Pharmacology Biochemistry & Behavior, Vol. 21, pp. 401–408, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Serotonergic Involvement in Phencyclidine-Induced Behaviors

T. NABESHIMA, K. YAMAGUCHI, M. HIRAMATSU, M. AMANO, H. FURUKAWA* AND T. KAMEYAMA

*Department of Chemical Pharmacology and *Department of Medicinal Chemistry Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan*

Received 25 November 1983

T. NABESHIMA, K. YAMAGUCHI, M. HIRAMATSU, M. AMANO, H. FURUKAWA AND T. KAMEYAMA. *Serotonergic involvement in phencyclidine-induced behaviors.* PHARMACOL BIOCHEM BEHAV 21(3) 401–408, 1984.—Administration of 5–10 mg/kg of phencyclidine (PCP) caused stereotyped behaviors including sniffing, backpedalling, head weaving and turning in rats. The PCP-induced stereotyped behaviors (backpedalling, head weaving and turning) were attenuated by serotonin (5-HT) depleters [reserpine, *p*-chlorophenylalanine, *p*-chloroamphetamine (PCA)] and 5-HT receptor antagonist (cyproheptadine). PCP-induced head weaving and turning were potentiated by 5-HT precursor (tryptophan) and 5-HT releaser (PCA). PCP-induced head weaving were potentiated also by monoamine oxidase inhibitor (pargyline) and 5-HT reuptake inhibitor (imipramine). PCP 5–10 mg/kg significantly increased the content of 5-HT in the thalamus/hypothalamus at 30 and 60 min after the injection, except PCP 5 mg/kg at 60 min. PCP 7.5 and 10 mg/kg increased the rate of increment of 5-HT by pargyline in the thalamus/hypothalamus at 30 and 60 min after the injection, respectively. PCP 10 mg/kg significantly increased the contents of 5-HIAA in the striatum and thalamus/hypothalamus at 30 min, but decreased that of 5-HIAA in all discrete brain areas except the striatum at 60 min after the injection. PCP also significantly prevented the depletion of 5-HT by PCA in all discrete brain areas except the stratium at 60 min after the injection. From these results, PCP-induced stereotyped behaviors are related to an increased serotonergic neuronal activity due to 5-HT releasing action and/or inhibitory action of 5-HT uptake by this drug.

Phencyclidine Serotonergic Stereotyped behavior 5-HT release 5-HT uptake

PHENCYCLIDINE (PCP), a general anesthetic agent developed in the late 1950's has been reported to induce psychotomimetic reaction in man [12,13]. The locomotor activity and some aspects of the stereotyped behaviors produced by PCP appear similar to the effect of *d*-amphetamine and methylphenidate [2, 4, 6]. *d*-Amphetamine and methylphenidate are believed to produce their behavioral effects mainly through presynaptic actions on dopaminergic neuron. In general, drugs which increase dopaminergic function in the brain cause a well known pattern of behavior which includes increased locomotion and repetitive activity such as sniffing, nonspecific mouth movement, licking, biting and gnawing [10,19]. Furthermore, we have suggested that acute and chronic PCP administrations induce the functional changes in dopaminergic neuronal activity since dopamine metabolism is modified by their treatments [18]. Therefore, there is a possibility that PCP produces behavioral changes through dopaminergic neuron. However, we have reported also that the PCP-induced stereotyped behaviors (lateral head weaving, turning, backpedalling) are mediated by the straitum and medial raphe containing dopaminergic and serotonergic neurons, respectively, since PCP-induced stereotyped behaviors diminish after the lesion of these brain areas by electrocoagulation [16]. Our results suggest that PCP-induced stereotyped behaviors are mediated by not only dopaminergic but also serotonergic neurons. Although part of the behavioral syndrome produced by PCP is similar to dopamine (DA)-induced stereotyped behavors, part of the syndrome is not dopaminergic in nature [23]. These behaviors consist of reciprocal forepaw treading, lateral head weaving, hind limb abduction, turning and backpedalling. Furthermore, the behaviors such as reciprocal forepaw treading, lateral head weaving and hind limb abduction are like those produced by serotonin (5-HT) agonist [9]. In addition, a recent behavioral study showed that the serotonergic components of the stereotyped behaviors induced by a very large dose of PCP (50 mg/kg) could be blocked by the 5-HT antagonists such as cinanserin and cyproheptadine [14].

In the biochemical study, PCP is known to be a potent inhibitor of 5-HT uptake in a synaptosomal preparation [22]. However, its effects on 5-HT synthesis and metabolism are unclear. Previously, Tonge and Leonard [25] have reported that the ratio of 5-hydroxyindoleacetic acid (5-HIAA) to 5-HT in rat brain both increased and decreased in response to PCP (10 mg/kg) depending on the source of rat supplied. Turnover of 5-HT has been assessed in a paradigm which utilized the *p*-chlorophenylalanine-induced decline of 5-HT. Using this method, three times administrations of PCP (10 mg/kg, IP) at three-hour intervals appeared to decrease the turnover of 5-HT [24]. In the present study, in order to examine the effects of PCP on 5-HT neuron, we have investigated the effects of 5-HT-related drugs on PCP-induced stereotyped behaviors, changes in 5-HT and 5-HIAA contents and turnovers of 5-HT in discrete brain areas of rat after PCP administration. In addition, we have investigated the effects

of PCP on the depletion of brain 5-HT induced by *p*-chloroamphetamine (PCA) whether PCP could inhibit the uptake of 5-HT *in vivo*.

METHOD

Animals

Male Wistar rats (Kyoto Inst. Kitamyama Labo. Co. Ltd., Japan), weighing 200–250 g, were used. All animals were group housed with continuously available food and water on a 12:12 hr light-dark cycle (Light on 8:00 a.m.). The animals were 40–50 days of age at the start of the study. Temperature in the laboratory was maintained at 23±1°C. All animals were maintained in the laboratory for a minimum of 1 week prior to the start of the experiment.

Drugs

Phencyclidine hydrochloride (synthesized in our laboratory), *dl-p*-chloroamphetamine hydrochloride (Sigma), imipramine hydrochloride (Fujisawa), tranylcypromine hydrochloride (Sigma) and pargyline hydrochloride (Sigma) were dissolved in 0.9% saline. 5-Methoxy-N,N-dimethyltryptamine (Sigma) was dissolved in 0.9% saline containing ascorbic acid (0.5%). Reserpine (Roche) all *dl-p*-chlorophenylalanine methyl ester hydrochloride (PCPA, Sigma) were suspended with 0.3% carboxymethylcellulose in 0.9% saline. L-Tryptophan (Sigma) was dissolved in the minimal quantity of 0.5 N HCl and diluted with saline to appropriate volume. Cyproheptadine hydrochloride (Sigma) was dissolved in distilled H_2O since it was not entirely soluble in 0.9% saline at the dose and volume used. The volume of intraperitoneal or subcutaneous injection was 0.2 ml/100 g body weight except cyproheptadine (0.5 ml/100 g body weight).

Behavioral Studies

All behavioral experiments took place in a quiet room, at a temperature of 22–24°C between 10:00 and 15:00 hr. Observation of animals was done in a plastic cage with dimension of 30×35×17 cm. The animals were habituated to the plastic cage by placing them individually in the cage for 30 min before experiment. Twenty-four hours after this session, the animals were randomly assigned to different drug treatment conditions. Ratings were made by two of the authors, who were blind to drug treatment.

Measurement of PCP-induced stereotyped behaviors. Stereotyped behaviors induced by PCP were assessed by observational rating scales which were developed by the authors [16] and validated in dose response studies using PCP doses from 5 to 10 mg/kg [23]. Briefly, the stereotyped behaviors were rated as follows: sniffing (0: absent, 1: occasional, 2: frequent, 3: constant); backpedalling (the number of times the animal made backward locomotion); head weaving (the number of times the animal made slow, side to side or lateral head movements); turning (the number of times the animal circled laterally to left or right over 360° within a relatively small area). Stereotyped behavioral ratings were made for four periods of 15 min each at 0–15, 15–30, 30–45 and 45–60 min post-injection: One of two authors recorded the behavioral scores of head weaving and other author recorded those of sniffing, backpedalling and turning.

Measurement of 5-HT-mediated behaviors induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and PCA. After injection of drugs, 5-HT-mediated behaviors were recorded for 15 min by the method of Lee *et al.* [11]. Intermittent behaviors (backpedalling, turning, lateral head weaving, reciprocal forepaw treading and "wet dog" shake) were scored on a 0–4 scale (0: absent, 1: present one time, 2: present several times, 3: present frequently, 4: present continuously). Continuous behavioral responses (hind limb abduction, Straub tail reaction and tremor) were scored on a 0–4 scale of relative intensity (0: absent, 1: perceptible, 2: weak, 3: medium, 4: maximal). On the other hand, lateral head weaving, turning and "wet dog" shake induced by PCA (2.5–10 mg/kg, IP) were counted the number of times for four periods of 3 min each at 15–18, 30–33, 45–48 and 60–63 min post-injection.

Effects of 5-HT-related drugs on the PCP-induced stereotyped behaviors. Doses of PCP (5 and 7.5 mg/kg) were set at the dosage range which should be employed for studies of neurologic mechanisms underlying the more complex stereotyped behaviors [23]. The doses and pretreatment times for 5-HT-related drugs were comparable with those which could modify PCA (2.5 and 5 mg/kg)-induced 5-HT-mediated behaviors. Using PCP (7.5 mg/kg, IP), as a maximal dose which constantly produces stereotyped behaviors, a group of drugs reducing 5-HT or blocking 5-HT was investigated for their ability to inhibit PCP-induced stereotyped behaviors. Reserpine (2.5 and 5 mg/kg, IP), PCPA (300 mg/kg, IP), PCA (20 mg/kg, IP) and cyproheptadine (1 and 5 mg/kg, IP) were administrated to rats at 24 hr, 72 hr, 48 hr and 30 min prior to PCP injection, respectively. On the other hand, using PCP (5 mg/kg, IP), as a minimal dose which frequently produces PCP-induced stereotyped behaviors, a group of drugs increasing 5-HT was investigated for their ability to potentiate PCP-induced stereotyped behaviors. Imipramine (12.5 mg/kg, IP), tryptophan (50 mg/kg, IP), pargyline (50 mg/kg, IP) and PCA (2.5 mg/kg, IP) were administrated to rats at 2.5 hr, 30 min, 30 min and 15 min prior to PCP injection, respectively. The effects of these agents on PCP-induced stereotyped behaviors were assessed at 15–30 min following administration of PCP.

Biochemical Studies

Determination of 5-HT and 5-HIAA contents in the discrete brain areas of rat. Following administration of PCP 5, 7.5 and 10 mg/kg, IP to rats, the animals of 4–8 in each group were decapitated at 30 and 60 min after the PCP administration between 10:00 a.m. and 1:00 p.m. Each brain was dissected into 4 discrete areas including the cortex, striatum, thalamus/hypothalamus and midbrain/medulla oblongata according to the method of Glowinski and Iversen [8] with some modifications. The samples were rapidly frozen and stored in a deep freezer at −70°C until assayed. The 5-HT and 5-HIAA contents of discrete brain areas were determined by the method of Curzon and Green [3].

Effects of PCP on the pargyline-induced accumulation of 5-HT in the thalamus/hypothalamus of rat. Rats pretreated with pargyline 75 mg/kg, IP were given vehicle, PCP 5, 7.5 and 10 mg/kg, IP at 30 and 60 min before the decapitation. The animals were sacrificed at 60 min after the administration of pargyline and 5-HT content in the thalamus/hypothalamus was determined. Each group consisted of 5–6 rats.

Effects of PCP on the PCA-induced depletion of 5-HT in the discrete brain areas of rat. The ability of 5-HT uptake inhibitors to block the PCA-induced depletion of brain 5-HT has been described by Fuller *et al.* [7]. This method could be used to determine the effects of drugs on 5-HT uptake *in*

TABLE 1

PCP-INDUCED STEREOTYPED BEHAVIORS IN RELATION TO THE DOSE OF AND TIME AFTER PCP

Stereotyped behaviors	Dose of PCP (mg/kg)	Behavioral scores/15 min 0–15	15–30	30–45	45–60 min*
Stereotyped sniffing	0	1.2 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.2
	5	2.8 ± 0.2‡	2.5 ± 0.2‡	2.8 ± 0.2‡	1.5 ± 0.8
	7.5	2.9 ± 0.1‡	2.6 ± 0.2‡	2.8 ± 0.2‡	1.5 ± 0.2†
	10	2.6 ± 0.2‡	2.9 ± 0.1‡	2.9 ± 0.1‡	2.9 ± 0.1‡
Backpedalling	0	0	0	0	0
	5	4.3 ± 1.3‡	0.8 ± 0.4	0.1 ± 0.1	0
	7.5	14.6 ± 4.2‡	13.8 ± 3.9†	9.0 ± 1.3‡	1.8 ± 0.5†
	10	9.4 ± 4.5‡	11.9 ± 4.7‡	18.6 ± 3.0‡	11.4 ± 2.7‡
Head weaving	0	0	0	0	0
	5	134.8 ± 22.6‡	52.4 ± 13.0‡	14.6 ± 7.0‡	0.4 ± 0.4
	7.5	442.4 ± 75.8‡	322.5 ± 68.8‡	144.8 ± 38.2‡	50.6 ± 22.5†
	10	585.0 ± 69.0‡	506.0 ± 48.9‡	302.0 ± 55.9‡	155.0 ± 33.6‡
Turning	0	0	0	0	0
	5	2.9 ± 0.9‡	0.4 ± 0.4	0	0
	7.5	33.0 ± 5.8‡	20.4 ± 3.2‡	11.2 ± 2.1‡	1.3 ± 0.3†
	10	36.6 ± 4.7‡	29.9 ± 6.1‡	19.4 ± 5.1‡	6.3 ± 1.7‡

Values are the means ± S.E. from 8 animals.
*Time after administration of PCP.
†$p<0.05$, ‡$p<0.01$ vs. PCP 0 mg/kg.

vivo. Rats pretreated with PCA 5 mg/kg, SC were given vehicle and PCP 10 mg/kg, IP at 30 and 60 min and imipramine 12.5 mg/kg, SC at 180 min before the decapitation. The animals were sacrificed at 4 hr after the administration of PCA and 5-HT contents in discrete brain areas were determined. Each group consisted of 5–6 rats.

Assay of monoamine oxidase (MAO) activity. Effects of PCP on the MAO activity were determined using Weissbach's method [26] in the P_2 fraction of crude mitochondria prepared according to the method of Whittaker [27]. It is known that there are two types of MAO, A and B [20] and kynuramine is the substrate for both types of MAO. Therefore, kynuramine was used as substrate in present experiment.

Statistical Analysis

The results are expressed as the mean±S.E. and two tailed Student's *t*-test was used for statistical analysis of the data in biochemical experiments, except for the data in behavioral experiments for which the Mann-Whitney's *U*-test was applied.

RESULTS

PCP-Induced Stereotyped Behaviors in Rat

Administration of PCP 5–10 mg/kg to rats did not cause 5-HT-mediated behaviors such as "wet dog"shake, hind limb abduction and reciprocal forepaw treading and DA-dependent behaviors such as licking, gnawing and biting. Administration of PCP 2.5 mg/kg to rats produced exploratory behavior, sniffing and grooming, but not backpedalling, head weaving and turning. The scores of PCP-induced stereotyped behaviors were averaged for each 15 min interval and time course of the effects of PCP are shown in Table 1. PCP-induced stereotyped behaviors including sniffing, backpedalling, turning and head weaving were observed in a dose dependent manner in terms of maximal action. At the doses 5 and 7.5 mg/kg, PCP-induced stereotyped behaviors reached to a peak within the first and the second 15 min-time periods after the injection and rapidly decreased in following time periods. The effects of PCP 10 mg/kg continued longer than those of 5 and 7.5 mg/kg.

5-HT-Mediated Behaviors Induced by 5-MeODMT and PCA

Administration of 5-MeODMT 5 and 10 mg/kg to rats induced several behavioral responses including head weaving, backpedalling, turning, forepaw treading, Straub tail reaction, hind limb abduction and tremor. Forepaw treading was the most pronounced behavior (Table 2). On the other hand, administration of PCA 2.5–10 mg/kg to rats induced several behavioral responses including head weaving, turning and "wet dog" shake (Table 3). At the dose of PCA 20 mg/kg, the all behavioral responses induced by 5-MeODMT were also observed (Table 2).

Effects of 5-HT-Related Drugs on the PCP-Induced Stereotyped Behaviors in Rat

Effects of 5-HT-related drugs on the PCP-induced stereotyped behaviors were investigated at the second 15 min time-period after the injection of PCP.

Monoamine depleter. Treatment with reserpine 2.5 and 5 mg/kg at 24 hr before the injection of PCP 7.5 mg/kg greatly attenuated backpedalling, head weaving and turning. Reserpine 5 mg/kg also attenuated sniffing (Table 4). Reserpine 5

TABLE 2

5-HT-MEDIATED BEHAVIORS INDUCED BY 5-MeODMT AND PCA

Treatments		Behavioral scores/0–15 min							
Drugs	Dose (mg/kg)	Head weaving	Back-pedalling	Turning	"Wet dog" shake	Forepaw treading	Straub tail reaction	Hind limb abduction	Tremor
5-MeODMT	5	2.5 ± 0.2	0.8 ± 0.3	2.1 ± 0.2	0	3.8 ± 0.1	1.4 ± 0.5	1.4 ± 0.5	0
	10	2.3 ± 0.2	2.0 ± 0.0	0.8 ± 0.2	0	4.0 ± 0.0	2.3 ± 0.5	2.6 ± 0.2	3.8 ± 0.2
PCA	20	4.0 ± 0.0	1.3 ± 0.3	0.2 ± 0.2	2.0 ± 0.3	4.0 ± 0.0	1.5 ± 0.7	3.3 ± 0.2	1.7 ± 0.2

Values are the means ± S.E. from 6 to 8 animals.

TABLE 3

5-HT-MEDIATED BEHAVIORS IN RELATION TO THE DOSE OF AND TIME AFTER PCA

Stereotyped behaviors	Dose of PCA (mg/kg)	Behavioral scores/3 min			
		15–18	30–33	45–48	60–63*
Head weaving	2.5	3.4 ± 0.7	4.0 ± 1.1	3.4 ± 0.9	1.1 ± 0.7
	5	33.3 ± 2.6	27.9 ± 2.3	27.1 ± 1.5	21.1 ± 1.8
	10	17.5 ± 2.7	18.7 ± 2.0	22.0 ± 1.8	10.0 ± 1.6
Turning	2.5	0	0	0	0
	5	1.9 ± 0.4	2.8 ± 0.4	3.6 ± 0.8	2.6 ± 0.6
	10	5.8 ± 1.4	5.8 ± 2.1	3.8 ± 1.4	2.2 ± 0.7
"Wet dog" shake	2.5	1.5 ± 0.7	3.0 ± 0.9	2.5 ± 0.4	1.4 ± 0.4
	5	2.0 ± 0.5	3.5 ± 0.6	2.1 ± 0.3	1.4 ± 0.4
	10	3.2 ± 0.9	2.3 ± 0.6	1.5 ± 0.6	0.8 ± 0.3

Values are the means ± S.E. from 8 animals.
*Time after administration of PCA.

TABLE 4

EFFECTS OF DRUGS ATTENUATE 5-HT NEURONAL FUNCTION ON THE PCP-INDUCED STEREOTYPED BEHAVIORS IN RAT

Pretreatments			Stereotyped behaviors (scores/15 min)			
Drugs	Time* (hr)	Dose (mg/kg)	Stereotyped sniffing	Backpedalling	Head weaving	Turning
Vehicle	0.5	—	2.4 ± 0.1	24.3 ± 4.0	331.7 ± 36.5	31.6 ± 2.5
Reserpine	24	2.5	2.9 ± 0.1	5.7 ± 1.7‡	96.9 ± 10.6‡	4.5 ± 1.8‡
	24	5	1.3 ± 0.2‡	2.1 ± 0.8‡	120.6 ± 19.9‡	8.0 ± 2.7‡
PCPA	72	300	2.7 ± 0.2	2.7 ± 0.2‡	29.0 ± 1.3‡	5.5 ± 1.4‡
Cyproheptadine	0.5	1	1.6 ± 0.2‡	4.6 ± 1.2‡	110.6 ± 10.3‡	21.7 ± 3.7‡
	0.5	5	1.8 ± 0.2‡	5.1 ± 2.2‡	80.6 ± 19.2‡	7.9 ± 2.7‡
PCA	48	20	2.4 ± 0.2	2.0 ± 0.8‡	92.4 ± 23.4‡	3.7 ± 2.0‡

Values are the mean ± S.E. from 6 to 15 animals between 15 and 30 min after administration of PCP 7.5 mg/kg, IP.
*Time before administration of PCP.
†$p<0.05$, ‡$p<0.01$ vs. Vehicle.

TABLE 5

EFFECTS OF DRUGS POTENTIATE 5-HT NEURONAL FUNCTION ON THE PCP-INDUCED STEREOTYPED BEHAVIORS IN RAT

Pretreatments			Stereotyped behaviors (scores/15 min)			
Drugs	Time* (hr)	Dose (mg/kg)	Stereotyped sniffing	Backpedalling	Head weaving	Turning
Vehicle	0.5	—	2.6 ± 0.4	4.1 ± 0.1	93.7 ± 13.2	5.2 ± 1.3
Tryptophan	0.5	50	1.4 ± 0.2‡	6.9 ± 1.8	191.6 ± 15.7‡	14.5 ± 4.0‡
PCA	0.25	2.5	2.1 ± 0.1	6.3 ± 1.2	323.3 ± 32.8‡	16.4 ± 2.7‡
Imipramine	2.5	12.5	1.0 ± 0.0‡	8.6 ± 2.5	220.0 ± 23.4‡	9.6 ± 4.3
Pargyline	0.5	50	1.3 ± 0.2‡	4.2 ± 0.6	188.0 ± 18.4‡	4.8 ± 0.3

Values are the means ± S.E. from 6 to 15 animals between 15 and 30 min after administration of PCP 5.0 mg/kg, IP.
*Time before administration of PCP.
†$p<0.05$, ‡$p<0.01$ vs. Vehicle.

mg/kg alone depressed spontaneous behavior, but reserpine 2.5 mg/kg alone did not show any obvious behavioral effects.

5-HT depleter. PCP-induced backpedalling, head weaving and turning were significantly attenuated by the pretreatments with PCPA 300 mg/kg and PCA 20 mg/kg (Table 4). PCPA or PCA alone did not show any obvious behavioral effects.

5-HT receptor antagonist. Treatment with cyproheptadine 1 and 5 mg/kg at 30 min before the injection of PCP 7.5 mg/kg attenuated all the components of PCP-induced stereotyped behaviors (Table 4). Cyproheptadine itself did not show any obvious behavioral effects at the dose used.

Tryptophan and 5-HT releaser. Treatment with tryptophan 50 mg/kg at 30 min before the injection of PCP 5 mg/kg increased head weaving and turning, while attenuated sniffing. Treatment with PCA 2.5 mg/kg at 15 min before the injection of PCP 5 mg/kg also increased head weaving and turning (Table 5). Tryptophan alone did not show any obvious behavioral effects. PCA alone induced a weak head weaving and "wet dog" shake, but not turning (Table 2).

MAO inhibitor and 5-HT uptake inhibitor. Treatment with pargyline 50 mg/kg at 30 min before the injection of PCP 5 mg/kg increased head weaving, while attenuated sniffing. Treatment with imipramine 12.5 mg/kg at 2.5 hr before the injection of PCP 5 mg/kg increased head weaving, while attenuated sniffing (Table 5). Pargyline or imipramine alone did not show any obvious behavioral effects.

Effects of PCP on the Contents of 5-HT and 5-HIAA in the Discrete Brain Areas of Rat

As shown in Table 6, at the doses of 5–10 mg/kg, PCP significantly increased 5-HT content in the thalamus/hypothalamus at 30 and 60 min after the injection except PCP 5 mg/kg at 60 min. At the dose of 10 mg/kg, PCP significantly increased 5-HIAA contents in the thalamus/hypothalamus and striatum at 30 min after the injection. On the contrary, PCP 10 mg/kg significantly reduced 5-HIAA contents in the thalamus/hypothalamus and midbrain/medulla oblongata at 60 min after the injection. At the doses of 7.5 and 10 mg/kg, PCP significantly reduced 5-HIAA content in the cortex at 60 min.

Effects of PCP on the Pargyline-Induced Accumulation of 5-HT in the Thalamus/Hypothalamus of Rat

To clarify effects of PCP on 5-HT turnover rate in the thalamus/hypothalamus in which 5-HT and 5-HIAA contents were changed by PCP, the 5-HT contents were determined after the administration of PCP in the pargyline-treated rats (Table 7). Pargyline 75 mg/kg, IP increased the 5-HT content in the thalamus/hypothalamus to 141% of the control level at 30 min after the injection. At the dose of 7.5 mg/kg, PCP significantly increased the pargyline-induced accumulation of 5-HT in the thalamus/hypothalamus at 30 min after the injection. In addition, PCP 10 mg/kg also significantly increased the accumulation of 5-HT by pargyline in the thalamus/hypothalamus at 60 min after the injection.

Effects of PCP on the PCA-Induced Depletion of 5-HT in the Discrete Brain Areas of Rat

PCA 5 mg/kg, SC significantly decreased the 5-HT contents in the all discrete brain areas to 43–82% of control level at 4 hr after the injection. On the other hand, imipramine, a highly specific inhibitor of uptake in 5-HT neuron [1], significantly prevented the PCA-induced depletion of 5-HT in the all discrete brain areas of rat when imipramine was used at the dose and pretreatment time which inhibited head weaving, turning and "wet dog" shake of 5-HT-mediated behaviors induced by PCA 5 mg/kg, SC in rat (Table 8). At the dose of 10 mg/kg, PCP significantly prevented the PCA-induced depletion of 5-HT in the cortex, thalamus/hypothalamus and midbrain/medulla oblongata at 60 min after the injection, but not at 30 min (Table 8).

Effects of PCP on the MAO Activity in Rat Brain

The effects of PCP on the MAO activity were investigated in the following study. As shown in Fig. 1, a MAO inhibitor, tranylcypromine, showed a dose-dependent inhibition *in vitro* as follows: 12% at 10^{-8} M, 44% at 10^{-7} M and 97% at 10^{-6} M. Pargyline showed an equally strong inhibitory action of 29%, 51% and 76% with 10^{-8} M, 10^{-7} M and 10^{-6} M, respectively. In contrast to these agents, PCP exhibited only a weak inhibitory action of 11% with 10^{-4}. In addition, it had no MAO inhibitory effect *in vivo* at 30 and 60 min after the administration of the dose of 10 mg/kg, IP. On the contrary, pargyline and tranylcypromine strongly inhibited the MAO activity *in vivo* (Fig. 2).

DISCUSSION

The accumulated evidences have suggested that PCP,

TABLE 6

EFFECTS OF PCP ON THE CONTENTS OF 5-HT AND 5-HIAA IN THE DISCRETE BRAIN AREAS OF RAT

Brain areas	PCP Dose (mg/kg)	PCP Time (min)	5-HT (ng/g wet tissue)	5-HIAA (ng/g wet tissue)
Cortex	0	30	550.0 ± 24.2	414.5 ± 14.5
	5		578.3 ± 23.7	428.6 ± 8.3
	7.5		515.6 ± 41.7	371.4 ± 15.2
	10		559.3 ± 22.0	394.8 ± 37.9
	5	60	560.6 ± 56.6	368.6 ± 32.6
	7.5		532.9 ± 15.6	368.4 ± 12.3*
	10		571.5 ± 68.1	339.3 ± 10.0*
Striatum	0	30	906.7 ± 45.7	551.1 ± 13.0
	5		855.6 ± 33.8	533.4 ± 29.3
	7.5		998.4 ± 29.7	555.5 ± 22.9
	10		847.4 ± 72.7	667.8 ± 26.7†
	5	60	832.0 ± 38.0	497.8 ± 40.8
	7.5		874.1 ± 62.4	527.0 ± 42.9
	10		760.5 ± 82.3	575.8 ± 21.2
Thalamus/ Hypothalamus	0	30	786.7 ± 35.2	662.2 ± 17.6
	5		1024.3 ± 132.2*	621.3 ± 35.4
	7.5		1046.1 ± 101.6*	621.5 ± 31.1
	10		1118.9 ± 63.5†	772.2 ± 31.5†
	5	60	929.7 ± 90.1	695.6 ± 54.5
	7.5		1015.8 ± 53.9†	660.4 ± 45.5
	10		1036.1 ± 76.5†	489.7 ± 44.3†
Midbrain/ Medulla oblongata	0	30	1121.4 ± 53.3	734.7 ± 44.3
	5		1094.0 ± 108.3	682.7 ± 34.6
	7.5		943.0 ± 70.6	735.0 ± 73.3
	10		1142.9 ± 29.7	744.4 ± 44.8
	5	60	1014.3 ± 39.1	670.5 ± 12.9
	7.5		1080.9 ± 77.9	748.3 ± 100.8
	10		1115.8 ± 47.5	530.9 ± 25.4*

Values are the means ± S.E. from 4 to 8 samples.
*$p<0.05$, †$p<0.01$ vs. PCP 0 mg/kg.

TABLE 7

EFFECTS OF PCP ON THE PARGYLINE-INDUCED ACCUMULATION OF 5-HT IN THE THALAMUS/HYPOTHALAMUS OF RAT

Pretreatments Drug	Pretreatments Dose (mg/kg)	PCP Dose (mg/kg)	PCP Time (min)	5-HT (ng/g wet tissue) Thalamus/ Hypothalamus
Saline		0	30	716.9 ± 47.4
Pargyline	75	0	30	1008.7 ± 93.0*
		5		1179.1 ± 94.3
		7.5		1442.7 ± 153.0†
		10		1103.7 ± 127.6
		5	60	1174.4 ± 90.0
		7.5		1257.9 ± 94.3
		10		1348.4 ± 65.8†

Values are the means ± S.E. from 5 to 6 samples.
Rats were sacrificed at 60 min after the pargyline administration.
*$p<0.05$ vs. Saline; †$p<0.05$ vs. Pargyline alone.

TABLE 8

EFFECTS OF PCP ON THE PCA-INDUCED DEPLETION OF 5-HT IN DISCRETE BRAIN AREAS OF RAT

Treatments	Time (min)	5-HT (ng/g wet tissue) Cortex	Striatum	Thalamus/ Hypothalamus	Midbrain/ Medulla oblongata
Saline	60	538.4 ± 23.9	759.6 ± 33.2	1041.3 ± 88.2	915.5 ± 52.7
PCA + Saline	60	232.3 ± 7.6*	450.1 ± 19.9*	646.9 ± 25.6*	740.0 ± 56.0*
+ Imipramine 12.5 mg/kg	180	360.5 ± 31.5‡	549.7 ± 35.3†	858.5 ± 30.1‡	879.8 ± 31.6†
+ PCP 10 mg/kg	30	281.3 ± 22.0	465.1 ± 44.8	762.8 ± 58.2	904.9 ± 113.2
+ PCP 10 mg/kg	60	347.2 ± 26.8‡	485.1 ± 44.8	889.8 ± 87.0†	991.9 ± 69.5‡

Values are the mean ± S.E. from 5 to 6 samples.
*$p<0.01$ vs. Saline, †$p<0.05$, ‡$p<0.01$ vs. PCA alone.

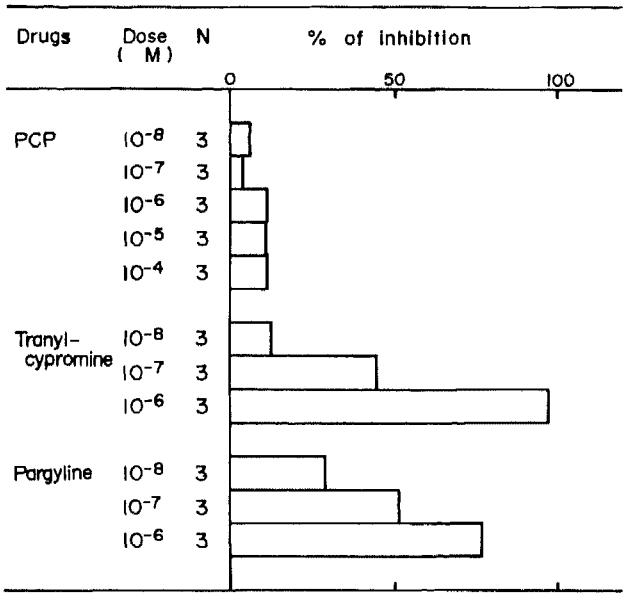

FIG. 1. Effects of PCP on the monoamine oxidase activity *in vitro* in crude mitochondria fraction in rat brain. Assay based on kynuramine disappearance (Weissbach *et al.* [26]). Control: 1.42 nmole/min/mg protein. Each point represents the average of 3 experiments.

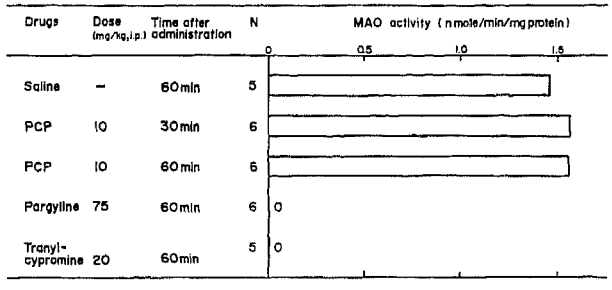

FIG. 2. Effects of PCP on the monoamine oxidase activity *in vivo* in crude mitochondria fraction in rat brain. Rats were decapitated at 30 or 60 min after the PCP, pargyline or tranylcypromine administration. Assay based on kynuramine disappearance (Weissbach *et al.* [26]). Each point represents the average of 5–6 experiments.

similar to amphetamine, produces stereotyped behaviors [4,15]. In this study, at the doses of 5–10 mg/kg, PCP produced stereotyped behaviors including sniffing, backpedalling, head weaving and turning within 30 min after the injection, in good agreement with previous results [16, 17, 23]. As described in Introduction, PCP-induced backpedalling, head weaving and turning may not be mediated by dopaminergic neuron. In addition, present experiment exhibited that these behaviors were also induced by 5-HT indirect agonist (PCA) and 5-HT direct agonist (5-MeODMT). PCP-induced backpedalling, head weaving and turning were prevented when endogenous 5-HT was depleted by reserpine, PCPA and PCA. Furthermore, these stereotyped behaviors were also prevented by 5-HT receptor antagonist. On the other hand, PCP-induced turning increased when 5-HT synthesis and 5-HT release were enhanced by tryptophan and PCA administration, respectively. PCP-induced head weaving increased when 5-HT neuron was activated by tryptophan (5-HT precursor), PCA (5-HT releaser), imipramine (5-HT reuptake inhibitor) and pargyline (MAO inhibitor), but not backpedalling. On the contrary, one of the dopaminergic components, sniffing was prevented by pretreatment of tryptophan, pargyline and imipramine. Recently, Solviter *et al.* [21] have reported that some behavioral syndromes induced by *d*-amphetamine are mediated by serotonergic neuron. That is, *d*-amphetamine (15–80 mg/kg, IP) causes numerous behavioral effects including simultaneous side to side head weaving, head tremor, forepaw paddling and splayed hindlimbs. These amphetamine syndromes have been prevented by 5-HT depletion with 5,7-dihydroxytryptamine or with PCPA. In addition, *d*-amphetamine (15 mg/kg, IP) also causes PCP like backward locomotion and circling/pivotting, these syndromes are increased when 5-HT synthesis is increased by tryptophan administration. On the contrary, these syndromes are decreased by an inhibitor of 5-HT synthesis, PCPA, and the 5-HT receptor blockers, metergoline and cyproheptadine [11]. Taken together with our present results, we could consider that PCP-induced backpedalling, head weaving and turning might be due to a pre-synaptic action of PCP on 5-HT neurons, and activation of 5-HT neuron is required for these behaviors to occur. The lack of

potentiation of the PCP-induced backpedalling may be related to less sensitivity to activate 5-HT neuron than to inhibit it.

On the other hand, in the biochemical study, PCP increased the contents of 5-HT in the thalamus/hypothalamus in parallel to PCP-induced sterotyped behaviors. At the dose of 10 mg/kg, PCP increased the contents of 5-HIAA in the thalamus/hypothalamus and striatum at 30 min after the injection. Furthermore, in the thalamus/hypothalamus, PCP increased the rate of increment of 5-HT folllowing the administration of pargyline. However, at the dose of 10 mg/kg, PCP decreased the contents of 5-HIAA in all brain areas except the striatum at 60 min after the injection. On the other hand, it is well known that the major mechanism for inactivation neuronally released 5-HT is re-uptake into 5-HT nerve ending. Furthermore, inhibition of 5-HT uptake results in decrement of 5-HIAA and increment of extraneuronal content of 5-HT [1]. Therefore, the decrement of 5-HIAA is also observed after administration of imipramine and chloroimipramine which are the strongest 5-HT uptake inhibitors [1]. In addition, PCP is also known to be a potent inhibitor of 5-HT uptake in a synaptosomal preparation [22]. Moreover, PCP prevented the depletion of 5-HT by PCA in all brain areas except the striatum at 60 min as strong as imipramine. However, it has no MAO inhibitory effect *in vivo* at the dose of 10 mg/kg. These results suggest that PCP increases 5-HT release and/or inhibits 5-HT uptake.

In conclusion, it has been demonstrated that PCP-induced stereotyped behaviors (backpedalling, head weaving and turning) are related to an increased serotonergic neuronal activity due to 5-HT releasing action and/or inhibitory action of 5-HT uptake by this drug.

ACKNOWLEDGEMENT

This study was supported in part by a grant No. 57570089 from the Ministry of Education, Science and Culture, Japan to T.N.

REFERENCES

1. Buus, Lassen, J., R. F. Squires, J. A. Christensen and L. Molander. Neurochemical and pharmacological studies on a new 5-HT-uptake inhibitor, FG 4963, with potential antidepressant properties. *Psychopharmacologia* **42:** 21–26, 1975.
2. Chen, G., C. R. Ensor, D. Russel and B. Bohner. The pharmacology of 1-(l-phenylcyclohexyl)-piperidine-HCl. *J Pharmacol Exp Ther* **127:** 241–250, 1959.
3. Curzon, G. and A. R. Green. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small region of rat brain. *Br J Pharmacol* **39:** 653–655, 1970.
4. 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.
5. Fessler, R. G., R. D. Sturgeon and H. Y. Meltzer. Phencyclidine induced ipsilateral rotation in rats with unilateral 6-hydroxydopamine-induced lesions of the substantia nigra. *Life Sci* **24:** 1281–1288, 1979.
6. Finnegan, K. T., M. I. Kanner and H. Y. Meltzer. Phencyclidine-induced rotational behavior in rats with nigrostraital lesions and its modulation by dopaminergic and cholinergic agents. *Pharmacol Biochem Behav* **5:** 651–660, 1976.
7. Fuller, R. W., K. W. Perry and B. B. Molloy. Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine. *Eur J Pharmacol* **33:** 119–124, 1974.
8. Glowinski, J. and L. L. Iversen. Regional studies of catecholamine in the rat brain-I: The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. *J Neurochem* **13:** 655–699, 1966.
9. Grahame-Smith, D. G. Studies *in vivo* on the relationships between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with monoamine oxidase inhibitor and L-tryptophan. *J Neurochem* **18:** 1053–1066, 1971.
10. Kelly, P. H. Drug-induced motor behaviour. In: *Drug, Neurotransmitters and Behaviour. 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.
11. Lee, A. J., J. C. R. Fernando and G. Curzon. Serotonergic involvement in behavioral responses to amphetamine at high dosage. *Neuropharmacology* **18:** 153–158, 1978.
12. Luby, E. D., B. O. Cohen, G. Rosenbaum, J. S. Gottlieb and R. Kelly. Study of a new schizophrenomimetic drug-sernyl. *Am Med Ass Arch Neurol Psychiatry* **31:** 363–369, 1959.
13. Ludy, 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.
14. Martin, J. R., M. H. Berman, I. Krewsun and S. F. Small. Phencyclidine-induced stereotyped behavior and serotonergic syndrome in rat. *Life Sci* **24:** 1699–1704, 1979.
15. Murray, T. F. and A. Horita. Phencyclidine-induced stereotyped behavior in rats: Dose response effects and antagonism by neuroleptics. *Life Sci* **24:** 2217–2225, 1979.
16. 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.
17. 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.
18. Nabeshima, T., M. Hiramatsu, M. Amano, H. Furukawa and T. Kameyama. Effects of acute and chronic administrations of phencyclidine on the levels of dopamine and metabolites in discrete brain areas of mouse. *Res Commun Sub Abuse* **4:** 273–287, 1983.
19. Randrup, A. and I. Munkvad. Biochemical, anatomical and psychological investigations of stereotyped behaviour induced by amphetamine. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garratini. New York: Raven Press, 1970, pp. 695–713.
20. Sander, M. and M. B. H. Youdim. Multiple forms of monoamines oxidase functional significance. *Pharmacol Rev* **24:** 331–348, 1972.
21. Solviter, R. S., E. G. Drust and J. D. Connor. Evidence that serotonin mediates some behavioral effects of amphetamine. *J Pharmacol Exp Ther* **206:** 348–352, 1978.
22. 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.
23. Sturgeon, R. D., R. G. Fessler and H. Y. Meltzer. Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. *Eur J Pharmacol* **59:** 169–179, 1979.
24. Tonge, S. R. and B. E. Leonard. The effects of some hallucinogenic drugs upon the metabolism of 5-hydroxytryptamine in the brain. *Life Sci* **8:** 805–814, 1969.
25. Tonge, S. R. and B. E. Leonard. Variation in hydroxytryptamine metabolism in the rat: effects on the neurochemical response to phencyclidine. *J Pharm Pharmacol* **23:** 711–712, 1971.
26. Weissbach, H., T. E. Smith, J. W. Daly, B. Witkop and S. Udenfriend. A rapid spectrophotometric assay of monoamine oxidase based on the rate of disappearance of kynuramine. *J Biol Chem* **235:** 1160–1169, 1960.
27. Whittaker, V. P. The application of subcellular fraction techniques to the study of brain function. *Prog Biophys Mol Biol* **15:** 39–96, 1965.