Effects of 5-Hydroxytryptamine$_2$ Receptor Antagonism on the Behavioral Activation and Immediate Early Gene Expression Induced by Dizocilpine

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ABSTRACT

The noncompetitive N-methyl-D-aspartate (NMDA) antagonists dizocilpine and phencyclidine cause behavioral changes in animals that can be blocked by antipsychotic agents, implicating NMDA receptors in the expression of schizophrenic symptoms. In the present study, we examined the effects of dizocilpine (0.1–3.0 mg/kg s.c.) on locomotor activity and on the expression of c-fos and hsp-70 immediate-early genes (IEGs) in mice. Results indicate that dizocilpine increases locomotor activity and selectively increases the expression of c-fos and hsp-70 in the posterior cingulate cortex. Haloperidol (0.01–0.1 mg/kg) and clozapine (0.6–1.25 mg/kg) block both the locomotor response and the increased IEG immunoreactivity induced by dizocilpine (0.6 mg/kg). The 5-HT$_2$ antagonists ritanserin (0.06–0.25 mg/kg), ketanserin (0.03–0.12 mg/kg) and amisergide (0.3–1.25 mg/kg) also significantly attenuated the locomotor response to dizocilpine. Haloperidol and clozapine suppressed the head weaving induced by dizocilpine, but ritanserin, as previously reported did not. Although some attenuation of the c-fos and hsp-70 immunoreactivity was seen with the 5-HT$_2$ antagonists it was less pronounced than that induced by haloperidol or clozapine. In conclusion, 5-HT$_2$ antagonists as well as antipsychotic compounds attenuate the locomotor response to dizocilpine in mice. Haloperidol and clozapine appear to be more effective, however, in attenuating the expression of c-fos and hsp-70 in the posterior cingulate gyrus than 5-HT$_2$ antagonists ritanserin, ketanserin or amisergide. We thus have seen a dissociation in the capacity of compounds to alter the effects on behavior and IEG expression after dizocilpine administration.

Dizocilpine (MK-801), ketamine and PCP are noncompetitive antagonists which act by blocking the channel mediating ion flux in the NMDA receptor complex (Lodge and Johnson, 1990). The compounds differ in that dizocilpine has higher affinity at the NMDA ion channel site than PCP or ketamine (Clineschmidt et al., 1982; Wong et al., 1986). Dizocilpine and PCP induce a very similar behavioral syndrome characterised by hyperactivity at lower doses and by ataxia, head weaving and body rolling at higher doses (Hiramatsu et al., 1989; Liljequist et al., 1991). The similarities in their behavioral syndromes suggest that they are mediated largely by a common mechanism, namely their noncompetitive antagonism of the NMDA receptor.

This behavioral syndrome in animals is of interest because abuse of PCP produces a syndrome in humans that resembles some of the symptoms of schizophrenia (see Javitt and Zukin, 1991, for review). Other stimulants, such as amphetamine, also induce syndromes resembling psychoses, but those induced by PCP are reported to mimic more completely the clinical features of schizophrenia. PCP induces both the positive (delusional/hallucinatory) symptoms as well as the negative (e.g., social withdrawal) symptoms, whereas these appear to be less commonly associated with abuse of other stimulants (Javitt and Zukin, 1991). This suggests that PCP-induced psychosis may more closely reflect the neuronal dysfunction underlying schizophrenic symptomatology. This is part of the basis of the hypoglutamatergic theory of schizophrenia, which, put simply, proposes that schizophrenic symptoms are related to reduced functioning of glutamate acting at NMDA receptors (e.g., Carlsson, 1995). A corollary of this theory is that the behavioral syndrome induced by noncompetitive NMDA antagonism in animals may model neurochemical abnormalities found in schizophrenia.

The neuronal mechanism by which noncompetitive NMDA antagonists induce hyperactivity is not clear. Selective blockade of either D$_1$ or D$_2$ dopamine receptors can attenuate the behavioral effects of dizocilpine in rats (Ouagazzal et al., 1993), suggesting that dopaminergic mechanisms underlie at least some of the behavioral effects of these noncompetitive NMDA antagonists. It has been observed, however, that the

ABBREVIATIONS: PCP, phencyclidine; NMDA, N-methyl-D-aspartate; 5-HT, 5-hydroxytryptamine; IEG, immediate early genes; Hsp, heat shock pattern; PBS, phosphate buffered saline; ANOVA, analysis of variance.
behavioral syndrome induced by dizocilpine does not show some features characteristic of stimulants acting via dopaminergic mechanisms. Hitri et al. (1993) have shown that while apomorphine induces intense gnawing and sniffing, dizocilpine does not. Furthermore, the head weaving and body rolling seen with NMDA antagonists have not been reported after treatment with dopaminergic stimulants such as apomorphine, amphetamine or cocaine. This suggests that mechanisms other than the dopaminergic system may be engaged in the mediation of the behavioral effects of noncompetitive NMDA antagonists.

A number of studies have reported that antipsychotic compounds reduce the locomotor response to noncompetitive NMDA antagonists. For example, Freed et al. (1980, 1984) showed that both haloperidol and clozapine reduced the locomotor response to PCP in mice and that both did so at doses in excess of those that inhibited spontaneous motor activity. Corbett et al. (1995) likewise found that haloperidol and clozapine reduced the locomotion and falling syndrome induced by dizocilpine in mice. These authors reported that the dose of haloperidol required to attenuate the locomotor effect of dizocilpine (0.5 mg/kg) was cataleptogenic, whereas clozapine did not induce catalepsy even at the highest dose tested (80 mg/kg). However, it has also been shown that dizocilpine can induce locomotor activity in animals that have been depleted of monoamines by pretreatment with reserpine and α-methyl-para-tyrosine (Carlsson and Carlsson, 1989), suggesting that dizocilpine’s behavioral effects are not mediated solely via monoaminergic mechanisms.

Different aspects of the behavioral effects of noncompetitive NMDA antagonists may also show a differential response to different treatments. Hoffman (1992) found that 1 mg/kg clozapine completely abolished dizocilpine-induced locomotion, whereas 10 mg/kg of clozapine was required to block the stereotyped sniffing induced by dizocilpine. The same doses of haloperidol that blocked stereotypy also blocked the locomotor response to dizocilpine. These findings suggest that the locomotor response to dizocilpine is more sensitive to atypical antipsychotics than are stereotyped responses. This is of particular interest in that the locomotor responses to stimulants are considered to be mediated by the A10 (ventral tegmental-nucleus accumbens) pathway, whereas stereotyped responding is more indicative of A9 (nigrostriatal) pathway activation. The lack of effect of clozapine on A9 nigrostriatal-mediated behavior is believed to reflect its relative lack of extrapyramidal side effects in schizophrenic patients (Kinon and Lieberman, 1996). Selective blockade of locomotor activity induced by dizocilpine without an alteration in other parameters may thus be indicative of an atypical antipsychotic profile. The question remains as to what aspect of clozapine’s pharmacology is responsible for inducing this atypical profile.

Clozapine has higher affinity for 5-HT2A receptors than it does for either D1 or D2 receptors, whereas haloperidol, which has high affinity for D1 and D2 receptors, has relatively weak activity at 5-HT2 receptors (Bymaster et al., 1996). Freed et al. (1984) found that methiothepin, which acts at a wide range of 5-HT receptors, potently blocked the locomotor effects of PCP in mice. Phencyclidine-induced hyperactivity has been shown to be blocked by a range of atypical neuroleptics (Maurel-Remy et al., 1995). In the latter studies, the rank order of potency in reducing the behavioral effects of phencyclidine correlated with the affinity of the compounds as 5-HT2 receptor antagonists. Furthermore, Gleason and Shannon (1997) tested a range of compounds with affinity for 5-HT2 receptors but without any activity at dopamine receptors. They showed that blockade of 5-HT2 receptors was sufficient to block the locomotor activation induced by PCP. The role of 5-HT2 receptors in the mediation of dizocilpine-induced hyperactivity has yet to be described. The present study set out to examine the effects of compounds that acted as antagonists at 5-HT2 receptors and to compare the effects of these compounds with the effects of the typical antipsychotic haloperidol and the atypical antipsychotic clozapine. Ritanserin, ketanserin and amesergide have all been shown to antagonize 5-HT2 receptors at low nanomolar concentrations (Hoyer, 1991; Cocco et al., 1996). Although PCP and dizocilpine appear to act via a common mechanism, there is evidence to suggest that their effects are not always mediated by the same neuronal substrates. Ogren and Goldstein (1994) showed that low doses of the D2/D3 agonist quinpirole reduced the locomotor response to dizocilpine but not to PCP, possibly due to differential presynaptic regulation by dopamine receptors. Hiramatsu et al. (1989) reported that PCP altered both dopamine and 5-HT turnover rates in the cortex and striatum, whereas dizocilpine altered dopamine levels only. Furthermore, the same author observed that PCP was >3 times more potent than dizocilpine in inhibiting [3H]5-HT uptake into synaptosomes, indicating that the compounds could differ substantially in their effects on serotonergic systems. Thus, although 5-HT2 receptors have been shown to mediate PCP locomotion, it was important to confirm that this was also the case for dizocilpine. For purposes of comparison, we additionally set out to evaluate the effects of the 5-HT2 antagonists and the antipsychotics haloperidol and clozapine under the same conditions.

In addition to the behavioral work, neurochemical studies with noncompetitive NMDA receptor antagonists also may help elucidate the neuronal substrate that may underlie schizophrenia. For example, studies by Olney and co-workers demonstrated that high doses of dizocilpine and PCP were neurotoxic in certain subpopulations of neurons in the CNS, in particular the neurons in the posterior cingulate gyrus and retrosplenial cortex (Olney et al., 1989, 1991). It has been suggested that overactivity of cingulate gyrus neurons may underlie the symptoms observed in schizophrenia and reflect the psychotomimetic effects observed with dizocilpine (Sharp et al., 1994).

The 2-deoxyglucose uptake method of Sokoloff et al. (1977) has been used to demonstrate the site of functional activity associated with the activation of neurotransmitter receptors. This method uses autoradiography to demonstrate the sites of uptake of radioactively labeled 2-deoxyglucose, which is thought to label metabolically active neurons. Using this technique, dizocilpine has been shown to cause a marked increase in glucose metabolism in the posterior cingulate and retrosplenial cortex of the rat brain (Kurumaji and McCulloch, 1989; Kurumaji et al., 1989).

More recently, the induction of immediate early genes has been proposed as an alternative means of mapping populations of activated neurons (Sagar et al., 1988; Sharp et al., 1994; Gass et al., 1993). The immunohistochemical or in situ hybridisation localisation of the expression of the immediate early gene c-fos has been extensively utilised to study neu-
It has been shown that the dizocilpine-induced increase in glucose metabolism in the posterior cingulate and retrosplenial cortex (Kurumaji and McCulloch, 1989; Kurumaji et al., 1989) coincides with the region where c-fos is induced in the rat brain (Gass et al., 1993). Heat shock proteins such as hsp-70 are induced after injury to neurons by cerebral ischaemia, trauma or seizures (Vass et al., 1988, 1989). Studies by Sharp and co-workers have shown that dizocilpine in doses of 0.2 to 5 mg/kg induced hsp-70 in the cingulate cortex of the rat brain (Sharp et al., 1991). Induction of hsp-70 by PCP was blocked by a range of compounds including haloperidol and clozapine (Sharp et al., 1994) and also compounds that are not antipsychotics such as diazepam and muscimol. In addition to behavioral studies, we set out to determine if any blockade of the locomotor response to dizocilpine was paralleled by a reversal of the immediate-early gene expression induced by dizocilpine. We examined the effects of 5-HT2 antagonists on both locomotor activity and immediate-early gene expression in the cingulate cortex after dizocilpine administration in mice.

Methods

Subjects

Female BKTO mice (25–30 g; Bantin and Kingman, Hull, UK) were housed in groups of 15 under standard conditions with a normal light cycle (lights, 7:00 a.m. to 7:00 p.m.). Animals were allowed food and water ad libitum. Experiments were performed between 10:00 a.m. and 5:00 p.m. Each mouse was used only once.

Drugs

Dizocilpine (RB1) was dissolved in distilled water. Amesergide, clozapine (both synthesized at Lilly Research Centre), ritanserin, haloperidol and ketanserin (Janssen) were dissolved or suspended in 25% β-cyclodextrin in distilled water. All drugs were administered subcutaneously (s.c.) in the scruff of the neck in a volume of 10 μl/kg.

Locomotor Activity

Apparatus. The locomotor activity was measured in clear Perspex boxes (30 × 30 × 30 cm) with a metal base with a 2 cm covering of fine sawdust. Each of the 15 cages had five, equally spaced horizontal photocell beams 5 cm above the cage floor. Each beam break was recorded as a photocell count. All the boxes were individually connected to a Compaq PC, and the photocell interruptions were recorded as number of counts using software provided by Misac Instruments Ltd. (UK).

Procedure. For the dose-response study for dizocilpine, the mice were habituated in pairs to the test boxes for 30 min before administration with either a dose of dizocilpine (0.1–3.0 mg/kg) or distilled water vehicle. Activity of each pair was then measured for 90 min.

In the antagonism studies, mice were injected with the appropriate dose of pretreatment (haloperidol, clozapine, ritanserin, ketanserin, amesergide) or vehicle and immediately placed in the locomotor activity boxes. Thirty minutes later, the mice were removed and then received either a dose of dizocilpine or distilled water vehicle as appropriate. Recording of activity was begun immediately and continued for 90 min. Again, two mice were tested per cage; a minimum of six pairs was tested per treatment group.

Head Weaving

Mice were habituated in stainless steel observation chambers (10 ×15 × 13 cm) with a clear Perspex top under normal lighting conditions. After 60 min of habituation, the mice were injected with dizocilpine and returned to the observation chambers for a further 60 min. In the antagonism studies, the animals were injected with the test compound, returned to the test chamber for 30 min and then injected with dizocilpine. Sixty minutes after the dizocilpine injection, the animals were observed for 3 min, and the number of head weaves (full side-to-side movements of the head) was counted.

Immunohistochemistry. At 4 and 24 hr after behavioral testing, the animals were administered a fatal dose of anesthetic and then were transecrally perfused with 0.9% saline followed by 10% buffered formalin. The brains were removed, processed and embedded in paraffin wax. Six-micron sections were cut using a sledge microtome (Leitz, 1400)

A monoclonal hsp-70 antibody (Biomen Diagnostics, Berkshire, UK) designed for specific localization of hsp-70 in formalin-fixed, paraffin-embedded tissue and a rabbit polyclonal serum to c-fos (Calbiochem-Novabiochem, Nottingham, UK) that detects c-fos (1: 200) in paraffin-embedded tissue were used in these studies.

After removing the paraffin with xylene, the sections were rehydrated and endogenous peroxide was quenched by incubating the sections in 0.3% H2O2 for 30 min. After three washes in 0.01 M PBS, the sections were incubated with pepsin (Sigma, UK) and then washed a further three times. Adjacent sections were then incubated with hsp-70 or c-fos antisera for 24 hr at 4°C.

After a further wash in PBS, the sections were incubated in biotinylated goat anti-rabbit or horse anti-mouse antiseraum (1:200; Vector Laboratories, UK). Immunoreactivity was then visualized by the avidin-biotin complex method as described previously (Gass et al. 1993). Sections were developed in 0.02% diaminobenzide with 0.02% hydrogen peroxide, dehydrated and overslipped.

The number of immunoreactive cells in an (0.08 mm × 0.08 mm) area was counted in posterior cingulate cortex, anterior cingulate cortex, arcuate medial hypothalamic nucleus, piriform cortex, nucleus accumbens core and caudate putamen. After the initial dose response and time course studies, only cells in the posterior cingulate, where the increase in immediate-early gene was most evident, were counted in both left and right hemispheres. The immediate-early gene cell count was averaged and expressed as cell counts per hemisphere. Data are expressed as means based on n = three or four brains per treatment group.

Data Analysis

Data were analyzed by PROC GLM ANOVA using SAS software, and differences determined by post hoc (least square means) analyses.

Results

Dizocilpine dose-dependently increased locomotor activity in mice [F(4,25) = 3.79, P < .05]. The minimum effective dose (0.3 mg/kg) produced an increase from ~30 min postinjection, which lasted for a further 50 min (fig. 1). Higher doses (1.0 and 3.0 mg/kg) caused ataxia, and hyperlocomotion became significant only at 60 min postinjection as the ataxia became less evident. The effects of dizocilpine on locomotor activity are most evident when the activity counts are totaled for the 90-min test period (table 1). Doses of 0.3 and 1.0 mg/kg both induced significantly elevated levels of activity. The highest dose (3.0 mg/kg), on the other hand, induced a lower level of locomotor activity, but instead induced pronounced body rolling and head weaving.

Groups of mice were killed at 4 and 24 hr postinjection. The hsp-70 immunostaining was more consistently evident at 24 hr than at 4 hr after drug administration, and this time point was used in all subsequent experiments. Dizocilpine also produced a dose-dependent increase in the number of c-fos immunoreactive cells in the posterior and anterior cingulate cortex and retrosplenial cortex 24 hr after drug administration.
induced expression of both c-fos and hsp-70 immunoreactive neurons in the cingulate gyri, which was dose-dependently attenuated by haloperidol (table 1). Although haloperidol did not significantly suppress locomotor activity for the entire habituation period, it did suppress spontaneous activity as measured in the 20- to 30-min time period \( F(4,29) = 3.45, P < .05 \). Post hoc tests for this time bin showed that 0.1 mg/kg significantly reduced spontaneous activity. Thus, the dose of haloperidol (0.1 mg/kg) that was most effective is suppressing the behavioral activation induced by dizocilpine also significantly reduced spontaneous locomotor activity in the habituation period (fig. 2a). Lower doses (0.01 and 0.03 mg/kg) attenuated but did not significantly reverse dizocilpine-induced behavioral activation.

Dizocilpine (0.6 mg/kg) produced an increase in the number of c-fos and hsp-70 immunoreactive neurons in the posterior cingulate gyrus, which was dose-dependently attenuated by haloperidol (table 1). In parallel with the behavioral data, the highest dose of haloperidol (0.1 mg/kg) completely blocked the effect of dizocilpine, but the lower dose of 0.03 mg/kg, which did not significantly reverse the locomotor effects of dizocilpine, did significantly attenuate dizocilpine-induced expression of both c-fos and hsp-70 in the cingulate cortex.

Clozapine also significantly and dose-dependently reversed the activity-enhancing effects of dizocilpine (table 1, fig. 2b), and clozapine also significantly decreased spontaneous activity in the habituation period \( F(4,29) = 5.77, P < .005 \). Post hoc tests showed that the 1.25 mg/kg dose significantly lowered activity compared with vehicle controls (fig 2b), but lower doses, which did not effect spontaneous activity (0.62 mg/kg), also significantly attenuated the locomotor response to dizocilpine, suggesting the effect was not merely due to a nonspecific suppression of locomotor activity.

Ritanserin completely abolished the locomotor response to dizocilpine at all doses tested (0.06–0.25 mg/kg) (fig. 3a, table 1). These doses had no effect on spontaneous activity in the habituation period (fig. 3a); this suggests that the doses that were effective in reducing the activity induced by dizocilpine were not producing a nonsel ective sedative effect in the animals.

Ritanserin also attenuated the effect of dizocilpine on c-fos and hsp-70 in the posterior cingulate. Post hoc tests showed that the lower doses (0.06, 0.125 mg/kg) had no effect on immediate-early gene expression, whereas the highest dose (0.25 mg/kg) of ritanserin produced a significant attenuation of the dizocilpine induced c-fos and hsp-70 (table 1).

Amesergide (0.31–1.25 mg/kg) reduced the activity induced by dizocilpine at all doses tested (table 1, fig. 3b). These doses had no effect on spontaneous activity in the habituation period \( F(4,29) = 0.22 \) (fig. 3b). Amesergide produced some attenuation of the dizocilpine induced c-fos and hsp-70 at all doses administered. The attenuation was small, however, and none of the doses tested were significant compared with the vehicle/dizocilpine group (table 1).

Ketanserin also significantly reduced the activity induced by dizocilpine at all doses tested (table 1, fig. 3c). None of the doses of ketanserin tested had any effect on spontaneous locomotor activity in the habituation period \( F(4,29) = 0.92 \) (fig. 3c). Ketanserin produced a slight reduction in the dizocilpine-induced c-fos and hsp-70 but no dose significantly attenuated the response compared with vehicle/dizocilpine treated controls (table 1).

**Head weaving.** It was observed in the course of the locomotor experiments that other behavioral effects of dizocilpine, most notably head weaving, were still evident even when locomotor activity had been attenuated by pre-treatment with the 5-HT<sub>2</sub> antagonists. A separate series of experiments was conducted to examine the effects of the test compounds on the head weaving observed in the animals in the locomotor activity boxes. Dizocilpine-induced head weaving at 0.6 mg/kg the minimum dose required to significantly elevate immediate-early gene expression levels (table 1). This dose was chosen for subsequent antagonism studies. The head weaving induced by this dose was probably not optimal as there was considerable day-to-day variation in the base-line numbers of head weaves (table 1). However, to have used a different dose in this series of experiments would have compromised the comparison with results from the other experiments reported here. Both clozapine and haloperidol attenuated the head weaving induced by dizocilpine (0.6 mg/kg). The effect was more
TABLE 1
Effects of haloperidol, clozapine and 5HT2 antagonists on locomotor activity, head weaving and immediate early gene responses to dizocilpine in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Locomotor activity</th>
<th>Head weaves</th>
<th>c-fos</th>
<th>Hap-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dizocilpine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2278 ± 611</td>
<td>0 ± 0</td>
<td>59 ± 3</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>0.1</td>
<td>2240 ± 691</td>
<td>0 ± 0</td>
<td>103 ± 16</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>0.3</td>
<td>6696 ± 1710^a</td>
<td>0 ± 0</td>
<td>108 ± 18</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>13.7 ± 2.7^c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>7015 ± 1455^b</td>
<td></td>
<td>156 ± 13^b</td>
<td>112 ± 15^b</td>
</tr>
<tr>
<td>3.0</td>
<td>5236 ± 918^b</td>
<td></td>
<td>130 ± 20^b</td>
<td>96 ± 17^b</td>
</tr>
<tr>
<td>Haloperidol</td>
<td></td>
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</tr>
<tr>
<td>Veh/Veh</td>
<td>813 ± 115</td>
<td>0 ± 0</td>
<td>63 ± 135</td>
<td>2 ± 12</td>
</tr>
<tr>
<td>Veh/Diz</td>
<td>8240 ± 2026^c</td>
<td>13.9 ± 4.7^b</td>
<td>125 ± 12^a</td>
<td>112 ± 15^b</td>
</tr>
<tr>
<td>0.03/Diz</td>
<td>5489 ± 1485^b</td>
<td>17.4 ± 2.3^b</td>
<td>114 ± 9</td>
<td>102 ± 8</td>
</tr>
<tr>
<td>0.03/Diz</td>
<td>5135 ± 931^c</td>
<td>4.9 ± 1.5</td>
<td>65 ± 19^d</td>
<td>62 ± 10^d</td>
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<tr>
<td>0.1/Diz</td>
<td>717 ± 519^b</td>
<td>7.1 ± 1.8</td>
<td>55 ± 18^d</td>
<td>43 ± 6^d</td>
</tr>
<tr>
<td>Clozapine</td>
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</tr>
<tr>
<td>Veh/Veh</td>
<td>766 ± 211</td>
<td>0 ± 0</td>
<td>32 ± 8</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>Veh/Diz</td>
<td>6024 ± 1872^c</td>
<td>5.8 ± 2.2^a</td>
<td>11 ± 5^a</td>
<td>78 ± 10^b</td>
</tr>
<tr>
<td>0.31/Diz</td>
<td>5256 ± 1283^c</td>
<td>7.6 ± 2.1^a</td>
<td>78 ± 18</td>
<td>61 ± 12</td>
</tr>
<tr>
<td>0.62/Diz</td>
<td>2284 ± 691^d</td>
<td>6.5 ± 2.7^a</td>
<td>68 ± 16</td>
<td>38 ± 3^d</td>
</tr>
<tr>
<td>1.25/Diz</td>
<td>306 ± 158^c</td>
<td>0.6 ± 0.3</td>
<td>58 ± 10^d</td>
<td>32 ± 7^d</td>
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<tr>
<td>Ritanserin</td>
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<tr>
<td>Veh/Veh</td>
<td>1967 ± 620</td>
<td>0 ± 0</td>
<td>46 ± 12</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Veh/Diz</td>
<td>9462 ± 2610^f</td>
<td>6.3 ± 1.7^a</td>
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<td>93 ± 7^a</td>
</tr>
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<td>0.06/Diz</td>
<td>4479 ± 1854^e</td>
<td>8.9 ± 2.1^a</td>
<td>85 ± 28</td>
<td>111 ± 21</td>
</tr>
<tr>
<td>0.12/Diz</td>
<td>4712 ± 873^g</td>
<td>6.1 ± 2.2^a</td>
<td>105 ± 30</td>
<td>71 ± 10</td>
</tr>
<tr>
<td>0.25/Diz</td>
<td>3523 ± 1705^g</td>
<td>7.9 ± 1.6^a</td>
<td>62 ± 14^d</td>
<td>66 ± 19</td>
</tr>
<tr>
<td>Amisulpride</td>
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</tr>
<tr>
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<td>1530 ± 393</td>
<td></td>
<td>52 ± 7</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>Veh/Diz</td>
<td>6640 ± 765^d</td>
<td></td>
<td>107 ± 4^a</td>
<td>89 ± 3^a</td>
</tr>
<tr>
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<td>3776 ± 742^d</td>
<td></td>
<td>97 ± 10</td>
<td>90 ± 6</td>
</tr>
<tr>
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<td>87 ± 10</td>
<td>72 ± 9</td>
</tr>
<tr>
<td>1.25/Diz</td>
<td>3003 ± 713^d</td>
<td></td>
<td>85 ± 9</td>
<td>74 ± 11</td>
</tr>
<tr>
<td>Ketanserin</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Veh/Veh</td>
<td>1429 ± 131</td>
<td></td>
<td>53 ± 7</td>
<td>47 ± 13</td>
</tr>
<tr>
<td>Veh/Diz</td>
<td>6259 ± 1496^c</td>
<td></td>
<td>109 ± 20^c</td>
<td>87 ± 12^c</td>
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<tr>
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<td>93 ± 34</td>
<td>86 ± 17</td>
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<tr>
<td>0.12/Diz</td>
<td>3867 ± 1203^d</td>
<td></td>
<td>92 ± 14</td>
<td>55 ± 12</td>
</tr>
<tr>
<td>0.25/Diz</td>
<td>1229 ± 270^d</td>
<td></td>
<td>78 ± 11</td>
<td>56 ± 15</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. Significant differences were determined by LSD test after a significant ANOVA.

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clearly dose related in the case of clozapine than with haloperidol. Ritanserin, in contrast, had no effect on dizocilpine-induced head weaving at any dose tested, although these same doses significantly reduced the locomotor activity induced by dizocilpine (table 1).

**Discussion**

Dizocilpine induced an increase in locomotor activation as previously reported (Clinesschmidt et al., 1982). This increase in activity was attenuated by haloperidol. The doses of haloperidol that decreased dizocilpine-induced hyperactivity also significantly suppressed spontaneous activity in the habituation period. Freed et al. (1984) found that haloperidol (0.5–2.0 mg/kg) reduced the stimulation induced by PCP in mice, but these doses also suppressed spontaneous activity. Verma and Kulkarni (1992) reversed the effects of MK-801 on locomotor activity in mice with a larger dose of haloperidol (0.5 mg/kg) than used in either the current study or by Freed et al. (1984). Corbett et al. (1995) showed that the ED<sub>50</sub>'s for haloperidol induction of catalepsy in rats and for the blockade of dizocilpine-induced locomotion and falling in mice were both 0.5 mg/kg.

Clozapine dose-dependently reversed the effect of dizocilpine on locomotor activity. The minimum effective dose of clozapine that was effective against dizocilpine was slightly less than the minimum effective dose for reduction of spontaneous activity in the habituation period (0.62 vs. 1.25 mg/kg). Corbett et al. (1995) found that clozapine reduced dizocilpine-induced locomotion and falling over an equivalent dose range to those tested in the current study (1.1 mg/kg ED<sub>50</sub>) but found that it did not induce catalepsy even at 80 mg/kg. Freed et al. (1984) found that clozapine (2.5–10 mg/kg) reduced the locomotor response to PCP in mice. These authors also reported that these doses significantly suppressed spontaneous locomotion in the habituation period as demonstrated in the present studies. Thus, it is difficult to dissociate the effects of the antipsychotic compounds on spontaneous locomotion from their effects on dizocilpine-induced hyperactivity as there was little separation between the doses active against both forms of activity.

It is clear, therefore, that both the typical antipsychotic haloperidol and the atypical antipsychotic clozapine reduce the locomotor response to dizocilpine. This finding is difficult to reconcile with claims that the syndrome induced in animals models by dizocilpine specifically models certain aspects of schizophrenic symptomatology such as negative symptoms that are resistant to typical neuroleptic treatment. The only difference observed between the two neuroleptic agents was that clozapine suppressed dizocilpine-induced locomotor activity at a slightly lower dose than was required to suppress spontaneous activity, whereas the converse was true for haloperidol. It is not yet known what aspect of
Clozapine’s pharmacology is responsible for its atypical profile. Clozapine has greater affinity for 5-HT$_2$ (K$_i$ = 4 nM for 5-HT$_{2A}$) than it does for either D$_1$ or D$_2$ receptors (K$_i$ = 85 and 125 nM, respectively). Haloperidol on the other hand has greater affinity for D$_2$ (Ki = 1 nM) and D$_1$ (K$_i$ = 25 nM) than for 5-HT$_2$ receptors (K$_i$ = 78 for 5-HT$_{2A}$) (Bymaster et al., 1996). The aim of the present was to examine the possible contribution of this greater affinity for 5-HT$_2$ receptors to the behavioral profile of clozapine. The locomotor activation induced by dizocilpine was also significantly attenuated by doses of the selective 5-HT$_2$ receptor antagonists amesergide, ketanserin and ritanserin at doses that had no effect on spontaneous locomotor activity. In previous studies, Maurel-Remy et al. (1995) showed that atypical antipsychotics clozapine, risperidone and the putative antipsychotic MDL100,907 blocked PCP-induced hyperlocomotion in mice, at doses lower than those necessary to block amphetamine-induced hyperactivity, whereas dopaminergic antagonists haloperidol and raclopride showed the reverse selectivity of effect. Moreover, these authors showed that the efficacy for blocking PCP-induced hyperactivity correlated significantly (r = 0.97) with the affinity for the 5-HT$_{2A}$ receptor. Similarly, Gleason and Shannon (1997) have shown that the novel atypical antipsychotic olanzapine as well as the selective 5-HT$_2$ antagonists LY53857, ketanserin and ritanserin all selectively reduced the hy-
peractivity induced by PCP in mice. These results, in combination with the findings of the current study, showing that the behavioral effects of a high dose of dizocilpine were also reversed by 5-HT<sub>2</sub>A antagonists strongly implicate 5-HT<sub>2</sub>A mechanisms in the mediation of the locomotor effects of NMDA antagonists.

In the present study, we noted that although the locomotor response was blocked by the 5-HT<sub>2</sub>A antagonists, body rolling and head weaving were still evident as the dizocilpine-treated animals were removed from the locomotor activity apparatus. Further studies were undertaken to characterize this effect of dizocilpine. The minimum effective dose for the induction of head weaving was 0.6 mg/kg. No head twitches were observed at the doses tested. The head-weaving induced by dizocilpine was attenuated by haloperidol. This is unlikely to be due to any direct interaction with 5-HT receptors as haloperidol does not interact with 5-HT receptors over the dose range tested (0.05–0.1 mg/kg). Clozapine (0.625–1.25 mg/kg) also reduced head weaving, whereas ritanserin (0.06–0.25 mg/kg) was without effect. Both haloperidol and clozapine reduced spontaneous activity over the effective dose range in this test. This may imply that a nonspecific sedative effect of the compounds may have contributed to the reduction in head weaving. Nabeshima et al. (1987) found that ritanserin reduced the head twitches induced by PCP but showed that pretreatment with ritanserin increased head weaving in PCP-treated mice. Hiramatsu et al. (1989) observed that ritanserin did not alter dizocilpine-induced stereotypy. These findings suggest that 5-HT<sub>2</sub>A receptor blockade selectively attenuates only part of the spectrum of behavioral consequences of noncompetitive NMDA receptor blockade, namely the locomotor activation, and that other aspects such as head weaving do not appear to involve 5-HT<sub>2</sub>A receptors.

In the present studies, dizocilpine caused a selective increase in the number of c-fos immunoreactive cells in the posterior cingulate cortex at doses higher than those required to induce significant increases in locomotor activity. Large increases in the number of hsp-70 immunoreactive cells in the posterior cingulate gyrus were also observed. This is in agreement with the work of Sharp and co-workers that has shown that dizocilpine in doses of 0.2 to 5 mg/kg induces hsp-70 in the cingulate cortex of the rat brain (Sharp et al., 1991). The present studies showed that the number of hsp-70 immunoreactive cells was larger in the 1.0 mg/kg than the 3.0 mg/kg-treated animals and that the expression levels of hsp-70 were higher at 24 than at 4 hr postdizocilpine treatment. This also concurs with the findings by Sharp et al. (1991), who observed that the hsp-70 induction was maximal at 1 to 3 days after dizocilpine and that the number of hsp-70 neurons induced with 5 mg/kg was less than that induced by 1 mg/kg. The authors suggest that this may be because cell death is reported with 5 mg/kg but not 1 mg/kg doses of dizocilpine (Fix et al., 1993).

The higher doses of haloperidol blocked the increases in the number of c-fos and hsp-70 immunoreactive cells in the cingulate cortex induced by dizocilpine. Haloperidol also prevented ketamine- and PCP-induced hsp-70 in the rat brain (Sharp et al., 1994; Nääkki et al., 1996). This suggests that haloperidol can reduce the induction of immediate-early gene expression induced by a range of noncompetitive NMDA antagonists and that, as with the suppression of dizocilpine-induced locomotor activity, the typical neuroleptic is also able to attenuate the neurochemical consequences of dizocilpine administration.

Clozapine also markedly attenuated the dizocilpine-induced c-fos and hsp-70 expression in the cingulate cortex. This concurs with the previous work of Sharp et al. (1994). In another recent study, Farber and co-workers demonstrated that clozapine, olanzapine and structurally related compounds such as fluperoxipine, loxapine and amoxapine prevent dizocilpine-induced neurotoxicity (Farber et al., 1996). Thus, it is evident that both typical and atypical neuroleptics can attenuate the expression of immediate-early genes induced by dizocilpine. It may not be possible, therefore, to differentiate between typical and atypical neuroleptics on the basis of their effect on immediate-early gene expression induced by dizocilpine.

In contrast to the antipsychotic compounds, the 5-HT<sub>2</sub>A receptor antagonists, ritanserin, amesergide and ketanserin provided only a modest attenuation of the c-fos and hsp-70 expression, whereas they almost completely blocked the increase in locomotor activity induced by dizocilpine. This lack of concordance between the effects on locomotor activity induced by dizocilpine and induction of immediate-early genes by 5-HT<sub>2</sub>A receptor blockade may suggest, as does the lack of correspondence between the locomotor response and the head weaving, that different components or markers for the neuronal response to dizocilpine may be mediated by different substrates. It is also possible that the head weaving may per se alter the expression of immediate-early genes in the cingulate. Likewise, it is noticeable that both haloperidol and clozapine suppressed spontaneous activity, whereas the 5-HT<sub>2</sub>A antagonists did not and that the neuroleptics also suppressed the expression of immediate-early genes while the 5-HT<sub>2</sub>A antagonists did not do so consistently. It remains to be determined if suppression of activity per se alters the expression of immediate-early genes. Compounds that do not act as antipsychotics can alter the neuronal response to noncompetitive NMDA antagonists. Diazepam and muscimol have been shown to reduce immediate-early gene expression induced by PCP (Sharp et al., 1994). Both diazepam and muscimol also suppress locomotor activity. Behrens and Gattaz (1992) showed that diazepam actually increased the stereotypies induced by dizocilpine. This observation further supports the possibility of differential regulation of the effects of dizocilpine on behavior and on gene expression.

The evidence presented here suggests that dizocilpine alters activity in a number of transmitter systems and that the consequences of this activation as measured by behavioral or neurochemical end points reflect the diversity and possibly the independence of these systems. Liljequist et al. (1991) studied the increase in locomotor activity induced by dizocilpine in relation to regional changes in neuronal function as measured by dopamine metabolism in mice. They found that the dose of dizocilpine that in their hands induced optimal levels of locomotion (0.2 mg/kg) did not significantly alter the rate of tyrosine hydroxylation in the limbic forebrain but did increase the concentration of DOPA in the striatum. It would appear that higher doses (0.5 mg/kg) were associated with changes in dopamine turnover in cortical areas and with ataxia and stereotypy (e.g., Hiramatsu et al., 1989). One must therefore not only consider the effects of dizocilpine on multiple neurotransmitters but also take into account differential effects on regional variation of neurotransmitter function within the same system. Dizocilpine preferentially elevates synaptic dopamine concentrations in the nucleus accumbens. This effect in turn is blocked by MDL 100,907, a selective 5-HT<sub>2A</sub> antagonist (Schmidt and Fayadel, 1996). MDL 100,907, a selective 5-HT<sub>2A</sub> antagonist (Schmidt and Fayadel, 1996).
100,907, however, did not alter dizocilpine-induced dopamine release in the medial prefrontal cortex. This suggests that the serotonergic modulation of the NMDA-glutamatergic interaction is highly regionally specific, which may also be reflected in differential behavioral outputs from the specific regions. Thus, while the neuronal substrates involved in the mediation of locomotion require the activation of 5-HT_{2A} receptors, those involved in head weaving or immediate-early gene expression do not. The different measures, locomotion, head weaving and immediate-early genes also showed different sensitivity to dizocilpine. Locomotion was significantly increased by a dose of 0.3 mg/kg, whereas 0.6 mg/kg was the minimum effective dose for the induction of both head weaving and immediate-early gene expression, suggesting that the later phenomena are triggered only when there is a more substantial blockade of NMDA receptors than that required to induce locomotor activity.

In conclusion, our findings show that blockade of 5-HT_{2} receptors is sufficient to attenuate the locomotor induced by the NMDA antagonist dizocilpine. The antipsychotics haloperidol and clozapine block the expression of c-fos and hsp-70 in the posterior cingulate gyrus, whereas selective 5-HT_{2A} antagonists produced a less marked attenuation of the dizocilpine effect. Our results suggest that the effects of dizocilpine on locomotor activity and immediate-early gene expression may rely on different neuronal substrates. The fact that ritanserin completely blocks dizocilpine-induced hyperactivity and yet showed only modest activity as an antipsychotic in clinical trials (Stefanski and Goldberg, 1997) suggests that this test may have limited predictive validity as an measure of antipsychotic potential for novel compounds. Likewise, although in our hands the antipsychotic compounds showed the best suppression of the induction of immediate-early genes by dizocilpine, previous work by Sharp et al. (1994) demonstrated that a range of compounds without antipsychotic activity, including SCH23390, diazepam and muscimol, reduced the induction of hsp70 by PCP. This suggests that this measure would have similar limitations as a predictor of antipsychotic activity.

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References


