Pharmacological Characterization of Locomotor Sensitization Induced by Chronic Phencyclidine Administration

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ABSTRACT

Phencyclidine (PCP) administration in rats acutely in high doses or chronically in lower doses causes neurotoxicity characterized by neuronal vacuolization and apoptotic neuronal death, respectively. The purpose of this study was to determine whether drugs that previously had been reported to prevent either type of neurotoxicity were also able to prevent locomotor sensitization following chronic PCP administration. PCP (5 or 20 mg/kg) was administered once a day for 5 days following drug pretreatment. After withdrawal, rats were challenged with 3.2 mg/kg PCP and locomotor activity was assessed. Haloperidol and clozapine significantly attenuated sensitization elicited by PCP (20 mg/kg). The D₁-like antagonist SCH23390 was much less effective than clozapine, showing a marginal inhibition. Risperidone, a D₂-serotonin (5-HT₂) antagonist, also resulted in a marginal attenuation of 15%. Ketanserin, a 5-HT₂ antagonist, had no effect. Atropine retarded sensitization by 35% and (+)-sulpiride caused a 50% reduction. The AMPA/kainate antagonist, 6,7-dinitroquinoxaline-2,3-dione, had no effect, but barbital sodium reduced sensitization by 54%. These data suggest that γ-aminobutyric acid A, D₂, and muscarinic receptors play a major role in the complex pathway underlying sensitization to PCP; whereas D₁, 5-HT₂ and AMPA receptors have little or no relevance in the behavioral sensitization produced by 20 mg/kg PCP. In a model using 5 mg/kg PCP, the effects of sulpiride and SCH23390 replicated those observed with 20 mg/kg PCP and further showed that acute locomotor activation is not a strict requirement for the development of sensitization. These data argue that there is overlap, but non-identity, between the mechanisms underlying PCP-induced sensitization and neurotoxicity.

The noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) is a drug of abuse that mimics both positive and negative signs of schizophrenia in humans (Javitt and Zukin, 1991). Acute PCP administration to rats leads to increased locomotor activity, ataxia, rearing, stereotypy, and head weaving (Castellani and Adams, 1981). Locomotor activation in rodents is postulated to predict the capacity of a drug to induce psychoses in humans (Wolf, 1998), and PCP-induced increases in locomotor activity are believed related to the clinical manifestations of schizophrenia (Steinpreis et al., 1994; Adams and Moghaddam, 1998). Acute administration of PCP also results in neurotoxicity characterized primarily by neuronal vacuolization (Olney et al., 1989, 1991). Neurotoxicity induced by PCP and other noncompetitive NMDA receptor antagonists such as MK-801 has been shown to be accompanied by the induction of heat shock protein 70 (Sharp et al., 1991), increased glial fibrillar acidic protein (Fix et al., 1993), and neuronal shrinkage and nuclear condensation (Zhang et al., 1996), perhaps suggestive of degeneration by both necrotic and apoptotic mechanisms. This occurs in many brain regions that are similar to those altered in schizophrenia (Olney and Farber, 1995). Neurotoxicity defined by vacuolization, heat shock protein, and glial fibrillar acidic protein has been demonstrated to be prevented by typical and atypical antipsychotics, dopamine D₁ receptors antagonists, non-NMDA glutamate receptor antagonists, muscarinic antagonists, sigma receptor ligands, α₂- receptor agonists, and γ-aminobutyric acid (GABA) receptor facilitators (Sharp et al., 1992, 1994a,b; Farber et al., 1995; Sharp and Petersen, 1995).

The pharmacological data described above, coupled with the finding of reduced interneuron density and increased GABA_A binding in the cingulate cortex of schizophrenic brains, led Olney to propose an NMDA receptor hypofunction hypothesis of schizophrenia (Olney and Farber, 1995). Simply stated, glutamate receptor activation of NMDA receptors located on GABAergic neurons maintains tonic inhibition over excitatory tone in the cortex. Blockade of this tonic inhibition via NMDA receptor antagonists leads to over excitation and cell death.

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; PCP, phencyclidine; DNQX, 6,7-dinitroquinoxaline-2,3-dione; SCH23390, R-(+)-8-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol; GABA_A, γ-aminobutyric acid A; MK-801, dizocilpine; 5-HT, serotonin.
Although acute PCP in humans sometimes produces psychoses reminiscent of schizophrenia, repetitive administration is more likely to result in schizophrenia-like behavioral alterations (Carlin et al., 1979; Lewis and Hordan, 1986; Cosgrove and Newell, 1991). Repeated administration of PCP in rats causes enhanced locomotor activation upon PCP challenge (Xu and Domino, 1994; Johnson et al., 1998). In one of these studies behavioral sensitization to chronic administration of PCP was associated with apoptotic degeneration of cortical neurons; both were prevented by clozapine pretreatment (Johnson et al., 1998), an atypical antipsychotic with antagonistic effects at multiple receptors. Locomotor sensitization following chronic administration is common among psychomotor stimulants, including cocaine and amphetamine. Interestingly, amphetamine and cocaine sensitization (Karler et al., 1989), but not sensitization to PCP (Xu and Domino, 1994), can be prevented by pretreatment with MK-801, an NMDA antagonist similar to PCP. As various forms of neuronal plasticity are dependent on NMDA receptors, sensitization to amphetamine and cocaine is thought to involve neuronal plasticity. However, sensitization to PCP may be fundamentally different in that it is itself an NMDA antagonist. It is possible that the neurotoxic effects of PCP could play a role in the evolution of long-lasting behavioral changes, including locomotor sensitization. As a working hypothesis, we proposed that PCP-induced behavioral sensitization and neurotoxicity involve a similar mechanism. The purpose of this study was to test the hypothesis that drugs that have been demonstrated to prevent neurodegeneration caused by NMDA antagonists also attenuate the locomotor-sensitizing effects of chronic PCP administration. Special interest was paid to drugs that mimic a particular action of clozapine.

Materials and Methods

Experimental Design. Adult female Sprague-Dawley rats (>90 days; 250 g) were housed two or three per cage with a regular 12-h/12-h light/dark cycle (lights on at 7:00 AM, off at 7:00 PM); food and water were available ad libitum. In most experiments, six to ten rats were used in each treatment group. (The exact N value is given in the appropriate figure legend.) Thirty minutes before administration of saline or PCP (20 mg/kg s.c.), rats were pretreated (s.c.) with either vehicle, 10 mg/kg clozapine, 1 mg/kg haloperidol, 20 mg/kg atropine, 20 mg/kg pentobarbital, 100 mg/kg barbital, 0.8 mg/kg ketanserin, 10 mg/kg 6,7-dinitroquinoxaline-2,3-dione (DNQX), 0.1 mg/kg \( R(+)-8,2,3,4,5\)-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SCH23390), or 100 mg/kg sulphide once a day for 5 consecutive days. The doses of atropine, haloperidol, DNQX, pentobarbital, and clozapine used were based on those used to prevent PCP-induced vacuolization and heat shock protein 70 (HSP70) induction (Sharp et al., 1992, 1994a,b; Olney and Farber, 1995; Sharp and Petersen, 1995). Those for SCH23390, sulphide, and ketanserin were based on the psychomotor stimulant literature. In a separate but parallel set of experiments, rats were pretreated with either 0.1 or 0.5 mg/kg SCH23390 or 50 or 100 mg/kg sulphide 30 min before chronic treatment with 5 mg/kg PCP. In the case of SCH23390, this drug was also administered 90 min after PCP to ensure blockade of \( D_1 \) receptors throughout the duration of action of PCP. In both series of experiments, rats were assessed for evidence of locomotor sensitization as described below 3 days (72–78 h) following the last injection.

Locomotor Activity Assessment. Rats were placed in locomotor activity boxes and allowed to habituate for 60 min before receiving a challenge dose of saline or 3.2 mg/kg PCP (i.p.). Locomotor activity was assessed for an additional 90 min. Locomotor activity was measured via an open-field activity system (San Diego Instruments, San Diego, CA) comprised of four individual Plexiglas enclosures (40 x 40 x 40 cm) consisting of a 4 x 4 photo beam matrix to measure central and peripheral activity. The number of horizontal (central + peripheral) activity photobeams interruptions were recorded in 5-min bins. Data were analyzed as horizontal (peripheral plus central) activity counts totaled for the 90-min test session following s.c. injection of PCP or its saline control. Because group comparisons were specifically defined before the start of the experiment, these planned comparisons were conducted in lieu of an overall F test in a multifactorial ANOVA; this statistical analysis has been supported in a number of statistical texts (e.g., Keppel, 1973). Thus, each experiment was subjected to a one way ANOVA with levels of the treatment factor corresponding to the three to four drug combinations administered in that experiment. Planned, pairwise comparisons of the treatment means were made with the help of SigmaStat statistical software (Jandel, San Rafael, CA). All statistical analyses were conducted with an experiment-wise error rate of \( \alpha = 0.05 \).

Drugs. PCP was acquired from the National Institute on Drug Abuse, Rockville, MD. SCH23390, \( R(+)-\) sulpiride, ketanserin, DNQX, risperidone, and atropine were obtained from RBI (Research Biochemicals International, Natick, MA). Clozapine was a gift from Sandoz Pharmaceuticals (East Hanover, NJ). Haloperidol (solution), pentobarbital (solution), and barbital sodium were obtained from the University of Texas Medical Branch pharmacy. PCP was dissolved in 0.9% NaCl. Atropine, ketanserin, risperidone, SCH23390, and barbital sodium were dissolved in water. Sulpiride was dissolved in water and 5% ethanol and adjusted to a pH of 7.0 using l-lactic acid (final lactic acid concentration = 0.1%). Clozapine was dissolved in 1% l-lactic acid and titrated to 0.1% l-lactic acid final concentration with 0.9% NaCl. Haloperidol solution was dissolved in water. Pentobarbital was diluted to the appropriate concentration with water and 1% ethanol. DNQX was dissolved in 10% dimethyl sulfoxide then titrated to the appropriate concentration (10 mg/ml) with double-distilled H\(_2\)O and NaOH (final pH = 8.0). One milliliter per kilogram of the corresponding vehicle controls was injected as appropriate.

Results

Acute administration of PCP in doses ranging from 2.5 to 20 mg/kg (i.p.) produced a monotonic increase in horizontal locomotor activity when assessed over the first 90 min following administration (Fig. 1). Observation of these animals after returning them to their home cage suggested that the duration of locomotor activation increased as a function of dose. For example, the effects of 5 mg/kg were evident for about 4 h, whereas 20 mg/kg PCP resulted in a hyperactive state that lasted about 5 h. Although not assessed quantitatively in this study, stereotypic activity was noticeable at 5 mg/kg and was prominent at 10 mg/kg. Similarly, ataxia was evident at 5 and 10 mg/kg and was pronounced at 20 mg/kg. We have previously reported locomotor sensitization to the effects of 20 mg/kg PCP in both males and females (Johnson et al., 1998; Hanania et al., 1999) but had not previously determined whether this response was produced in a dose-related manner. To make this determination, female rats were treated with either saline, 5, 10, or 15 mg/kg PCP (s.c.) once per day for 5 days; they were then challenged 3 days later with 3.2 mg/kg PCP (i.p.). Figure 2 shows that all three doses of PCP produced a robust sensitization to the PCP challenge (df = 3, \( F = 72.7, p < 0.05 \) versus saline). The response observed here was similar in magnitude to an earlier study of the effects of 20 mg/kg PCP in an identical
treatment protocol (Johnson et al., 1998). Although there were no apparent differences between PCP treatment groups, post hoc analysis indicated that the response of the 10 mg/kg group was significantly greater than that of the 15 mg/kg treatment group.

Because most of the previously published data on acute PCP neurotoxicity had used doses ranging from 18 to 80 mg/kg (see Discussion), we chose to pharmacologically characterize the effects of 20 mg/kg PCP in the first part of this study. Figure 3A shows the results of experiments in which rats were treated chronically with either clozapine vehicle plus saline, vehicle plus 20 mg/kg PCP, 10 mg/kg clozapine plus saline, or clozapine plus PCP (s.c.). Clozapine or its vehicle was administered 1 h before PCP. Clozapine pretreatment significantly reduced (60%) the locomotor activity elicited by the PCP challenge following chronic PCP administration (n = 8 for each group; df = 3; F = 133.8; p < 0.05). These data are very similar to those obtained in an earlier preliminary study with only three rats per group (Johnson et al., 1998). As we observed in the earlier study, chronic clozapine administration alone appeared to enhance the locomotor activating effects of PCP challenge. In a similar paradigm, pretreatment with 1 mg/kg haloperidol (df = 3, F = 58.7, p < 0.05) or 1 mg/kg risperidone (df = 3, F = 84.8, p < 0.05) also significantly attenuated PCP-induced behavioral sensitization (Fig. 3, B and C). Although haloperidol was particularly effective at this dose, the same dose of risperidone caused only a modest 15% reduction in sensitization.

The selective dopamine D_1 receptor antagonist SCH23390 (0.1 mg/kg) and the dopamine D_2 receptor antagonist (+)-sulpiride (100 mg/kg) also attenuated the development of locomotor sensitization caused by chronic PCP administration (Fig. 4, A and B). However, it was evident that the effect of sulpiride pretreatment (df = 3, F = 165, p < 0.05) was much greater than that of SCH23390 (df = 3, F = 54, p < 0.05) in attenuating the response. Like clozapine, chronic SCH23390 administration alone also resulted in a sensitized
locomotor response to the 3.2 mg/kg PCP challenge ($p < 0.05$).

Because of the possible confounding effects of sedation in the experiments with haloperidol and SCH23390, we decided to test the effects of pentobarbital in this paradigm. The results of this experiment were also of interest because of the report that diazepam blocked the neurotoxic effects of MK-801 (Olney et al., 1989), an NMDA antagonist similar in many respects to PCP. Despite the sedative effects of sodium pentobarbital, a dose of 20 mg/kg did not attenuate the development to sensitization caused by chronic PCP administration (Fig. 5A). A potential confound here is that the acute effects of PCP during the chronic regimen outlasted the effects of pentobarbital by 1 to 2 h. Thus, the lack of effect on sensitization could be because the modest locomotor activating effects of PCP after the sedative effect of pentobarbital subsided could be sufficient to cause sensitization. Thus, the longer-acting barbital sodium was utilized. A dose of barbital sodium (100 mg/kg) that was markedly sedative throughout the course of action of PCP attenuated the locomotor sensitizing effects of PCP by approximately 60% (Fig. 5B; $df = 3, F = 224, p < 0.05$) but did not completely block the development of sensitization.

Because of the potential role of muscarinic, 5-HT2A, and AMPA receptors in MK-801 toxicity, we determined the effects of single doses of atropine, ketanserin, and DNQX on PCP-induced sensitization. Figure 6A demonstrates that 20 mg/kg atropine pretreatment was effective in reducing (~50%) the locomotor sensitization elicited by chronic PCP administration ($df = 3, F = 118.3, p < 0.05$). Pretreatment with either ketanserin (0.8 mg/kg) or DNQX (10 mg/kg) had no effect on the development of sensitization to chronic PCP administration (Fig. 6, B and C). It should be noted that DNQX was used at a dose known to prevent acute high-dose PCP neurotoxicity (Sharp and Petersen, 1995), and ketanserin was used at a dose similar to those shown in the literature to prevent PCP-induced locomotor activity (e.g., Krebs-Thomson et al., 1998). These data are summarized in Table 1.

In the second part of this study, we sought to determine whether sensitization resulting from a lower, more behaviorally relevant dose of PCP was similarly affected by dopamine receptor antagonists. Also, we wanted to determine whether the acute locomotor activating effect of PCP was differentially affected by D1-like and D2-like antagonists. For these studies we chose 5 mg/kg PCP as the chronic dose and kept the challenge dose at 3.2 mg/kg. Figure 7 shows the effect of pretreatment with two doses of SCH23390 on the development of sensitization to PCP following 5 days of treatment with 5 mg/kg PCP. Neither 0.1 nor 0.5 mg/kg SCH23390 had any significant effect on the development of sensitization to PCP, although both doses of SCH23390 when given alone produced a moderate sensitization to PCP challenge. In opposition, pretreatment with 50 and 100 mg/kg sulpiride produced a dose-related inhibition of the development of sensitization (Fig. 8; $df = 3, F = 111, p < 0.05$ and $df = 3, F = 145$, $p < 0.05$).
It should be noted that sensitization development was not completely blocked by sulpiride in that the high dose produced only about 75% blockade.

Finally, Fig. 9 shows that the D₁-like and D₂-like antagonists had nearly the exact opposite effects on the acute locomotor activating effects of 5 mg/kg PCP. That is, the low dose of SCH23390 (0.1 mg/kg), which had no effect on the development of sensitization, inhibited the acute effect of PCP by about 75% (top panel, df = 3, F = 73, p < 0.05). SCH23390 at 0.5 mg/kg had a similar effect (df = 3, F = 54, p < 0.05, data not shown). Furthermore, 50 mg/kg sulpiride, which inhibited sensitization development by about 45%, had no significant effect on the acute effect of PCP (middle panel). However, this apparent separation between the roles of D₁-like and D₂-like receptors in mediating the acute locomotor effects of PCP and the development of sensitization, respectively, is incomplete in that the high dose of sulpiride affected both measures. In this regard, it should be noted that although sulpiride at 100 mg/kg blocked the acute locomotor effect of PCP by about 35% (bottom panel, df = 3, F = 35, p < 0.05), it blocked the development of sensitization by about 75%, suggesting a preferential action against the latter mechanism. The data from the second part of this study are also summarized in Table 1.

![Image](https://via.placeholder.com/150)

**Fig. 6.** The effect of atropine (A; N = 8/group), ketanserin (B; N = 3/group), and DNQX (C; N = 3/group) on the development of locomotor sensitization induced by chronic PCP administration. In all experiments, rats were pretreated with vehicle or drug 0.5 h before chronic administration of saline or 20 mg/kg PCP. Neither ketanserin nor DNQX had a significant effect, but atropine caused a significant reduction in locomotor activity following challenge with 3.2 mg/kg PCP (p < 0.05).

**Fig. 7.** The effect of SCH23390 on the development of locomotor sensitization induced by chronic PCP administration. In both experiments, rats were pretreated with vehicle or drug 0.5 h before chronic administration of saline or 5 mg/kg PCP and then again 90 min later. Pretreatment with SCH23390 at either 0.1 mg/kg (A; N = 6/group) or 0.5 mg/kg (B; N = 6/group) failed to attenuate sensitization, but both doses caused a significantly enhanced response to acute PCP challenge (p < 0.05).

**TABLE 1. Summary of antagonism of PCP-induced locomotor sensitization**

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Dose (mg/kg)</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Part 1 (20 mg/kg PCP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>10</td>
<td>-60</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>1</td>
<td>-40</td>
</tr>
<tr>
<td>Risperidone</td>
<td>1</td>
<td>-15</td>
</tr>
<tr>
<td>SCH23390</td>
<td>0.1</td>
<td>-17</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>100</td>
<td>-50</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>20</td>
<td>-35</td>
</tr>
<tr>
<td>DNQX</td>
<td>0.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Atropine</td>
<td>100</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>50</td>
<td>-30</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>100</td>
<td>-53</td>
</tr>
<tr>
<td>Part 2 (5 mg/kg PCP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCH23390</td>
<td>0.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>SCH23390</td>
<td>0.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>50</td>
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<tr>
<td>Sulpiride</td>
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Acute administration of high doses of PCP primarily causes neurotoxicity in the posterior cingulate/retrosplenial cortex (Olney et al., 1991). However, repeated administration of the NMDA receptor antagonists MK-801 and PCP caused neurotoxicity in additional areas, including the anterior cingulate, parietal, temporal, piriform, entorhinal cortices, hippocampus, and amygdala (Olney and Farber, 1995). Neurotoxicity in these chronic studies was defined as cytoplasmic vacuolization associated with necrosis (Olney et al., 1991). More recently, this laboratory has reported that the same PCP regimen as used in this study caused apoptosis in the olfactory tubercle and piriform cortex when measured 3 days after administration (Johnson et al., 1998) and in the anterior cingulate and dorsolateral prefrontal cortex when measured 24 h after administration of the chronic regimen (C. Wang and K. M. Johnson, unpublished observations). The behavioral effects of toxic regimens of PCP or other NMDA antagonists such as MK-801 have not been well studied. We postulated that we could gain insight into the mechanisms underlying the neurotoxicity and the subsequent behavioral changes, as well as the potential relationship between them, by comparing the pharmacological antagonist profile of these two variables.

Atropine, pentobarbital, clozapine, haloperidol, and DNQX have all been reported to prevent NMDA antagonist-induced vacuolization (Olney et al., 1991; Olney and Farber, 1994; Sharp et al., 1994a; Sharp and Petersen, 1995). Based on these and other experiments, including site-specific injections of antagonists into the posterior cingulate/retrosplenial cortex, Olney proposed a model in which excitatory glutamatergic and cholinergic input onto vulnerable cells was regulated by NMDA receptive GABAergic interneurons, which were driven by glutamatergic neurons. In this way PCP blockade of NMDA receptors on GABAergic interneurons would activate the excitatory glutamatergic and cholinergic input onto the vulnerable neurons. Thus, either facilitation of GABAergic transmission or inhibition of cholinergic or non-NMDA glutamatergic transmission could prevent the neurotoxicity caused by PCP-like drugs (Olney et al., 1991). This model was expanded to include an inhibitory α2-adrenergic modulation of the cholinergic neuron as well as an excitatory interneuron that utilized neuropeptide Y or some other sigma receptor-activating substance (Olney and Farber, 1995). The latter modifications were made to accommodate the protective effects of an α2-antagonist and haloperidol, which was presumed to be acting as a sigma receptor antagonist. However, no role was proposed for dopaminergic trans-
mission, presumably because of the extreme sparsity of dopaminergic input into the posterior cingulate/retrosplenial cortex.

PCP and its shorter acting analog, ketamine, have been known for about four decades to reproduce many of the positive symptoms of schizophrenia (Luby et al., 1962). More recently, it has been demonstrated that repetitive ingestion of PCP also results in long-lasting negative symptoms of schizophrenia (Carlin et al., 1979; Lewis and Hordan, 1986; Cosgrove and Newell, 1991). PCP-induced neurotoxicity has been proposed as a model of the negative symptoms of schizophrenia because of the similarity in brain regions affected by PCP and those in which abnormalities were found in schizophrenic brains (Olney and Farber, 1995). This was of particular interest to us because of the superiority of clozapine in treating the negative symptoms of schizophrenia and its protective effect against both the necrotic and apoptotic effects of chronic PCP or MK-801 described above. In addition, clozapine has been reported to block hyperlocomotion induced by acute PCP administration (Maurel-Remy et al., 1995). Thus, we repeated our earlier experiment with clozapine and again observed that clozapine partially antagonized the effect of chronic PCP on the development of sensitization. Furthermore, when administered alone, it produced a partial sensitization to PCP challenge. Thus, the partial antagonism observed could have been blunted by its own sensitizing effect. As clozapine is known to interact with many neurotransmitter receptors, the mechanism underlying these effects is uncertain.

Among the receptors blocked by clozapine are D1-like, D2-like (with an especially high affinity for D2 receptors), 5-HT2A, and muscarinic (Lieberman, 1993). Haloperidol is a classic antipsychotic drug that is thought to act primarily through its blockade of D2-like receptors, although it also has a high affinity for sigma receptors and a more modest affinity for D1-like receptors. At the rather high dose used here to almost completely block the development of sensitization, it is possible that this effect could have been derived from antagonism of any of these, including some combination. It is also possible that this action could be partly dependent on the reported ability of haloperidol to antagonize increases in locomotor activity induced by acute PCP (Castellani and Adams, 1981; Kitaichi et al., 1994; Maurel-Remy, 1995; Tsutsumi et al., 1995). Another atypical antischizophrenic drug whose pharmacology partially overlaps both haloperidol and clozapine is risperidone. This drug is thought to act primarily by blocking 5-HT2A and D2 receptors. Risperidone (0.8–2.4 mg/kg p.o.) also has been demonstrated to inhibit acute hyperlocomotion elicited by 5 mg/kg PCP (Kitaichi et al., 1994; Maurel-Remy, 1995). It has been demonstrated that pretreatment with 2.4 mg/kg risperidone (p.o.) blocked the development of PCP-induced locomotor supersensitivity (Kitaichi et al., 1995). Our studies show that pretreatment with 1 mg/kg (i.p.) risperidone, a D2/5-HT2A receptor antagonist, attenuated the locomotor-sensitizing effects of PCP, but the effect was not robust. The difference between this experiment and that of Kitaichi’s group could be related to the fact that the locomotor sensitization paradigms used are slightly different. Although Kitaichi et al. used 10 mg/kg PCP for 10 days, we used 20 mg/kg for 5 days. Although the total dose was identical, Kitaichi et al. did not use a withdrawal period before PCP challenge. That is, Kitaichi’s group examined the daily increase in locomotor activity upon PCP injection without a withdrawal period. However, it seems unlikely that the sensitized response elicited following 3 days of withdrawal is of fundamentally different origin than that after only 24 h of withdrawal. Although we chose our 1 mg/kg (i.p.) dose in an attempt to parallel the oral dose of 2.4 mg/kg, it is possible that the bioavailability of risperidone following 2.4 mg/kg (p.o.) is greater than that following 1 mg/kg (i.p.). Although these doses would appear to be very similar, risperidone is notorious in the clinic for having a very narrow difference between its “atypical” effects and its “typical” effects. That is, it is sometimes referred to as a “quantitatively atypical” antipsychotic agent, because its extrapyramidal side effects are limited in most patients only when the dose is below 6 mg, although it is commonly used at 8 and sometimes 16 mg/day (Baldessarini, 1996). This suggests the possibility that 5-HT2A receptors are preferentially blocked at lower doses (e.g., 1 mg/kg i.p.), but at higher doses (e.g., 2.4 mg/kg p.o.), D2 receptors are also blocked. If so, this would suggest that 5-HT2A receptors may not be important in the behavioral sensitization observed here, but the D2 blockade by haloperidol, clozapine, and high dose risperidone may be more relevant. This conclusion is supported by the lack of effect of ketanserin, a selective 5-HT2A receptor antagonist (Fig. 6B).

The role of D2-like and D1-like dopamine receptors was further addressed by comparing the effects of selective antagonists of these receptors on the development of sensitization to PCP administration at both 5 and 20 mg/kg. The relative effectiveness of sulpiride and SCH23390 in both paradigms suggests that D2-like receptors play a prominent role in the development of sensitization, but that D1-like receptors do not. However, it is interesting to note that, like clozapine, SCH23390 treatment alone also produced an apparent sensitized response to PCP challenge. An explanation is suggested by the observation that chronic clozapine administration increases dopamine D1 receptor density in various basal ganglia and mesolimbic areas, including the striatum and nucleus accumbens (Coward, 1992). PCP administration has been reported to cause an increase in dopamine release from the nucleus accumbens as well as an increase in firing of dopamine neurons in the substantia nigra and ventral tegmental area (Freeman and Bunney, 1984; French and Ceci, 1990). Thus, assuming that chronic SCH23390 also up-regulates D1 receptors, PCP challenge would be expected to cause an increase in behaviors mediated by D1-like receptors in the striatum and accumbens. The foregoing suggests that D1 receptors are important in mediating the acute locomotor effects of PCP. This is completely consistent with our observations on the effects of sulpiride and SCH23390 on the increase in locomotor activity following acute administration of 5 mg/kg PCP. That is, 0.1 mg/kg SCH23390 almost completely attenuated the acute effect of PCP, whereas 50 mg/kg sulpiride, which effectively antagonized sensitization, was inactive against the acute effect of PCP and 100 mg/kg sulpiride attenuated the behavior by only about 35%. This is not to say that D2 receptors have no role in mediating the acute effects of PCP on motor activity. There is ample literature suggesting the importance of D2 receptors in stereotypic behaviors (Schlemmer et al., 1978; Murray and Horita, 1979), but their role in PCP-induced locomotor activity is less clear (Balster, 1987). One reason is that most of these studies relied on relatively high doses of nonselective antagonists...
such as haloperidol (e.g., Castellani and Adams, 1981). The antagonistic effect of SCH23390 and the incomplete blockade of acute PCP-induced locomotor activity by a high dose of a selective D₂ antagonist (100 mg/kg sulpiride) generally support the concept that D₁-like receptors are more important than D₂-like receptors in mediating the locomotor activating effects of acute PCP. It is interesting to note that the roles of D₁ and D₂ receptors in sensitization to PCP are quite different from those described in sensitization to cocaine (Mattingly et al., 1994; Steketee, 1998; White et al., 1998), amphetamine, and morphine (Vezina and Stewart, 1989). That is, D₁, but not D₂, antagonists prevent the development of locomotor sensitization to amphetamine and morphine, whereas neither class affects the development of sensitization to cocaine. These data suggest that locomotor sensitization to psychomotor stimulants can be mediated by several fundamentally distinct mechanisms.

Another question addressed by these data concerns the role of acute behavioral activation in the development of behavioral sensitization. Since SCH23390 dramatically blunts the acute effect of PCP but has a modest effect on sensitization development, one might conclude that acute activation is not critical to the ultimate development of sensitization. This in turn implies that the mechanisms underlying acute behavioral activation and the development of sensitization are substantially different from each other. This hypothesis is supported by two additional experiments. First, although the effect of risperidone on acute PCP-induced locomotor activation was not measured, we observed that it almost completely inhibited the acute daily effects of PCP administration during the chronic paradigm. However, risperidone pretreatment resulted in minimal inhibition of the locomotor sensitization revealed on the day of challenge. Similarly, pentobarbital markedly reduced the acute locomotor-activating effects of PCP, but it also had no effect on the locomotor sensitization produced by PCP. Together these data strongly argue that acute behavioral activation is not a strict requirement for the ultimate development of sensitization.

This being said, the interpretation of the experiment with barbital sodium is somewhat difficult in this framework. That is, this experiment was originally conducted because we observed that pentobarbital did not completely inhibit the acute effects of 20 mg/kg PCP. This was most evident during the last 1 to 2 h of the 4- to 5-h period of PCP effect. Although the level of activity during this time frame was diminished relative to saline pretreatment, it was not completely attenuated. In contrast, we observed that barbital sodium completely prevented the acute effects of PCP at this dose. That is, barbital sodium pretreated rats showed very little to no motor activity during the 5 h after acute PCP administration. It also attenuated the development of sensitization by more than half, but it is important to note that it did not completely inhibit this process. Although this question may require additional experiments to definitively answer, it is reasonable to suggest that these data in sum demonstrate that acute locomotor activation is not a requirement for the development of sensitization. Furthermore, the barbital sodium data suggest that blockade of NMDA receptors on GABAergic neurons that activate GABA receptors are an important part of the pathway underlying the development of sensitization to chronic PCP. Additionally, since Olney et al. (1991) showed that pentobarbital prevented MK-801-induced vacuolization with an ED₅₀ of 13 mg/kg, the lack of effect of 25 mg/kg pentobarbital in these experiments suggests that the mechanism(s) underlying vacuolization and the development of behavioral sensitization to NMDA antagonists are probably not identical. Similar to the pentobarbital data discussed above, DNQX (Olney and Farber, 1995; Sharp and Petersen, 1995) has been reported to inhibit NMDA antagonist-induced neurotoxicity, but they showed no ability in the experiments reported here to prevent the development of sensitization. This dissociation seems to support the idea that neurotoxicity characterized by vacuolization and behavioral sensitization has different underlying mechanisms.

However, this dissociation of mechanisms is not complete. In addition to the similar effects of haloperidol and clozapine on neurotoxicity and sensitization discussed above, atropine has been reported to prevent neuronal vacuolization (Olney et al., 1991) and we demonstrate here that it attenuates sensitization. The dose of atropine used here was based on the report that atropine had an ED₅₀ value of 2.7 mg/kg in preventing MK-801 vacuolization. Thus we used about 7.5 times the ED₅₀ in an attempt to estimate an ED₈₀ value. This antagonistic effect of atropine on PCP-induced sensitization may also be relevant to the antagonistic properties of clozapine, as it is well known to possess antimuscarinic activity. Thus, these data support the role of cholinergic neurons acting on muscarinic receptors in both sensitization and neuronal vacuolization following chronic PCP administration.

In summary, this study has found both dissociation and overlap in the mechanisms that underlie behavioral sensitization and neuronal vacuolization following chronic high dose administration of PCP (or MK-801). It seems that activation of D₂ dopamine receptors, GABA receptors, and muscarinic acetylcholine receptors probably play a significant role in mediating the events downstream of PCP blockade of NMDA receptors that lead to sensitization and neurotoxicity. On the other hand, these data do not support a role for D₁, 5-HT₂, or non-NMDA glutamate receptors in behavioral sensitization following chronic PCP. Additionally, these data suggest that the development of sensitization is not critically dependent on the acute behavioral activation process that accompanies PCP administration during the chronic dosing regimen. This implies that the mechanisms underlying sensitization are distinct from those underlying acute behavioral activation. This distinction was also evident in a study of sensitization following a modest dose of PCP in which D₁ receptors were preferentially involved in the acute locomotor effects of PCP, whereas D₂ receptors were preferentially involved in the mechanisms mediating behavior sensitization to chronic PCP.

References


