fig. 2, Mann-Whitney U-test). However, at the dose of 7.5 mg/kg PCP, there was no difference in the behavioral scores of the vehicle and HAL-pretreated rats (Fig. 2, Mann-Whitney U-test).

2. Effects of Acute HAL Treatment on PCP-Induced DA-Dependent Behaviors After Withdrawal From Chronic HAL-Treatment

The dose employed for acute HAL-treatment (0.25 mg/kg) was sufficient to antagonized stereotyped behaviors including licking and gnawing induced by 1 mg/kg APO [21]. The acute dose of PCP was 2.5 mg/kg which mainly induced sniffing, licking and biting throughout the observation-period of 60 min in the HAL-pretreated rats. As shown in Fig. 2, significantly higher DA-dependent behavioral scores were obtained for the duration of 60 min after the injection of PCP in the HAL-pretreated rats compared with the vehicle-pretreated rats (Mann-Whitney U-test). There were highly significant drug-effect, $F(1,72)=106.03$, $p<0.01$, and time×drug interaction, $F(3,72)=2.96$, $p<0.05$ (ANOVA). On the other hand, the acute HAL treatment significantly attenuated the PCP-induced DA-dependent behavior in the HAL-pretreated rats (Mann-Whitney U-test). There were highly significant drug-effect, $F(1,72)=197.28$, $p<0.01$, and time-effect, $F(3,72)=4.08$, $p<0.01$ (ANOVA).

3. Effects of Withdrawal From Chronic HAL-Pretreatment on MAP-Induced DA-Dependent Behaviors

MAP-induced DA-dependent behaviors in rats on the 4th day of withdrawal from chronic HAL injection are shown in

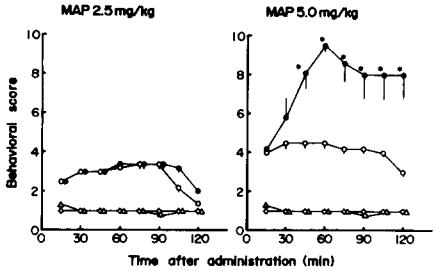

FIG. 3. Time courses of MAP-induced DA-dependent behaviors in HAL- and vehicle-pretreated rats. Each group of 5 rats received a daily injection of HAL (4 mg/kg, IP) or vehicle for 7 days. Four days after the last injection, rats were given MAP (0, 2.5 and 5 mg/kg, IP). The DA-dependent behaviors induced by MAP were evaluated for 1-min periods at 15 min intervals after MAP injection by scoring system described in the Method section. values are the mean±S.E. △—△ Chronic vehicle + Saline; ◇—◇ Chronic HAL + Saline; ○—○ Chronic vehicle + MAP; ●—● Chronic HAL + MAP, *$p<0.05$ vs. Chronic vehicle + MAP (Mann-Whitney U-test). The effects of doses and times after MAP administration on the stereotyped behavior were compared by Mann-Whitney U-test for data where Kruskal-Wallis H scores were associated with a probability of less than 5%. Time-effect: vehicle-pretreated groups; MAP 2.5 mg/kg, 30 min vs. 105 and 120 min, $p<0.05$, 60 min vs. 105 and 120 min, $p<0.05$, 75 min vs. 120 min, $p<0.05$, MAP 5 mg/kg, 15, 30, 45 and 60 min, $p<0.05$, HAL-pretreated groups; MAP 2.5 mg/kg, 30 min vs. 45 min, $p<0.05$, 60 and 105 min vs. 120 min, $p<0.05$, MAP 5 mg/kg 15 min vs. 30, 45, 60, 75, 90, 105 and 120 min, $p<0.05$ (Mann-Whitney U-test). Dose-effect: vehicle-pretreated groups; MAP 0 mg/kg vs. MAP 2.5 mg/kg, 15, 30, 45, 60, 75, 90, and 105 min, $p<0.05$, MAP 2.5 mg/kg vs. MAP 5 mg/kg, 15, 30, 45, 60 and 105 min, $p<0.05$, HAL-pretreated groups; MAP 0 mg/kg vs. MAP 2.5 and 5 mg/kg, all points, $p<0.05$, MAP 2.5 mg/kg vs. MAP 5 mg/kg, all points, $p<0.05$ (Mann-Whitney U-test).

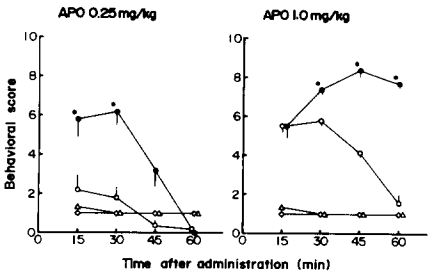

FIG. 4. Time courses of APO-induced DA-dependent behaviors in HAL- and vehicle-pretreated rats. Each group of 5 rats received a daily injection of HAL (4 mg/kg, IP) or vehicle for 7 days. Four days after the last injection, rats were given APO (0, 0.25 and 1 mg/kg, IP). The DA-dependent behaviors induced by APO were evaluated for 1-min periods at 15 min intervals after APO injection by scoring system described in the Method section. Values are the mean±S.E. △—△ Chronic vehicle + Saline; ◇—◇ Chronic HAL + Saline; ○—○ Chronic vehicle + APO; ●—● Chronic HAL + APO, *$p<0.05$ vs. Chronic vehicle + APO (Mann-Whitney U-test). The effects of doses and times after APO administration on the stereotyped behavior were compared by Mann-Whitney U-test for data where Kruskal-Wallis H scores were associated with a probability of less than 5%. Time-effect: vehicle-pretreated groups; APO 1 mg/kg, 15 min vs. 60 min, $p<0.05$, 30 min vs. 45 and 60 min, $p<0.05$, 45 min vs. 60 min, $p<0.05$, HAL-pretreated groups; APO 0.25 mg/kg, 15 min vs. 60 min, $p<0.05$, 30 min vs. 45 and 60 min, $p<0.05$, 45 min vs. 60 min, $p<0.05$, APO 1 mg/kg, 15 min vs. 45 min, $p<0.05$ (Mann-Whitney U-test). Dose-effect: vehicle-pretreated groups; APO 0 mg/kg vs. APO 2.5 mg/kg, 60 min, $p<0.05$, APO 0 mg/kg vs. APO 1 mg/kg, 15, 30 and 45 min, $p<0.05$, APO 2.5 mg/kg vs. APO 5 mg/kg, all points, $p<0.05$, HAL-pretreated groups; APO 0 mg/kg vs. APO 2.5 mg/kg, 15, 30 and 45 min, $p<0.05$, APO 0 mg/kg vs. APO 1 mg/kg, all points, $p<0.05$, APO 0.25 mg/kg vs. APO 1 mg/kg, 45 and 60 min, $p<0.05$ (Mann-Whitney U-test).

Fig. 3. In the vehicle-pretreated rats, the DA-dependent behaviors induced by 2.5 and 5 mg/kg MAP consisted mainly of rearing and sniffing with occasional licking, respectively. At the dose of 2.5 mg/kg MAP, the DA-dependent behaviors in the HAL-pretreated rats did not differ from those of the vehicle-pretreated rats. In contrast, at the dose of 5 mg/kg MAP, the DA-dependent behaviors in the HAL-pretreated rats consisted mainly of biting and self-biting, which were not observed in the vehicle-pretreated rats, with occasional licking. In the HAL-pretreated rats, significantly higher DA-dependent behavioral scores were observed for the duration of 45–120 min after the injection of 5 mg/kg MAP, compared with the vehicle-pretreated rats (Mann-Whitney U-test). There were highly significant time-effect, $F(7,64)=3.47$, $p<0.01$, drug-effect, $F(1,64)=101.43$, $p<0.01$, and time×drug interaction, $F(7,64)=2.88$, $p<0.01$ (ANOVA).

4. Effects of Withdrawal From Chronic HAL-Pretreatment on APO-Induced DA-Dependent Behaviors

APO-induced DA-dependent behaviors in rats on the 4th day of withdrawal from chronic HAL injection are shown in Fig. 4. In the vehicle-pretreated rats, the DA-dependent behaviors induced by 0.25 and 1 mg/kg APO consisted mainly of locomotor activity and licking with occasional gnawing, respectively. In contrast, the DA-dependent behaviors induced by APO at the same dose range in the HAL-pretreated rats consisted mainly of gnawing and biting with occasional licking and gnawing, respectively. In the HAL-pretreated rats, significantly higher DA-dependent behavioral scores were observed for the duration of 15–30 min and 30–60 min after the injection of 0.25 and 1 mg/kg APO, respectively, compared with the vehicle-pretreated rats (Mann-Whitney U-test). There were highly significant time-effect (0.25 mg/kg, $F(3,32)=18.49$, $p<0.01$; 1 mg/kg, $F(3,32)=5.89$, $p<0.01$), drug-effect (2.5 mg/kg, $F(1,32)=36.25$, $p<0.01$; 1 mg/kg, $F(1,32)=77.36$, $p<0.01$) and time×drug interaction (0.25 mg/kg, $F(3,32)=17.21$, $p<0.01$; 1 mg/kg, $F(3.32)=17.21$, $p<0.01$) (ANOVA).

5. Effects of Withdrawal From Chronic HAL-Pretreatment on PCP-Induced Stereotyped Behaviors

PCP-induced stereotyped behaviors in rats on the 4th day of withdrawal from chronic HAL injection are shown in Fig. 5. In the HAL-pretreated rats, significantly higher head weaving scores were observed at all points after the injection of 5 mg/kg PCP, compared with the vehicle-pretreated rats (Mann-Whitney U-test). There were highly significant time-effect, $F(3,72)=7.75$, $p<0.01$, and dose-effect (5 mg/kg, $F(1,72)=31.51$, $p<0.01$; 7.5 mg/kg, $F(1,72)=2.97$, $p<0.05$) (ANOVA). Furthermore, in the HAL-pretreated rats, significantly higher backpedalling scores were observed at 45–60 min after the injection of 7.5 mg/kg PCP, compared with the vehicle-pretreated rats (Mann-Whitney U-test). There were highly significant time-effect, $F(3,72)=7.42$, $p<0.01$, drug-effect, $F(1,72)=5.20$, $p<0.05$, and time×drug interaction,

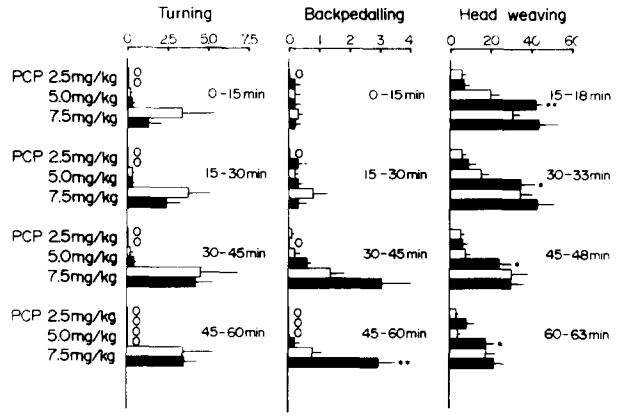

FIG. 5. Time courses of PCP-induced stereotyped behaviors in HAL- and vehicle-pretreated rats. Each group of 10 rats received a daily injection of HAL (4 mg/kg, IP) or vehicle for 7 days. Four days after the last injection, rats were given PCP (2.5, 5 and 7.5 mg/kg, IP). The stereotyped behaviors induced by PCP were evaluated by scoring system as described in the Method section. Values are the mean±S.E. Open columns: Chronic vehicle + PCP; Closed columns: Chronic HAL + PCP, $*p<0.05$, $**p<0.01$ vs. Chronic vehicle + PCP (Mann-Whitney U-test). The effects of doses and times after PCP administration on the stereotyped behavior were compared by Mann-Whitney U-test for data where Kruskal-Wallis H scores were associated with a probability of less than 5%.Turning, dose-effect: vehicle-pretreated groups; PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, 0–15 and 15–30 min, $p<0.01$, PCP 5 mg/kg vs. PCP 7.5 mg/kg, 0–15 min, $p<0.05$, 15–30 min, $p<0.01$, HAL-pretreated groups; PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, 15–30, 30–45 and 45–60 min, $p<0.01$, PCP 5.0 mg/kg vs. 7.5 mg/kg, 30–45 and 45–60 min, $p<0.01$ (Mann-Whitney U-test). Backpedalling, time-effect: HAL-pretreated groups; PCP 7.5 mg/kg, 0–15 min vs. 30–45 min, $p<0.01$, 0–15 min vs. 45–60 min, $p<0.01$, 15–30 min vs. 30–45 min, $p<0.01$, 15–30 min vs. 45–60 min, $p<0.01$, dose-effect: vehicle-pretreated groups; PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, 30–45 min, $p<0.05$, PCP 5 mg/kg vs. PCP 7.5 mg/kg, 30–45 min, $p<0.05$, HAL-pretreated groups; PCP 2.5 mg/kg vs. PCP 5.0 mg/kg, 30–45 min, $p<0.01$, PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, 30–45 and 45–60 min, $p<0.01$, PCP 5 mg/kg vs. 7.5 mg/kg, 45–60 min, $p<0.01$ (Mann-Whitney U-test). Head weaving, time-effect: vehicle-pretreated groups; PCP 5 mg/kg, 15–18 min vs. 45–48 min, $p<0.05$, 15–18 min vs. 60–63 min, $p<0.01$, 30–33 min vs. 60–63 min, $p<0.05$, HAL-pretreated groups; PCP 5 mg/kg, 15–18 min vs. 45–48 min, $p<0.05$,15–18 min vs. 60–63 min, $p<0.01$, dose-effect: vehicle-pretreated groups; PCP 2.5 mg/kg vs. PCP 5 mg/kg, 15–18 and 30–33 min, $p<0.05$, PCP 2.5 mg/kg vs. PCP 7.5 mg/kg, 15–18, 30–33 and 45–48 min, $p<0.01$, 60–63 min, $p<0.05$, PCP 5 mg/kg vs. PCP 7.5 mg/kg, 30–33, 45–48 and 60–63 min, $p<0.05$, HAL-pretreated groups; PCP 2.5 mg/kg vs. 5 mg/kg, 15–18, 30–33 and 45–48 min, $p<0.01$, PCP 2.5 mg/kg vs. 7.5 mg/kg, 15–18, 30–33 and 45–48 min, $p<0.01$ (Mann-Whitney U-test).

$F(3,72)=3.36$, $p<0.05$ (ANOVA). However, PCP-induced turning in the HAL-pretreated rats did not differ from that of the vehicle-pretreated rats at the dosage range used.

6. Effects of Withdrawal From Chronic HAL-Treatment on the Contents of Monoamines and These Metabolites in the Whole Brain

As shown in Fig. 6, the brain levels of DA, DOPAC and HVA in rats were significantly low on the 4th day of withdrawal from chronic HAL-treatment. In contrast, the brain level of 5-HIAA was significantly high on the 4th day of withdrawal from chronic HAL-treatment, but not the 5-HT content.

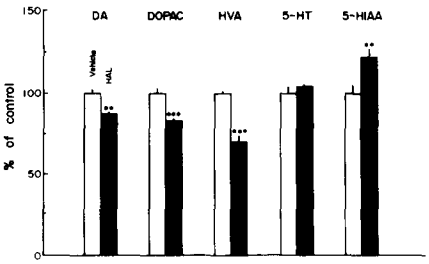

FIG. 6. Effects of withdrawal from chronic HAL-pretreatment on the contents of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the rat brain. Schedule of pretreatment of vehicle and HAL was described in the Method section. Contents of monoamines and their metabolites were expressed as % of control. Values are the mean±S.E. from 10 rats. $**p<0.01$, $***p<0.001$ vs. vehicle-pretreated group (two-tailed Student's t-test).

DISCUSSION

A number of lines of evidence from rodent studies points to similarities in the effects of PCP and sympathomimetic stimulants. PCP produced hyperactivity and a form of stereotyped behavior similar to those produced by d-amphetamine. In addition, these effects of PCP are enhanced by d-amphetamine [2]. Studies comparing the effects of PCP and d-amphetamine on operant behavior in mice and rats have also consistently found marked similarities [1,34].

On the other hand, in the biochemical study, Martres *et al.* [13] have demonstrated that following pretreatment with the DA receptor blocking agent HAL (4 mg/kg, IP), a biphasic modification in responsiveness to APO as regards climbing behavior is observed together with a biphasic variation in the striatal HVA level. Initially, the blockade of DA receptors by HAL results in the suppression of the APO-induced behavior as well as in the marked increase of the striatal HVA level. This probably reflects an increased DA release, as a consequence of the disinhibition of the neuronal feedback loop [13]. Furthermore, both effects disappear about 2 days after withdrawal from HAL and are followed, without any time lag, by a markedly increased behavioral responsiveness to APO and a significant decrease in the HVA level. Thus, as soon as the blockade of DA receptors ceases, hypersensitivity can be revealed behaviorally. The decreased HVA level reflects a diminished activity in the nigrostriatal DA neurones and is probably due to an increased activity of the feedback loop when the postsynaptic cells are hypersensitive to DA [13]. Moreover, Burt *et al.* [3] have found an increased DA receptor ([^{3}H] HAL) binding activity in the striatal homogenates and an increased APO-induced stereotyped behaviors one week after withdrawal from chronic neuroleptic treatment for 3 weeks in rats. In the present experiment, the decreased HVA level was observed in the chronic HAL-pretreated rat brain. Furthermore, PCP (2.5 and 5 mg/kg) produced the DA-dependent behaviors such as licking, gnawing, biting and self-biting suggestive of a state of hypersensitivity of DA receptors after the withdrawal from chronic HAL-treatment, since PCP failed to produce the DA-dependent behaviors in the control rats. In addition, these PCP-induced DA-dependent behaviors were attenuated by the acute pretreatment with HAL. We also found that the DA-dependent behaviors produced by PCP in the chronic HAL-treated rats

were similar to those of MAP. These findings suggest an interaction between PCP and DA neurones and an important role of DA neurons in the PCP-induced behavioral responses. Licking, gnawing and biting induced by APO and MAP were also potentiated in a state of hypersensitivity of postsynaptic DA receptors after withdrawal from chronic HAL-treatment. In addition, self-biting was also observed by PCP and MAP, but not APO in the chronic HAL-treated rats. Furthermore, Yamaguchi *et al.* (unpublished results) found that PCP (2.5, 5 and 7.5 mg/kg, IP) causes only biting when endogenous 5-HT was depleted by the 5-HT depleters, *p*-chloroamphetamine or *p*-chlorophenylalanine. Therefore, the PCP-induced DA-dependent behaviors can be observed in rats when serotonergic neuronal activity is depressed or dopaminergic neuronal activity is activated. In other words, activation of serotonergic neuronal activity is greater than that of dopaminergic neuronal activity in the PCP-treated normal rats.

On the other hand, we have reported that acute and chronic PCP administration changes DA metabolism in mice [15]. It is well known that amphetamine or MAP produces an ipsilateral rotation due to the release of DA from the presynaptic site in the normal side of the striatum or substantia nigra in mice or rats with 6-hydroxydopamine-induced lesion in the unilateral striatum or substantia nigra [30,33]. APO produces the contralateral rotation due to the stimulation of DA receptors in a state of denervation supersensitivity in the denervated striatum or substantia nigra in mice or rats [31,33]. PCP also produces ipsilateral rotation in the rat with unilateral lesion of the substantia nigra [7]. From these reports and our results, it is suggested that PCP stimulates DA receptors through the release of DA from the presynaptic site in the normal side of the striatum or substantia nigra as well as MAP [14].

Although part of the behavioral syndrome produced by PCP is similar to the DA-dependent stereotyped behavior, part of the syndrome is not dopaminergic in nature. The syndrome consists of stereotyped sniffing, backpedalling, turning and head weaving in rats [17,28]. In addition, Nabeshima *et al.* [18,19] have suggested that not only dopaminergic but also serotonergic and other systems in the striatum may play important roles in the PCP-induced stereotyped behaviors. Therefore, the lack of potentiation of the DA-dependent behaviors at the dose of 7.5 mg/kg PCP in the chronically HAL-treated rat may be related to a release of another amine induced by this dose.

In conclusion, the present results show that low doses of PCP (2.5 and 5 mg/kg) produce stereotyped licking, gnawing, biting and self-biting after the withdrawal from chronic HAL-treatment. The PCP-induced behaviors are attenuated by the acute pretreatment of DA antagonist, HAL. These behaviors are similar to DA-dependent behaviors produced by MAP and APO after withdrawal from chronic HAL-treatment. It appears that dopaminergic neuronal systems play a role in PCP-induced behaviors (at least in part). Furthermore, at the doses of 5 and 7.5 mg/kg, PCP-induced head weaving and backpedalling (which were developed through both DA and 5-HT neuronal systems) also increased after the withdrawal from chronic HAL-treatment.

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REFERENCES

1. Balster, R. L. and J. B. Baird. Effects of phencyclidine, *d*-amphetamine and pentobarbital on spaced responding in mice. *Pharmacol Biochem Behav* **11:** 617–623, 1979.
2. Balster, R. L. and L. D. Chait. The effects of phencyclidine on *d*-amphetamine stereotypy in rats. *Eur J Pharmacol* **48:** 445–450, 1978.
3. Burt, D. R., I. Creese and S. H. Snyder. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. *Science* **196:** 326–328, 1977.
4. Chen, G., C. R. Ensor, D. Russell and B. Bohner. The pharmacology of 1-(1-phenylcyclohexyl)-piperidine HCl. *J Pharmacol Exp Ther* **127:** 241–250, 1959.
5. Cole, S. O. Brain mechanisms of amphetamine-induced anorexia, locomotion and stereotypy: A review. *Neurosci Biobehav Rev* **2:** 89–100, 1978.
6. Fessler, R. G., R. D. Sturgeon and H. Y. Meltzer. Effects of phencyclidine and methylphenidate on *d*-amphetamine induced behaviors in reserpine pretreated rats. *Pharmacol Biochem Behav* **13:** 835–842, 1979.
7. Finnegan, K. Y., M. I. Kanner and H. Y. Meltzer. Phencyclidine-induced rotational behavior in rats with nigrostriatal lesions and its modulation by dopaminergic and cholinergic agents. *Pharmacol Biochem Behav* **5:** 651–660, 1976.
8. Garey, R. E. and R. G. Heath. The effects of phencyclidine on the uptake of ^{3}H-catecholamines by rat striatal and hypothalamic synaptosomes. *Life Sci* **18:** 1105-1110, 1976.
9. Kelly, P. H. Drug-induced motor behavior. In: *Drug Neurotransmitters and Behavior. Handbook of Psychopharmacology*, vol 2, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1977, pp. 295–751.
10. Klawans, H. L. and H. Rubovits. An experimental model of tardive dyskinesia. *J Neural Transm* **33:** 235–246, 1972.
11. Luby, E. D., J. S. Gottlieb, B. D. Cohen, G. Rosenbaum and E. F. Domino. Model psychoses and schizophrenia. *Am J Psychiatry* **119:** 61–67, 1962.
12. Luby, E. D., B. D. Cohen, G. Rosenbaum, J. S. Gottlieb and R. Kelly. Study of a new schizophrenomimetic drug—Sernyl. *Am Med Assoc Arch Neurol Psychiatry* **31:** 363–369, 1959.
13. Martres, M. P., J. Costentin, M. Baudry, H. Marcais, P. Protais and J. C. Schwarz. Long-term changes in the sensitivity of pre- and postsynaptic dopamine receptors in mouse striatum as evidenced by behavioural and biochemical studies. *Brain Res* **136:** 319–339, 1977.
14. Meltzer, H. Y., R. D. Sturgeon, M. Simonovic and R. G. Fessler. Phencyclidine as an indirect dopamine agonist. In: *PCP (Phencyclidine): Historical and Current Perspectives*, edited by E. F. Domino. Michigan: NPP Book, 1981, pp. 207–241.
15. Nabeshima, T., M. Hiramatsu, M. Amano, H. Furukawa and T. Kameyama. Effects of acute and chronic administration of phencyclidine on the levels of dopamine and metabolites in discrete brain areas of mouse. *Res Commun Subst Abuse* **4:** 273–287, 1983.
16. Nabeshima, T., M. Hiramatsu, S. Noma, M. Ukai, M. Amano and T. Kameyama. Determination of methionine-enkephalin, norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) in brain by high-pressure liquid chromatography with electrochemical detector. *Res Commun Chem Pathol Pharmacol* **35:** 421–442, 1982.

17. Nabeshima, T., K. Yamada, K. Yamaguchi, M. Hiramatsu, H. Furukawa and T. Kameyama. Effect of lesions in the striatum, nucleus accumbens and medial raphe on phencyclidine-induced stereotyped behaviors and hyperactivity in rats. *Eur J Pharmacol* **91:** 455–462, 1983.
18. Nabeshima, T., K. Yamaguchi, M. Hiramatsu, M. Amano, H. Furukawa and T. Kameyama. Serotonergic involvement in phencyclidine-induced behaviors. *Pharmacol Biochem Behav* **21:** 401–408, 1984.
19. Nabeshima, T., K. Yamaguchi, K. Yamada, M. Hiramatsu, H. Furukawa and T. Kameyama. Phencyclidine-induced stereotyped behaviors in rats following specific neurotoxin lesions of the striatum. *Eur J Pharmacol* **93:** 229–234, 1983.
20. Price, M. T. C. and H. C. Fibiger. Apomorphine and amphetamine stereotypy after 6-hydroxydopamine lesions of the substantia nigra. *Eur J Pharmacol* **29:** 249–252, 1974.
21. Puech, A. J., P. Rioux, M. Poncelet, D. Brochet, R. Chermat and P. Simon. Pharmacological properties of new antipsychotic agents: Use of animal models. *Neuropharmacology* **20:** 1279–1284, 1981.
22. Randrup, A. and I. Munkvad. Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamine. In: *Amphetamines and Related Compounds*, edited by E. Costa and S. Garratini. New York: Raven Press, 1970, pp. 695–713.
23. Rupniak, N. M. J., S. Boyce, P. Jenner and C. D. Marsden. Interpretation of changes in apomorphine-induced stereotyped behavior in rats receiving continuous administration of trifluoperazine for 15 months. *Neuropharmacology* **23:** 893–898, 1984.
24. Sayers, A. C., H. R. Burki, W. Ruch and H. Asper. Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. *Phsychopharmacologia* **41:** 97–104, 1975.
25. Smith, R. C. and J. M. Davis. Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. *Psychopharmacol Commun* **1:** 285–293, 1975.
26. Smith, R. C., H. Y. Meltzer, R. C. Arora and J. M. Davis. Effects of phencyclidine on [^{3}H]catecholamine and [^{3}H]serotonin uptake in synaptosomal preparations from rat brain. *Biochem Pharmacol* **26:** 1435–1439, 1977.
27. Smith, R. C., C. Biggs, G. Vroulis and S. Brikman. Effects of chronic administration of phencyclidine on stereotyped and ataxic behaviors in rat. *Life Sci* **28:** 1163–1174, 1981.
28. Sturgeon, R. D., R. G. Fessler and H. Y. Meltzer. Behavioral rating scales assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. *Eur J Pharmacol* **59:** 169–179, 1979.
29. Tarsy, D. and R. J. Baldessarini. Behavioural supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. *Neuropharmacology* **13:** 927–940, 1974.
30. Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. *Acta Physiol Scand (Suppl)* **367:** 49–68, 1971.
31. Voigtlander, P. F. V. and K. E. Moore. Turning behaviour of mice with unilateral 6-hydroxydopamine lesions in the striatum: Effects of apomorphine, L-dopa, amantadine, amphetamine and other psychomotor stimulants. *Neuropharmacology* **12:** 451–462, 1973.
32. Watanabe, H., S. Nakano and N. Ogawa. Pharmacokinetic study of apomorphine-induced stereotypy in food deprived rats. *Pharmacol Biochem Behav* **14:** 493–496, 1981.
33. Watanabe, H., K. Watanabe and K. Hagino. The involvement of catecholamine in scopolamine-induced locomotor activation and rotational behaviour in mice. *Jpn J Pharmacol* **28:** 465–472, 1978.
34. Wenger, G. R. and P. B. Dews. The effects of phencyclidine, ketamine, *d*-amphetamine and pentobarbital on schedule-controlled behavior in the mouse. *J Pharmacol Exp Ther* **196:** 616–624, 1976.