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Antagonism of a PCP Drug Discrimination by Hallucinogens and Related Drugs

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Drugs such as PCP and MK-801 can cause psychotic reactions in humans by antagonizing NMDA receptors. This action is ultimately toxic to certain cortical neurons and may be one mechanism underlying neurodegenerative diseases, including schizophrenia. It has been reported that hallucinogens such as LSD, DOM, and DOI can block the neurotoxic effects of NMDA antagonists, possibly by activating inhibitory 5-HT2A receptors on GABAergic interneurons that normally inhibit glutamatergic projections to the retrosplenial and cingulate cortexes. The purpose of this experiment was to determine the extent to which similar drugs might also alter the behavioral effects of one NMDA antagonist, PCP. Rats were trained to discriminate this compound (2.5 mg/kg) from saline and were then given a series of antagonist tests. It was found that LSD (0.32 mg/kg) and DOM (4.0 mg/kg) blocked the PCP cue completely; DMT (8.0 mg/kg) and a structural congener of LSD, lisuride (LHM; 0.4 mg/kg), blocked the effects of PCP partially. The 5-HT/DA antagonists spiperone and ritanserin had no effect on the PCP cue. These data suggest that LSD, DOM, and, less effectively, DMT and LHM can block the behavioral as well as the neurotoxic effects of NMDA antagonists most likely through agonist actions at 5-HT2 receptors.
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Phencyclidine (PCP) was synthesized by Parke, Davis & Company in 1957 and used as an anesthetic under the name Sernyl until the drug was withdrawn in 1965. In normal humans, PCP appeared to induce schizophrenomimetic and other psychotic like effects that persisted long after recovery from anesthesia (Luby et al. 1959). In addition, patients reported changes in body perceptions and seemed to "dissociate" from their normal mental state while remaining conscious (Luby et al. 1959). In hospitalized schizophrenics, PCP reinstated acute, exacerbated psychotic symptoms (Luby et al. 1959, 1962).

Although PCP did not prove to be clinically useful, it generated a wealth of literature, particularly on the relationship between schizophrenia and glutamate. The drug is thought to be bind to a site on the NMDA receptor complex that inhibits excitatory glutamatergic transmission (Lodge and Anis 1982). Indeed, the correlation between reports of psychotic effects and affinity of PCP-like drugs for this site led to the hypothesis that antagonism of the NMDA receptor complex is psychotogenic, if not schizophrenogenic (Javitt and Zukin 1991).

Drug discrimination has been used to study the mechanism of action of PCP and arguably serves as an animal model of the subjective effects of this compound in humans (Koek 1999). Studies using this technique have indicated that there is a strong positive correlation between the potency of ligands that bind to the PCP-binding site on the NMDA receptor complex and ability to substitute for PCP (Koek and Woods 1988). However, the extent to which NMDA antagonists that do not bind to the PCP site may also have psychotomimetic effects is not clear.

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While animal studies have shown that drugs such as CPP (3-[(+/−)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid) and CGS 19755 (cis-4-phosphonomethyl-2-piperidine-carboxylic acid) have PCP-like biochemical and discriminative stimulus effects at high doses (Balster 1991; Koek et al. 1990), clinical trials do not suggest that either of these compounds induce psychotic-like effects (Grotta et al. 1995; Kristensen et al. 1992).

NMDA receptor hypofunction is one mechanism that has been proposed to mediate both the neurotoxic and psychotogenic effects of NMDA antagonists. PCP, MK-801 (dizocilpine), and ketamine have been shown to cause vacuoles in tissue comprised primarily of glutamatergic pyramidal neurons located in the posterior cingulate and retrosplenial cortexes (Olney et al. 1989, 1991). Of particular relevance to the present research is a report that 5-HT2 agonists such as LSD, DOM, DOI, and DOB (Appel et al. 1982; Branchek et al. 1990; Fiorella et al. 1995a,b; Glennon et al. 1983; Krebs-Thomson et al. 1998) inhibit the neurotoxic effects of MK-801 (Farber et al. 1998). In addition, ritanserin (a relatively non-selective 5-HT2A/2C and DA antagonist) and SDZ SER-082 (a putatively more selective 5-HT2C antagonist) were given in combination with both DOI and MK-801; ritanserin, but not SDZ SER-082, blocked the neuroprotective effects of DOI. These findings suggest that agonistic action at 5-HT2A receptors might protect against neuronal damage caused by NMDA antagonists (Farber et al. 1998).

The present experiment was designed to examine the extent to which the discriminative stimulus properties of PCP can be antagonized by LSD, DOM, DMT, LHM, ritanserin, and spiperone.

MATERIALS AND METHODS

Subjects

Experimentally naïve 90-day old male Sprague-Dawley rats (n = 16) weighing approximately 350 g were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were housed individually in a colony maintained on a 12 h light-dark schedule (lights on at 0700 h, off at 1900 h). Temperature and humidity were held constant at 20–22°C and 40–50%, respectively. Initially, animals had free access to both food and water. During this period they were handled at least once a day. Five days before training, water was restricted to one hour per day. Access to water was then restricted to training sessions, weekends (Friday evening to Sunday morning), and a 10-min period following test sessions.

Apparatus

Eight commercially available experimental chambers (MED Associates ENV 018) housed in light- and sound-attenuating shells (MED Associates ENV 008) were used. Each chamber contained two retractable levers and a dipper that was programmed to deliver 0.1 ml of water for 0.3 sec whenever a reinforcer was scheduled. IBM compatible computers using MED State software were used to control experimental chambers.

Training Procedure

Animals were trained to discriminate phencyclidine HCl (2.5 mg/kg) from saline (0.9% NaCl) in a manner described by Cunningham and Appel (1987). Training injections were given intraperitoneally (i.p.), 15 min before daily (Monday to Friday) experimental sessions. During the training stage of an experiment, only the drug-appropriate lever was present (PCP or saline). Rats were randomly assigned to levers in order to control for lever bias. The order of stimulus (drug) presentation was also random with the restriction that neither drug was administered for more than three consecutive sessions. Lever pressing was initially maintained under a fixed-ratio (FR 1) schedule of reinforcement; as response rates stabilized, the ratio was raised gradually to FR 20.

Discrimination Training

After all rats were responding reliably under the FR 20 schedule, both levers were presented simultaneously. Responses on the incorrect lever were recorded but had no additional consequences. Training continued until an animal reached the criterion of at least 80% responding on the correct lever for seven consecutive sessions.

Testing

Rats were pretreated with either vehicle (control) or antagonist at either 60 or 90 min before behavioral testing; 45 or 75 min before PCP, as indicated in Table 1. Tests terminated as soon as 20 responses were completed on either lever, and were conducted under extinction conditions one to two times a week. Only rats maintaining accurate discrimination during intervening training sessions (a minimum of three sessions at 80% correct) continued to be tested.

Table 1. Effects of Six Drugs Given at the Times Indicated in Combination with PCP (2.5 mg/kg) to Rats Trained to Discriminate PCP from Saline

Drug	Doses (mg/kg)	N	Time (min)	AD_{50} (mg/kg)	95% CI
LSD	0.08, 0.16, 0.32	16	60	0.19	0.04–0.31
DMT	2, 4, 8	16	60	7.57	4.22–9.80
LHM	0.1, 0.2, 0.4	16	60	0.22	0.06–0.85
DOM	1, 2, 4	16	90	1.37	0.87–2.15
Spiperone	0.1, 0.2, 0.4	11	90	—	
Ritanserin	2.5, 5, 10	14	90	—	

Drugs

As indicated in Table 1, the following compounds were tested in combination with the training drug [PCP; phencyclidine hydrochloride, 2.5 mg/kg]: LSD, d-lysergic acid diethylamide bitartrate; DMT, N,N-dimethyltryptamine oxalate; or LHM, lisuride hydrogen maleate; DOM, ±2,5 dimethoxy-4-methyl-amphetamine sulfate; spiperone HCL; and ritanserin. All drugs were dissolved in 0.9% saline and were given i.p. in a volume of 1 ml/kg except ritanserin which was first dissolved in a few drops of 3 M glacial acetic acid. LSD was obtained from the National Institute on Drug Abuse (NIDA; Rockville, MD); all other compounds were purchased from Research Biochemicals, Inc. (Natick, MA).

Data Analysis

Antagonist Dose (AD_{50}) and 95% confidence intervals (CI) were determined in a manner described by Tallarida and Murray (1987). To be included in the analysis of the effects of each test drug, each animal had to make at least 10 responses on either lever following all doses of the respective antagonist (see Table 1). Complete antagonism was defined as less than 20% responding on PCP-appropriate lever. Responding between 20% and 49% on the PCP-lever was considered partial antagonism.

Rates of responding prior to completion of the first 20 responses on one lever were analyzed for each test drug using a repeated measures ANOVA. For significant comparisons ($p < .05$), *post hoc* analyses were performed using the Bonferroni All Pairwise method of comparison.

RESULTS

Figure 1 shows that the PCP-saline discrimination was acquired in an average of 35 days. Once acquired, performance remained at criterion levels of accuracy (⩾ 80%) for the duration of the experiment.

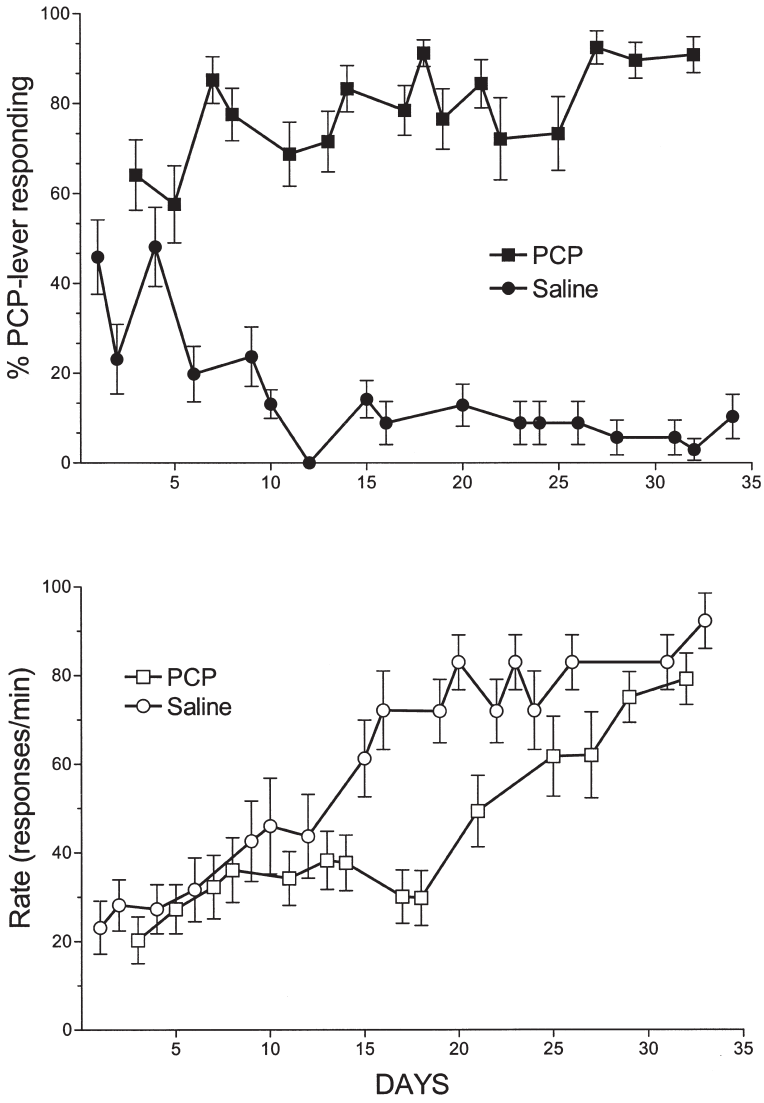

Figure 1. Acquisition of a discrimination between intraperitoneal injections of PCP (2.5 mg/kg) and saline, given 15 min prior to training in 16 rats. The top curve shows the average percent of responding on the drug-appropriate lever under each training condition (PCP or saline) and the bottom curve shows the average rate of responding between the onset of discrimination training until combination testing began.

The results of combination tests with LSD and DOM are shown in Figure 2. Pre-treatment with LSD antagonized the PCP cue completely (18%) with AD_{50} = 0.19 mg/kg. In addition, this antagonism occurred without any significant disruption of response rate [F(3,45) = 1.182, p = .327]. Pretreatment with DOM also blocked the discriminative stimulus properties of PCP completely (AD_{50} = 1.373). At the highest dose of DOM (4.0 mg/kg), PCP-lever responding was reduced to an average of 19.75%. Rate of response was significantly different from control [F(3,45) = 4.52, $p < .01$]. *Post hoc* testing showed that the 4.0 mg/kg dose accounted for this difference.

Figure 3 shows the effects of LHM and DMT on the PCP-cue. LHM reduced PCP-lever responding, but only to 48% (AD_{50} = 0.22 mg/kg). Unlike LSD, LHM disrupted rate of responding significantly [F(3,45) = 10.00, $p < .01$]. The highest dose (0.4 mg/kg) reduced the rate to nine responses/min; this was significantly different from all other doses tested.

DMT had an AD_{50} = 7.57 but, like LHM, failed to block the PCP discrimination completely (46% PCP-lever responding). DMT also had significant effects on response rate [F(3,45) = 3.975, p = .014]. The highest dose lowered rate of response to 19.8 responses/min; an effect that was significantly lower than control.

The effects of spiperone and ritanserin on the PCP-discrimination are shown in Figure 4. Spiperone failed to reduce PCP-lever responding below 80%. However, the rate of responding was significantly reduced [F(3,30) = 17.84, $p < .01$] and doses of 0.2 and 0.4 mg/kg had rate-depressing effects that were significantly lower than control. Ritanserin also failed to reduce PCP-lever responding and reduced the rate of response in a dose dependent manner [F(3,39) = 5.68, $p < .01$];

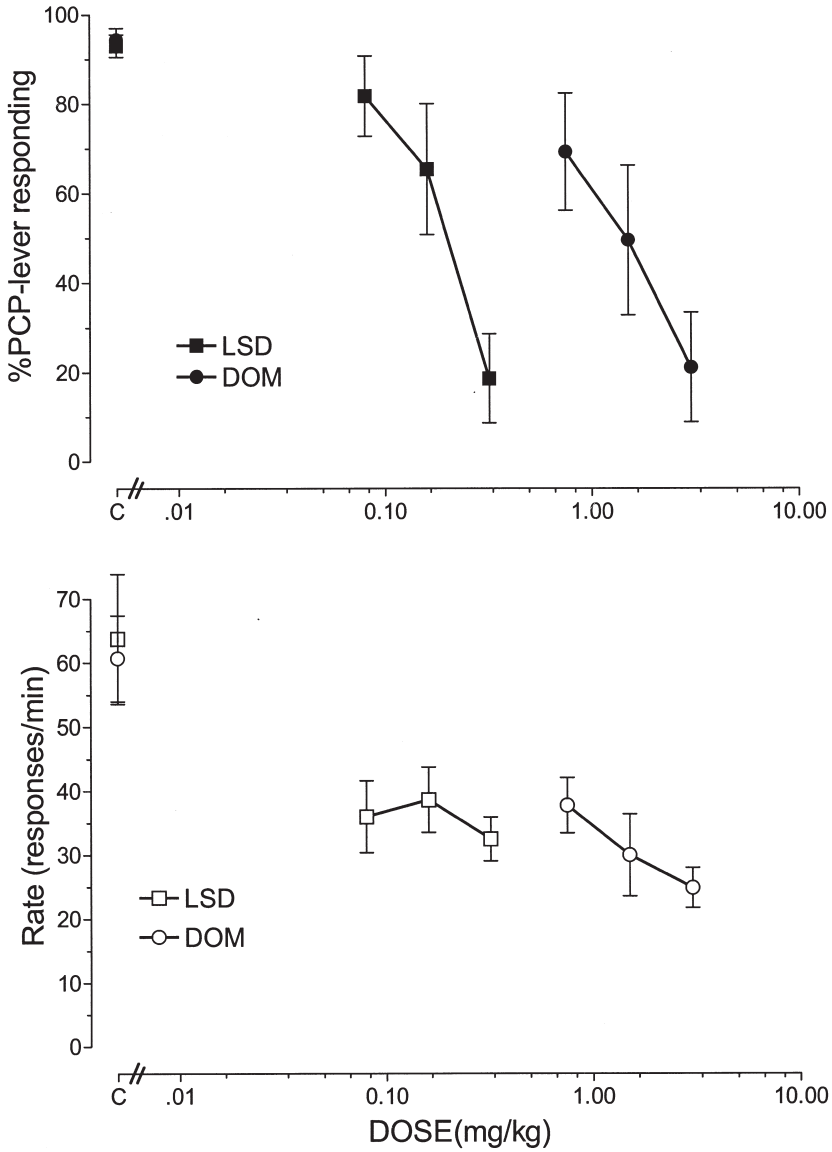

Figure 2. Results of combination tests with two indoleamine hallucinogens, LSD (given 60 min prior to testing; 45 min prior to PCP) or DOM (given 90 min prior to testing; 75 min prior to PCP), and PCP, in 16 rats trained to discriminate PCP (2.5 mg/kg) from saline. Average effects of PCP in combination with vehicle controls on both percent responding on the drug appropriate lever (top figure) and rate of responding (bottom figure) are shown on the y axis of each graph.

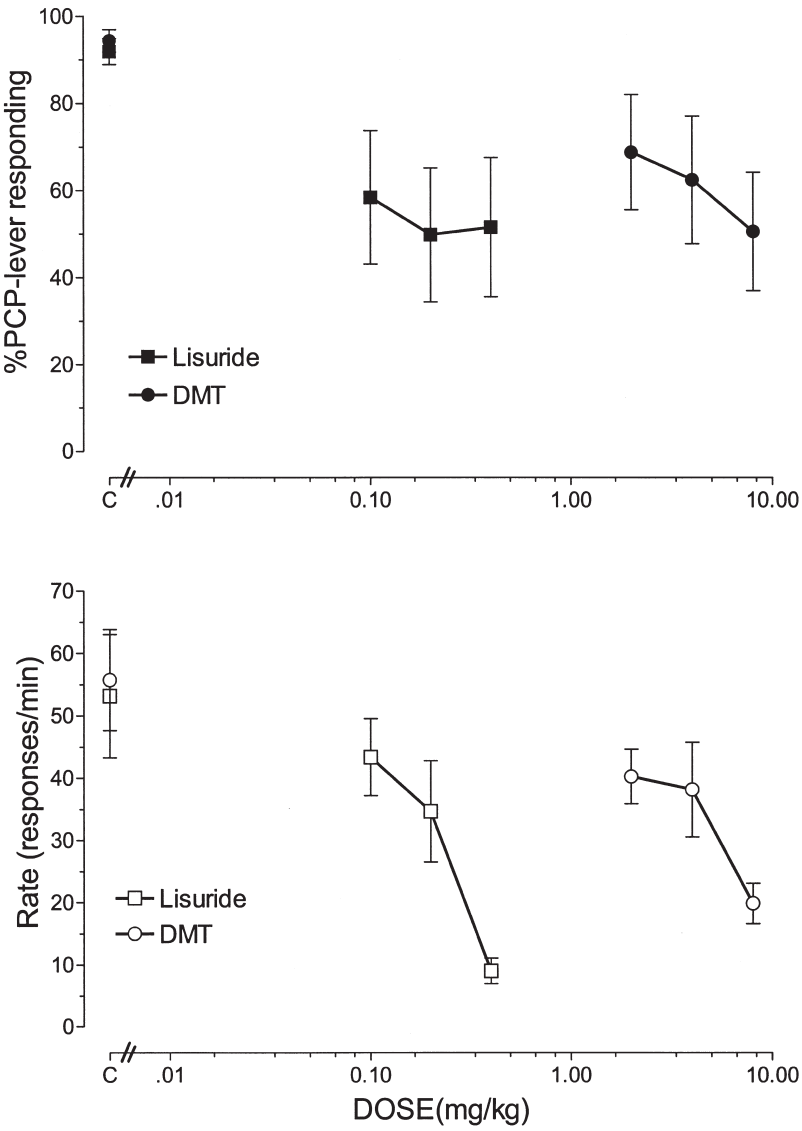

Figure 3. Results of combination tests with lisuride or DMT (both given 60 min prior to testing) and PCP, in 16 rats trained to discriminate PCP from saline. Average effects of PCP in combination with vehicle controls on both percent responding on the drug appropriate lever (top figure) and rate of responding (bottom figure) are shown on the y axis of each graph.

the highest dose tested (10 mg/kg) had effects on rate that were significantly lower than control.

DISCUSSION

In the present study, LSD and DOM antagonized the PCP-discrimination completely; with LSD, this antagonism occurred without any concurrent disruption of response rate. LHM and DMT blocked the PCP cue partially; spiperone and ritanserin had no significant effects on ability to discriminate PCP from saline. In addition, LHM, DMT, spiperone, and ritanserin disrupted response rates significantly. These findings suggest that at least one behavioral effect of PCP (the PCP cue) can, like the neurotoxic effects of this NMDA antagonist (Farber et al. 1998), be attenuated by 5-HT2 agonists.

Many drugs with agonist properties at 5-HT2A receptors also have agonist properties at 5-HT2C and other receptor subtypes; this is clearly the case with LSD (Marek and Aghajanian 1996), DOM (Glennon et al. 1992), and DMT (Smith et al. 1998). Therefore, it is difficult to attribute the behavioral or neurotoxic effects of these compounds exclusively to their actions at the 5-HT2A receptor. Lisuride may be a notable exception because its action at 5-HT2C receptors has been reported to be *antagonistic* (Burris et al. 1991). However, a recent study indicates that lisuride may act as an agonist at both 5-HT2A and 5-HT2C, as do other LSD-like drugs (Egan et al. 1998).

The present experiment found that lisuride blocked the discriminative stimulus properties of PCP partially. As mentioned previously, it is difficult to isolate the contribution of each 5-HT2 receptor subtype to this effect. Nonetheless, because spiperone and ritanserin failed to block the PCP-cue, it is likely that antagonist

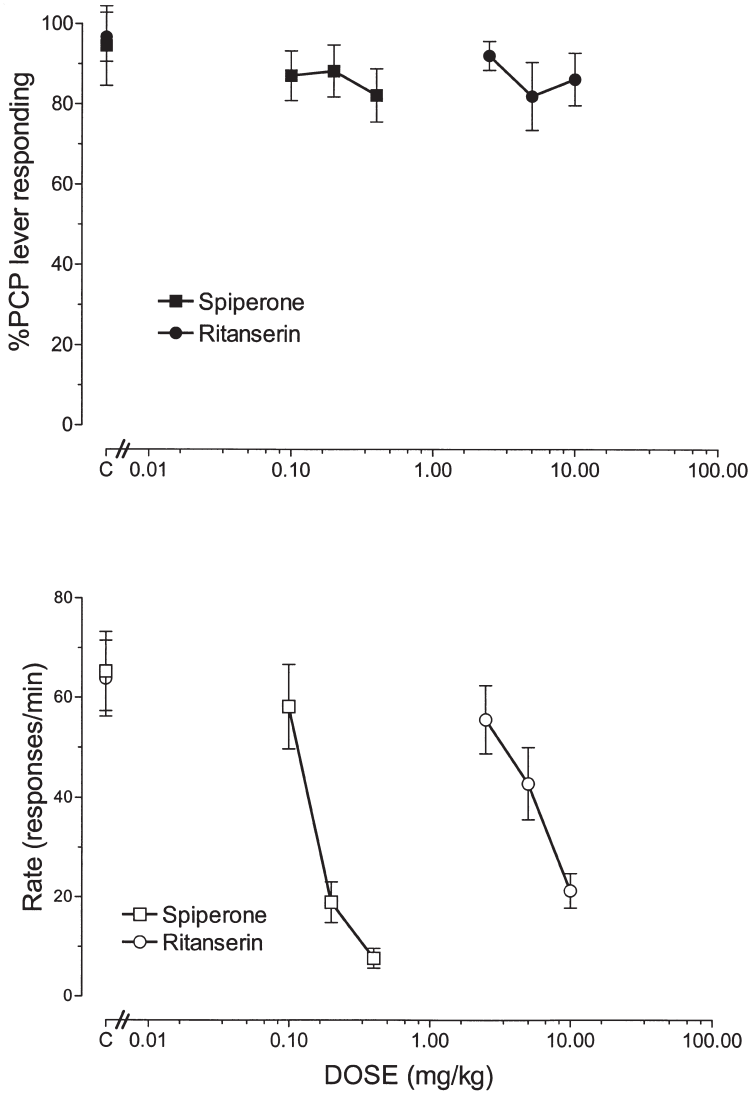

Figure 4. Results of combination tests with spiperone or (both given 90 min prior to testing; 45 min prior to PCP) and PCP, in 16 rats trained to discriminate PCP from saline. Average effects of PCP in combination with vehicle controls on both percent responding on the drug appropriate lever (top figure) and rate of responding (bottom figure) are shown on the y axis of each graph. The disruptive effects of these two drugs reduced the number of animals to 11 (spiperone) and 14 (ritanserin). Asterisks denote $p < .05$ (Bonferroni pairwise method of comparison).

action at 5-HT2 receptors is less important than agonist action. In addition, the reduction in rate of responding following combination tests with LSD, DOM, DMT, and LHM suggests that something more than simple agonistic action of 5-HT2 receptors is involved in the attenuation of the PCP-cue.

In light of the findings of Farber et al. (1998), the results of this study suggest that the behavioral (discriminative stimulus) and neurotoxic effects of PCP are mediated by similar mechanisms, which might involve a circuit originally described by Olney and Farber (1995a, 1995b). These investigators suggested that NMDA receptor hypofunction is ultimately involved in the reinstatement of excitatory functions of neurons in the cingulate cortex. This disinhibition is thought to be a result of NMDA antagonists that decrease GABAergic input to otherwise excitatory cell bodies. Cholinergic neurons in the cingulate cortex are likely to be innervated by projections from basal forebrain and glutamatergic neurons in the anterior thalamus. With less inhibitory (GABA) influence on these excitatory neurons, an increase in excitatory transmission would be expected in regions such as the posterior cingulate and retrosplenial cortexes (Fix et al. 1995; Olney and Farber 1995b).

GABAergic interneurons in rat piriform cortex were shown to have significant concentrations of 5-HT2A receptors (Sheldon and Aghajanian 1990; Marek and Aghajanian 1996). Farber et al. (1998) proposed that activation of these receptors on GABAergic interneurons might restore inhibition of the NMDA receptor hypofunction-neurotoxicity circuit, thus preventing some of the damage caused by NMDA antagonists in the posterior cingulate and retrosplenial cortexes. Indeed, Farber et al. (1998) showed that pretreatment with hallucinogens such as LSD and DOM significantly reduced the neurotoxic effects of MK-801. Lisuride, a congener of LSD that is not known to produce hallucinations in humans (White and Appel 1982) was also able to block the

neurotoxic effects of MK-801 (Farber et al. 1998). However, because lisuride has been reported to be a 5-HT2C agonist (Egan et al. 1998) and possibly a 5-HT2C antagonist, at least in the choroid plexus (Burris et al. 1991), determining the contribution of 5-HT2C in regards to the discriminative or neuroprotective properties of lisuride is, at best, difficult. Nonetheless, it appears that agonistic activity at the 5-HT2 receptors protects against the effects of NMDA antagonists—a result remarkably similar to that of the present experiment.

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