Involvement of Dopaminergic System in Phencyclidine-Induced Place Preference in Mice Pretreated with Phencyclidine Repeatedly

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ABSTRACT

In the conditioned place preference test, phencyclidine (PCP) produces place aversion in naive rats, whereas PCP produces place preference in rats treated with PCP repeatedly. Although the PCP-induced place aversion is thought to involve the serotonergic system, the mechanisms of the PCP-induced place preference are unclear. We investigated whether the dopaminergic system is involved in place preference induced by PCP in mice repeatedly treated with PCP, because it is well known that the dopaminergic system plays an important role in the rewarding effect of drugs. PCP (2–8 mg/kg s.c.) induced a dose-dependent place aversion in naive mice, whereas PCP (2–8 mg/kg s.c.) induced a dose-dependent place preference in mice pretreated with PCP (10 mg/kg/day s.c.) for 28 days. The place preference induced by PCP (8 mg/kg s.c.) was attenuated significantly by α-methyl-p-tyrosine (100 mg/kg i.p.), a tyrosine hydroxylase inhibitor, 6-hydroxydopamine (100 μg/mouse i.c.v.), a dopaminergic neurotoxin, and R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (0.5 mg/kg s.c.), a dopamine D1 receptor antagonist. These agents themselves produced neither the place preference nor aversion. In contrast to the attenuating effects of these agents, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (30 mg/kg i.p.), a noradrenergic neurotoxin, ritanserin (1 mg/kg i.p.), a serotonin2 receptor antagonist, and (–)-sulpiride (50 and 100 mg/kg i.p.), a dopamine D2 receptor antagonist, failed to affect the PCP-induced place preference. In mice pretreated with methamphetamine (1 mg/kg/day s.c.) for 14 days, PCP (8 mg/kg s.c.) induced the place preference, but not aversion. These results demonstrate that the PCP-induced place preference depends on dopaminergic, but not on serotoninergic and noradrenergic, neuronal systems and suggest a role for D1 receptors in the mediation of the PCP-induced place preference.

A place conditioning paradigm is used widely for determining both rewarding and aversive properties of drugs in animals (see review, Schechter and Calcagnetti, 1993). In this paradigm, many drugs of abuse such as amphetamines, cocaine and benzodiazepines produce a place preference in animals (Acquas et al., 1989; Schechter and Calcagnetti, 1993; Cervo and Sambanis, 1995).

PCP has been a common drug of abuse in the United States during the past two decades, because it produces both physical and psychological dependence in humans (Petersen and Stillman, 1978). Like many other abused drugs, PCP reinforces drug self-administration behavior (Marquis and Moreton, 1987). In the place conditioning paradigm, PCP produces place aversion in naive animals (Barr et al., 1985; Kitaichi et al., 1996; Nabeshima et al., 1996), whereas it produces place preference in rats pretreated with PCP repeatedly (Kitaichi et al., 1996; Nabeshima et al., 1996). These phenomena observed in rats are similar to those in humans; although a single use of PCP produces aversive effects, long-term use of it causes abuse in humans (Isaacs et al., 1986). We previously found that the PCP-induced place aversion on the CPP in rats is attributed to interaction with the serotonergic system, particularly, the postsynaptic 5-HT2 receptors (Nabeshima et al., 1996). However, few reports have addressed the pharmacological mechanisms of PCP-induced place preference.

ABBREVIATION: PCP, phencyclidine [1-(1-phenylcyclohexyl)piperidine]; CPP, conditioned place preference; 5-HT, serotonin (5-hydroxytryptamine); DA, dopamine; AMPF, α-methyl-p-tyrosine; 6-OHDA, 6-hydroxydopamine; (+) SCH-23390, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; DSP-4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; HPLC, high-performance liquid chromatography; NA, noradrenaline; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; NMDA, N-methyl-D-aspartate.
PCP interacts with the dopaminergic system directly through an inhibition of dopamine reuptake (Smith et al., 1977) or, to a lesser extent, through a stimulation of DA release (Bowyer et al., 1984) in vitro. Several in vivo microdialysis studies have confirmed the ability of PCP to increase DA efflux in the nucleus accumbens (Bristow et al., 1993) and medial prefrontal cortex (Rao et al., 1989; Hondo et al., 1994). Further, long-term treatment with PCP produces the development of sensitization to PCP-induced hyperlocomotion and rearing (Nabeshima et al., 1987; Noda et al., 1996) and the increased turnover of DA in the brain (Nabeshima et al., 1987). These effects are antagonized by DA antagonists, which suggests that the dopaminergic system is involved in behavioral sensitization.

Several lines of evidence suggest that the dopaminergic system plays an important role in the rewarding and abuse properties of drugs in the place conditioning paradigm. Blockade of DA receptors reduces or abolishes the place preference induced by amphetamine and opiates (Leone and Di Chiara, 1987). A recent study has shown that cocaine administered i.p. increases extracellular concentrations of DA in the nucleus accumbens and induces CPP (Hemby et al., 1994), which suggests that the mesolimbic DA neurons mediate the rewarding properties of various drugs of abuse (Di Chiara and Imperato, 1988). These findings, together with the pharmacological properties of PCP, suggest that the dopaminergic system is involved in PCP-induced place preference in rats pretreated with PCP repeatedly.

Accordingly, in the present study we investigated the pharmacological characterization of PCP-induced place preference in the CPP test. First, we investigated whether PCP-induced place preference in mice was modified by hypofunction or overfunction of the dopaminergic system. Second, selective DA receptor antagonists then were tested to delineate the role of D1 vs. D2 receptors in the mediation of the PCP-induced place preference. Finally, we examined the effect of a 5-HT2 receptor antagonist, ritanserin, on PCP-induced place preference, because ritanserin antagonizes the PCP-induced place aversion (Nabeshima et al., 1996).

Methods

Animals. Male mice of the ddY strain (Japan SLC Inc., Shizuoka, Japan), weighing 25 to 27 g at the beginning of the experiments, were used. The animals were housed in plastic cages and were kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity), with a 12/12 hr light-dark cycle (light on at 8:00 A.M.). Food (CE2, Clea Japan Inc. Tokyo, Japan) and tap water were available ad libitum.

All experiments were performed in accordance with the Guidelines for Animal Experiments of the Nagoya University School of Medicine. The procedures involving animals and their care were conducted in conformity with the international guidelines "Principles of Laboratory Animal Care" (NIH publication no. 85–23, revised 1985).

Drugs. Phencyclidine HCl (PCP) was synthesized by the authors according to the method of Maddox et al. (1965) and was checked for purity. AMPT, 6-OHDA and desipramine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Methamphetamine HCl (Philopine) was purchased from Dai-nippon Pharmaceutical Co. Ltd. (Osaka, Japan). (+) SCH-23390 HCl, (+) sulpiride and DSP-4 HCl were purchased from Funakoshi (Tokyo, Japan). Ritanserin was kindly provided from Janssen Kyowa Co. Ltd. (Tokyo, Japan). Other agents were obtained by standard commercial sources.

PCP, methamphetamine, DSP-4 and desipramine were dissolved in saline, 6-OHDA in saline containing 0.1% ascorbic acid and ritanserin in water containing 1% tartaric acid. AMPT was suspended in saline containing 0.3% (w/v) carboxymethyl cellulose sodium salt. (+) SCH-23390 and (+) sulpiride initially were dissolved in a minimum volume of 0.1 N HCl and then were diluted with distilled water (the pH of the solutions was adjusted to about 4 with NaHCO3).

Apparatus. The apparatus used for the place conditioning task consisted of two compartments: a black Plexiglas box and a transparent Plexiglas box (both 15 × 15 × 15 cm high) with metal gird floor. To enable the mice to distinguish easily the transparent box from the black one, the floor of the transparent and black boxes were covered with white plastic mesh and with black frosting Plexiglas, respectively. Each box could be divided by a sliding door (10 × 15 cm high).

Preconditioning test. The place conditioning paradigm was performed according to the method of Kitaichi et al. (1996), with a minor modification. In the preconditioning test, the sliding door was opened and the mouse was allowed to move freely between both boxes for 15 min once a day for 3 days. On the third day of the preconditioning test, we measured the time that the mouse spent in the black and transparent boxes with use of Scanet SV-10 LD (Toyo Sango Co. Ltd., Toyama, Japan). The box in which the mouse spent the most time was referred to as the "preferred side," and the other box the "nonpreferred side."

Conditioning. Conditioning was performed during 6 successive days. Mice were given drugs or vehicle in the apparatus with the sliding door closed. That is, a mouse was given PCP and put in its preferred (for investigating the PCP-induced place aversion) or nonpreferred (for the PCP-induced place preference) side for 20 min. The next day, the mouse was given saline, and placed opposite the drug conditioning site for 20 min. These treatments were repeated for three cycles (6 days).

Postconditioning test. In the postconditioning test, the sliding door was opened, and we measured the time that the mice spent in the black and transparent boxes for 15 min with use of Scanet SV-10 LD.

Data analysis. Place conditioning behaviors were expressed by Post – Pre, which was calculated as: [(postvalue) – (prevalue)], where post- and prevalues were the difference in time spent in the drug conditioning and the saline conditioning sides in the postconditioning and preconditioning tests, respectively.

Drug administration. PCP (2–8 mg/kg s.c.) was injected immediately before the conditioning. Ritanserin (0.3 and 1 mg/kg i.p.), AMPT (50 and 100 mg/kg i.p.), (+) SCH-23390 (0.1 and 0.5 mg/kg s.c.) and (+) sulpiride (50 and 100 mg/kg i.p.) were administered 60, 180, 15 and 60 min, respectively, before every treatment with PCP (8 mg/kg). These drugs were administered for 3 alternating days in the 6-day conditioning period, and corresponding vehicles were administered for the other 3 days. 6-OHDA (100 μg/mouse i.c.v.) and DSP-4 (30 mg/kg i.p.) were administered 7 and 3 days, respectively, before the preconditioning test. To prevent the destruction of noradrenergic neurons, mice were administered desipramine (25 mg/kg i.p.) 30 min before 6-OHDA treatment.

In the experiment of repeated administration of PCP, mice were administered PCP (10 mg/kg/day) for 28 days as in a previous report (Kitaichi et al., 1996). In the experiment of repeated administration of methamphetamine, mice were administered methamphetamine (1 mg/kg/day) for 7 or 14 days. One day after the last treatment with PCP or methamphetamine, the place conditioning test including preconditioning, conditioning and postconditioning tests was commenced.

Determination of monoamine contents. Immediately after the postconditioning test, the 6-OHDA-, AMPT- and DSP-4-treated and control mice were sacrificed. Brains were removed rapidly, and the prefrontal cortex and striatum were dissected out on an ice-cold plate according to the method of Glowinski and Iversen (1966). Each tissue sample was frozen quickly and stored in a deep freezer at −80°C until assayed.
The contents of monoamines were determined by a HPLC system with an electrochemical detector (Eicom, Kyoto, Japan) as described (Noda et al., 1997). Each frozen tissue sample was weighed, then homogenized with an ultrasonic processor (475 W, model XL2020, Heat Systems Inc., New York, New York) in 350 μl of 0.2 M perchloric acid containing isoproterenol (internal standard). The homogenate was placed in ice for 30 min and then centrifuged at 20,000 × g for 15 min at 4°C. The supernatant was mixed with 1 M sodium acetate to adjust the pH to 3.0 and then injected into a liquid chromatography system equipped with a reversed-phase ODS-column (4.6 × 150 mm, Eicom MA-5 ODS (diameter of stationary phase grains, 5 μm), Eicom, Kyoto, Japan) and an electrochemical detector (model ECD-100, Eicom). The column temperature was maintained at 25°C, and the detector potential was set at +750 mV. The mobile phase was 0.1 M citric acid and 0.1 M sodium acetate, pH 3.9, containing 14% methanol, 160 mg/l sodium-l-octanesulfonate and 5 mg/l ethylenediaminetetraacetic acid; the flow rate was 1 ml/min.

Statistics. All data were expressed as means ± S.E. Statistical differences among values for individual groups were determined with the Student-Newmann-Keuls multiple comparisons test.

Results

Effect of PCP on the performance in the place conditioning paradigm. We have confirmed our previous results that PCP has both aversion and preference in the place conditioning paradigm depending on the treatment schedule. In the naive mice, PCP (2–8 mg/kg) showed place aversion in a dose-dependent manner (fig. 1A). In contrast to this finding, in mice pretreated with PCP (10 mg/kg/day s.c.) for 28 days, PCP (2–8 mg/kg) produced place preference in a dose-dependent manner (fig. 1B).

Because significant PCP-induced place aversion and preference were obtained at 8 mg/kg, this dose was used in all the subsequent experiments.

Effect of AMPT and 6-OHDA on the PCP-induced place preference. The contents of DA in the prefrontal cortex and striatum of AMPT-treated mice were decreased significantly to 40.6% and 63.5%, respectively, compared with those in the vehicle-treated mice (table 1). In contrast, the contents of NA and 5-HT in both regions remained unaffected (table 1). When 6-OHDA (100 μg/mouse) was administered 7 days before starting the preconditioning test, the PCP (8 mg/kg)-induced place preference was attenuated (fig. 2B). 6-OHDA (100 μg/mouse) itself did not produce either place preference or aversion (fig. 2A).

The contents of DA in the prefrontal cortex and striatum of 6-OHDA-treated mice were decreased remarkably to 28.3% and 51.0%, respectively, compared with those in the vehicle-treated mice (table 1). In contrast, the contents of NA and 5-HT in both regions remained unaffected (table 1). When 6-OHDA (100 μg/mouse) was administered 7 days before starting the preconditioning test, the PCP (8 mg/kg)-induced place preference was attenuated (fig. 2B). 6-OHDA (100 μg/mouse) itself did not produce either place preference or aversion (fig. 2A).

Effects of repeated methamphetamine treatment on the motivational properties of PCP in mice. PCP (8 mg/kg) significantly produced place aversion in mice pretreated with saline repeatedly. In mice pretreated with methamphetamine (1 mg/kg/day) for 7 days, however, PCP (8 mg/kg) produced neither place aversion nor preference. On the other hand, PCP (8 mg/kg) significantly induced place preference in mice treated with methamphetamine (1 mg/kg/day) for 14 days (fig. 3).

Effects of (+)-SCH-23390 and (-)-sulpiride on the PCP-induced place preference. As shown in figure 4, (+)-SCH-23390 (0.5 mg/kg) significantly attenuated place preference produced by PCP in mice pretreated with PCP repeatedly. (+)-SCH-23390 (0.5 mg/kg) itself did not produce either place preference or place aversion (fig. 4). However, (-)-sulpiride (50 and 100 mg/kg) failed to modify the place preference produced by PCP.

Effect of ritanserin and DSP-4 on the PCP-induced place preference. In agreement with a previous report (Nabeshima et al., 1996), ritanserin (0.3 and 1 mg/kg) inhibited the place aversion produced by PCP in naive mice (fig. 5). However, it (1 mg/kg) failed to inhibit the place preference produced by PCP in mice pretreated with PCP repeatedly (fig. 6A). Ritanserin (1 mg/kg) itself did not produce either place preference or aversion (fig. 5).

The contents of NA in the prefrontal cortex and striatum of DSP-4-treated mice were decreased to 34.2% and 42.0%, respectively, compared with those in the vehicle-treated mice (table 1). In contrast, the contents of DA and 5-HT in both regions remained unaffected (table 1). PCP (8 mg/kg)-induced place preference was not affected by DSP-4 (30 mg/kg) (fig. 6B).

![Fig. 1. Effect of PCP on the performance in the place conditioning paradigm in naive mice (A) and in mice pretreated with PCP (10 mg/kg/day) for 28 days (B). The experimental protocol is described in the text. Each column represents the mean ± S.E.M. Numbers in the parentheses are the number of animals used. *P < .05, **P < .01 vs. corresponding saline-treated group.](attachment:image)
In the present study, PCP produced a dose-dependent place preference in mice pretreated with PCP for 28 days, as reported previously (Kitaichi et al., 1996; Nabeshima et al., 1996). This finding that PCP has rewarding properties is consistent with the results of several studies that PCP is self-administered by animals (Marquis and Moreton, 1987). Further, such a phenomenon also has been observed in humans; although single use of PCP produces aversive effects, long-term use of it causes abuse (Isaacs et al., 1986). Thus, these findings suggest that some functional changes induced by repeated PCP treatment play a critical role in PCP-induced place preference.

The dopaminergic system plays an important role in the rewarding and abuse properties of drugs in the place conditioning paradigm (Leone and Di Chiara, 1987). Several in vivo microdialysis studies have confirmed the ability of PCP to increase DA efflux in the nucleus accumbens (Bristow et al., 1993) and medial prefrontal cortex (Rao et al., 1989; Hondo et al., 1994). Repeated PCP treatment produces the behavioral sensitization (Nabeshima et al., 1987; Noda et al., 1996) and the increase of DA turnover in the striatum and nucleus accumbens (Nabeshima et al., 1987). Taken together, the dopaminergic system may be involved in PCP-induced place preference in rats pretreated with PCP repeatedly. The present study showed that PCP-induced place preference in mice pretreated with PCP for 28 days was blocked by coadministration of a tyrosine hydroxylase inhibitor, AMPT, and lesion of the dopaminergic system by 6-OHDA. Analysis of the neurochemical effects of 6-OHDA and AMPT treatment revealed a marked decrease of DA level and no reduction of NA and 5-HT levels in the brain. Although AMPT depletes both NA and DA contents in the brain, the reasons for which no depletion of NA was obtained by AMPT in the present biochemical study are unclear.

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>NA (ng/g wet tissue)</th>
<th>DA (ng/g wet tissue)</th>
<th>5-HT (ng/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>327.9 ± 22.1</td>
<td>50.3 ± 8.5</td>
<td>450.3 ± 32.5</td>
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<tr>
<td>PCP</td>
<td>7</td>
<td>326.7 ± 13.4</td>
<td>69.0 ± 20.9</td>
<td>417.9 ± 31.0</td>
</tr>
<tr>
<td>AMPT (100 mg/kg)</td>
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<td>255.2 ± 29.2</td>
<td>20.4 ± 6.3*</td>
<td>438.9 ± 19.8</td>
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<tr>
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<td>337.7 ± 21.6</td>
<td>75.5 ± 2.7</td>
<td>399.8 ± 50.0</td>
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<tr>
<td>PCP</td>
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<td>277.0 ± 26.9</td>
<td>78.1 ± 9.1</td>
<td>355.6 ± 66.8</td>
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<tr>
<td>6-OHDA (100 µg/mouse)</td>
<td>8</td>
<td>233.6 ± 47.7</td>
<td>21.4 ± 4.2**</td>
<td>431.2 ± 22.1</td>
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<tr>
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<td>366.1 ± 8.1</td>
<td>57.7 ± 6.3</td>
<td>415.1 ± 14.8</td>
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<tr>
<td>PCP</td>
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<td>69.7 ± 24.0</td>
<td>403.5 ± 21.0</td>
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<td>DSP-4 (30 mg/kg)</td>
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<td>125.3 ± 22.4**</td>
<td>50.7 ± 5.8</td>
<td>413.0 ± 17.5</td>
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<tr>
<td>Striatum</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>6</td>
<td>167.5 ± 23.2</td>
<td>10753.9 ± 1170.2</td>
<td>431.9 ± 54.6</td>
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<tr>
<td>PCP</td>
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<td>6829.4 ± 838.7*</td>
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<tr>
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<td>526.8 ± 118.7</td>
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<td>84.7 ± 15.9***</td>
<td>9407.7 ± 451.4</td>
<td>435.2 ± 29.8</td>
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*Values are expressed as the mean ± S.E.M. *P < .05, **P < .01 vs. control group.

Discussion

In the present study, PCP produced a dose-dependent place preference in mice pretreated with PCP for 28 days, as reported previously (Kitaichi et al., 1996; Nabeshima et al., 1996). This finding that PCP has rewarding properties is consistent with the results of several studies that PCP is self-administered by animals (Marquis and Moreton, 1987). Further, such a phenomenon also has been observed in humans; although single use of PCP produces aversive effects, long-term use of it causes abuse (Isaacs et al., 1986). Thus, these findings suggest that some functional changes induced by repeated PCP treatment play a critical role in PCP-induced place preference.

The dopaminergic system plays an important role in the rewarding and abuse properties of drugs in the place conditioning paradigm (Leone and Di Chiara, 1987). Several in vivo microdialysis studies have confirmed the ability of PCP to increase DA efflux in the nucleus accumbens (Bristow et al., 1993) and medial prefrontal cortex (Rao et al., 1989; Hondo et al., 1994). Repeated PCP treatment produces the behavioral sensitization (Nabeshima et al., 1987; Noda et al., 1996) and the increase of DA turnover in the striatum and nucleus accumbens (Nabeshima et al., 1987). Taken together, the dopaminergic system may be involved in PCP-induced place preference in rats pretreated with PCP repeatedly. The present study showed that PCP-induced place preference in mice pretreated with PCP for 28 days was blocked by coadministration of a tyrosine hydroxylase inhibitor, AMPT, and lesion of the dopaminergic system by 6-OHDA. Analysis of the neurochemical effects of 6-OHDA and AMPT treatment revealed a marked decrease of DA level and no reduction of NA and 5-HT levels in the brain. Although AMPT depletes both NA and DA contents in the brain, the reasons for which no depletion of NA was obtained by AMPT in the present biochemical study are unclear. A possible explanation is that the doses of AMPT used are lower than those of the depleted.
NA contents. DSP-4, a NA neurotoxin, at the dose of 30 mg/kg which caused significant depletion of NA, but not of DA and 5-HT, in the brain, failed to affect the PCP-induced place preference. Thus, it is suggested that the abolition of PCP-induced place preference results specifically from the destruction of DA neurons.

A 5-HT$_2$ receptor antagonist, ritanserin, attenuated the PCP-induced place aversion in mice pretreated with PCP repeatedly. Thus, it is possible that 5-HT$_2$ receptors may be activated by the PCP-induced behaviors, including 5-HT$_{1A}$ receptor-mediated behaviors, are decreased (Nabeshima et al., 1987). Therefore, it can be hypothesized that the activity of DA, but not of NA and 5-HT, neurons may be necessary for the expression of the rewarding effects of PCP.

This hypothesis also is supported by the other present finding. Namely, in mice pretreated with methamphetamine repeatedly, PCP induced the place preference, but not aversion. Repeated treatment with amphetamine or methamphetamine enhances several behaviors (hyperlocomotion and stereotypy) and the DA efflux produced by these drugs in rats either in vitro and in vivo (Nabeshima et al., 1987; Yamada et al., 1988; Robinson et al., 1988; Kazahaya et al., 1989). These findings have demonstrated that hyperfunction of the dopaminergic system is produced by repeated methamphetamine treatment. Thus, in view of the repeated PCP-induced hyperfunction in the dopaminergic system, together with the critical importance of the dopaminergic system to the rewarding properties of drugs, it is suggested that the place preference induced by repeated administration of PCP for 28 days is caused by functional changes in the dopaminergic system.

A recent study showed that cocaine administered i.p. increases extracellular concentrations of DA in the nucleus accumbens and induces CPP (Hemby et al., 1994), which suggests that the mesolimbic DA neurons mediate the rewarding properties of various drugs of abuse (Di Chiara and Imperato, 1988). Repeated PCP treatment was demonstrated by an increase of DA turnover in the striatum and nucleus accumbens of rats treated with PCP repeatedly (Nabeshima et al., 1987). Thus, it is suggested that both PCP- and cocaine-induced place preferences are mediated via the mesolimbic DA neurons. However, we could not determine the changes of DA turnover in the nucleus accumbens of mice showing PCP-induced place preference, because it is difficult to dissect exactly only the nucleus accumbens in mice. Our recent in vivo microdialysis study in rats has found that DA turnover is increased in the nucleus accumbens of rats showing the place preference induced by PCP, compared with that in control rats, which suggests that the hyperfunction in the mesolimbic dopaminergic neurons is involved in the expression of PCP-induced place preference.
Another possibility is that the serotonergic system, in particular 5-HT$_2$ receptors, mediating the aversion may become desensitized by repeated PCP treatment because some biochemical studies have demonstrated a decrease of 5-HT turnover and 5-HT$_2$ receptor density in the brain of rats treated with PCP repeatedly (Nabeshima et al., 1985, 1987). Previously our finding showed that PCP-induced place aversion is diminished in rats pretreated with PCP for 14 days (Nabeshima et al., 1996), which indicates that PCP induces neither place aversion nor preference. In such rats, PCP-induced head-twitch behavior, which may be mediated by 5-HT$_2$ receptors, also was diminished (Nabeshima et al., 1996), which suggests that such a regimen produces the down-regulation of 5-HT$_2$ receptors. In the present study, we found that PCP induced place preference in mice pretreated with methamphetamine repeatedly. Although repeated methamphetamine treatment produces the hyperfunction of the dopaminergic system, there is little evidence that the serotonergic system is desensitized by methamphetamine. Thus, the present result, that PCP induces place preference in mice pretreated with PCP repeatedly, may not be caused only by desensitization of the serotonergic system by repeated PCP treatment. However, further studies are necessary to clarify the significance of desensitized serotonergic system.

Recent studies with selective D$_1$ and D$_2$ receptor antagonists have demonstrated that blockade of D$_1$, but not D$_2$ receptors prevents behavioral sensitization to amphetamine (Drew and Glick, 1990). An increase in D$_1$ receptor density in the brain has been found after daily treatment with methamphetamine (Ujike et al., 1991). Further, amphetamine regulates the expression of several genes, including c-fos, via D$_1$ receptors in the rat brain (Nguyen et al., 1992). Considering these findings and the present finding that PCP induced the place preference in rats pretreated with methamphetamine repeatedly, there is a possibility that the rewarding effect of PCP is mediated via D$_1$ receptors. The present results showed that the selective D$_1$ receptor antagonist (+) SCH-23390 blocked the PCP-induced place preference. In contrast, the selective D$_2$ receptor antagonist, (-) sulpiride failed to block it, although we used enough doses to block the central D$_2$ receptors (Ljungberg and Ungerstedt, 1978). The doses of (+) SCH-23390 used in this study were ineffective as a conditioning stimulus, and SCH-23390 has been shown to attenuate the drug-conditioned place preference as well as drug-conditioned place aversion (Acquas et al., 1989). Thus, the attenuation of PCP place conditioning cannot be attributed to an aversive action of the antagonist by itself. A similar effect of SCH-23390 has been observed in the cocaine-induced place preference in rats as well as the PCP-induced place preference. Namely, Cervo and Samanin (1995) demonstrated that SCH-23390 administered before cocaine during the conditioning phase significantly blocked the establishment of place conditioning, whereas (-) sulpiride had no effect. These findings suggest that D$_1$ receptors play a more important role in cocaine place conditioning as well as that of PCP. It is unlikely that the relatively high affinity of SCH-23390 for 5-HT$_2$ receptors (Bischoff et al., 1986) plays a role, because (+) SCH-23390 at the doses used in the present study does not affect the in vivo binding of [3H]spiperone in the rat prefrontal cortex (Bischoff et al., 1986). In addition, in the present study, ritanserin, a selective 5-HT$_2$ receptor antagonist, failed to affect the PCP-induced place preference in mice pretreated with PCP repeatedly. Taken together with these findings, the present results suggest that PCP can sensitize D$_1$ receptors to DA, enabling them to act independently from D$_2$ receptors, as observed in some cases of sensitization (Breese et al., 1985), and that D$_1$ receptors are involved in the conditioning of the rewarding effect of PCP.

Abundant evidence exists that SCH-23390 impairs learning and memory in rodents. Thus, it is possible that the effect on place conditioning may be resulted from a generalized disruption of behavior rather than a specific motivational deficits. This possibility, however, is unlikely because SCH-23390 has failed to modify the place preference induced by [D-Ala$_2$]deltorphine II, a selective delta-1 opioid receptor agonist (Suzuki et al., 1996). Therefore, (+) SCH-23390 may not disrupt learning, but directly affect a reward process in our present experiment.

(+)-SCH-23390 blocks the PCP-induced place preference by the involvement of D$_1$ receptors in mediating the rewarding effects of PCP as assessed by expression of place preference. Kobayashi and Inoue (1993) reported that the systemic administration of MK-801, which is a noncompetitive NMDA receptor antagonist, as well as PCP, enhances the in vivo

![Fig. 6. Effect of ritanserin (A) and DSP-4 (B) on the place preference induced by PCP in mice. The experimental protocol is described in the text. Each column represents the mean ± S.E.M. Numbers in the parentheses are the number of animals used. *P < .05, **P < .01 vs. (vehicle + saline)-treated group.](image-url)
binding of \(^{3}H\)SCH-23390 in the striatum. In addition, MK-801 enhances the stimulant effect of a D
al.
801 enhances the stimulant effect of a D 1, but not a D 2, receptor agonist, in monoamine-depleted mice (Goodwin et al., 1992; Svensson et al., 1992). On the basis of these results, we speculate that the functional changes in the dopaminergic system, particularly in D 1 receptors, were produced during repeated PCP treatment for 28 days, and then, the rewarding effect of PCP resulted from an increase in D 1 receptor activation, secondary to an increase of DA release. If such is the case, then the administration of (+) SCH-23390 before the conditioning phase would attenuate the motivational effect of PCP by masking PCP-induced activation of D 1 receptors. However, studies that could associate the neurochemical and behavioral actions of PCP should be performed to elucidate the mechanisms of PCP abuse.

The conditioned place preference consists of an acquisition or development phase during which the animals receive the drug in one distinctive environment, and a test or expression phase in which drug-free animals are tested for their preference of the environment previously paired with the drug. Blockade of D 1 receptors blocks the acquisition and expression of amphetamine-induced place preference (Hiroi and White, 1991), whereas it blocks only acquisition of cocaine-induced place preference (Cervo and Samanin, 1995). In the present study, (+) SCH-23390, administered before PCP during the conditioning phase, blocked the acquisition, whereas it is unclear whether (+) SCH-23390 blocks the expression of PCP-induced place preference. Thus, the significance of D 1 receptors and/or others in expression of PCP-induced place preference should be clarified by further study with use of D 1 receptors and/or other antagonists, because it has been suggested that different neurochemical mechanisms appear to mediate the acquisition and expression of this incentive learning (Hiroi and White, 1991).

In summary, the present results indicate that the repeated administration of PCP produces place preference, and that dopaminergic systems, but not serotoninergic and/or noradrenergic systems, are involved in PCP-induced place preference and that some changes in dopaminergic systems induced by repeated PCP treatment play a critical role in the addiction of this drug. Further, the present study demonstrates an involvement of D 1 receptors in the conditioning of the rewarding effect of PCP. The previously documented (Leone and DiChiera, 1987; Shippenberg et al., 1993) effectiveness of systemically administered (+) SCH-23390 in attenuating the rewarding effects of opioids and other drugs of abuse suggests that D 1 receptor ligands may be useful therapeutic agents for the treatment of drug addiction.

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