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BRIEF COMMUNICATION

Clozapine But Not Haloperidol Antagonizes an MK-801 Discriminative Stimulus Cue

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CORBETT, R. *Clozapine but not haloperidol antagonizes an MK-801 discriminative stimulus cue.* PHARMACOL BIOCHEM BEHAV 51(2/3) 561-564, 1995.—The noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist dizocilpine (MK-801) produced an interoceptive stimulus cue in rats trained to discriminate between MK-801 (0.075 mg/kg) and saline in a two-choice, discrete trial avoidance paradigm. Haloperidol (0.03–0.3 mg/kg) failed to antagonize the discriminative stimulus cue of MK-801, with all rats choosing the MK-801-appropriate choice lever. Higher doses of haloperidol (1.0 mg/kg) produced significant sedation such that the rats were unable to complete all the trials. In contrast, clozapine dose dependently antagonized the discriminative stimulus cue produced by MK-801. Clozapine at a dose of 3.0 mg/kg completely antagonized the stimulus cue produced by MK-801. Therefore, the discriminative stimulus cue produced by the noncompetitive NMDA antagonist MK-801 may be useful as an animal model for selecting novel drugs with potential efficacy for treatment-resistent schizophrenia.

Drug discrimination Noncompetitive NMDA antagonists Schizophrenia Atypical antipsychotic Clozapine

THE NONCOMPETITIVE *N*-methyl-D-aspartate (NMDA) antagonists phencyclidine (PCP) and dizocilpine (MK-801) induce stereotyped behaviors such as locomotion and ataxia in rodents which are similar but not identical to amphetamine mediated behaviors (4,13,20). Although it was initially proposed that PCP- and MK-801-induced stereotyped behaviors were due to the inhibition of dopamine uptake (17), further studies revealed that these behaviors were the result of a catecholamine-independent mechanism (20).

The glutamate hypothesis of schizophrenia suggests a relative deficiency of glutamate transmission (5,8,28) that may result in a dysregulated dopamine system. Numerous studies have shown an interaction between the dopaminergic and glutamatergic systems in subcortical regions (15,21,26,31,34). The descending glutamatergic projections originating from areas of the limbic cortex and the ascending dopaminergic projections originating from the ventral tegmental area (VTA) converge on a common neuronal target that is predominantly the GABAergic medium-size spiny projection neurons in the nucleus accumbens (26). These findings suggest that the glutamatergic and dopaminergic systems functionally oppose each other in terms of their regulation of the GAGAergic output projections in this region. This pathway innervates the ventral pallidum and represents the main output from the nucleus accumbens, which is considered crucial in the regulation of psychomotor function, including the rewarding properties of drugs and the therapeutic effects of antipsychotic drugs. This interaction may account for the ability of amphetamine, PCP, and MK-801 to induce similar behavioral effects in rodents.

It has previously been demonstrated that antipsychotic agents including haloperidol, chlorpromazine, and clozapine antagonized PCP-induced locomotion and stereotypies (9,10). More recently, it has been shown that haloperidol nonselectively antagonized MK-801-induced stereotyped sniffing as well as spontaneous sniffing, whereas clozapine was shown to selectively antagonize the MK-801-induced sniffing (29). Selective antagonism of these behaviors has been proposed as an animal model of neuroleptic-resistent schizophrenia (5). The purpose of the present investigation was to compare the ability of the antipsychotic agents haloperidol and clozapine to antagonize an MK-801 interoceptive discriminative stimulus cue.

METHOD

Male Wistar rats (225–250 g; Charles River, Wilmington, MA) were housed under standard laboratory conditions as outlined in the NIH guide for the care and use of laboratory animals (National Institute of Health Publications No.85-23,

revised 1985) with a 12 L : 12 D cycle and allowed free access to food and water.

Phencyclidine (Sigma Chemical Co., St. Louis, MO), MK-801, and clozapine (Research Biochemicals, Inc., Natick, MA) and haloperidol (McNeil Pharmaceuticals, Fort Washington, PA) were administered in these studies. Compounds were either dissolved or suspended in distilled water with a drop of Tween-80. Clozapine was initially dissolved in a drop of glacial acetic acid then made up to the correct concentration with deionized water and adjusted to pH 6.5 using 1 N NaOH. The final volume was prepared to account for salt content, and the dosage was expressed as 100% base. The rats were administered compounds in a dosage volume of 1 ml/kg.

For the drug discrimination assay, rats were trained to discriminate between MK-801 (0.075 mg/kg) and saline in a two-choice, discrete trial avoidance paradigm in a test chamber housed within a ventilated and sound attenuating enclosure (27). The onset of a trial was signalled by simultaneous illumination of the house light and the presentation of white noise. At this time, the rat was required to press the observing lever on one wall of the chamber, turn, and press one of the two choice levers on the opposite wall of the chamber. The first of the two responses (observing response) of the trial terminated the white noise, and the second response (appropriate choice response) extinguished the house light and ended the trial. Beginning 5 s after the onset of the trial, a 0.1-mA shock was delivered to the grid floor of the chamber every 3 s in 0.5-s pulses until the two-response chain was completed. The intertrial interval was 30 s, during which time the chamber was dimly illuminated by a red light. Experimental sessions ended after 20 trials or 30 min, whichever came first. Training sessions were conducted 5 days/week, with two sessions/day, at least 3 h apart. Either MK-801 or vehicle was injected IP 30 min before both training sessions on each day. Training continued until rats could complete reliably at least 18 of 20 trials (i.e., 90%) on the appropriate choice lever under each condition. Drug test sessions were conducted on Tuesdays and Fridays provided the rats satisfied the performance criterion in training sessions on days before drug testing. During test sessions, both levers were activated so that a response on either choice lever after the observing response terminated the trial. Test sessions and training sessions were identical in all other aspects. Challenge compounds were administered 60 min before test sessions, and MK-801 was administered 30 min before testing.

The data were analyzed in terms of the number of trials completed on the MK-801-appropriate choice lever. All animals had to complete all trials of every session to be included in the analysis. A dose of test compound was considered to produce discriminative stimulus comparable to those produced by the training dose of MK-801 (0.075 mg/kg) if a rat completed at least 18 of 20 trials on the MK-801-appropriate choice lever. Antagonism tests consisted of administering the test compond 30 min before MK-801, and then 30 min later testing for antagonism of the MK-801 interoceptive cue.

RESULTS

Figure 1 shows the dose-response effects of MK-801 and PCP in producing an interoceptive discriminative stimulus cue. The lowest dose of MK-801 that reliably produced at least 18 of 20 trials (90%) on the MK-801-appropriate choice lever was 0.075 mg/kg. Rats were then given PCP and all test subjects reliably produced > 90% correct responding on the MK-801-appropriate choice lever at a dose of 0.75 mg/kg.

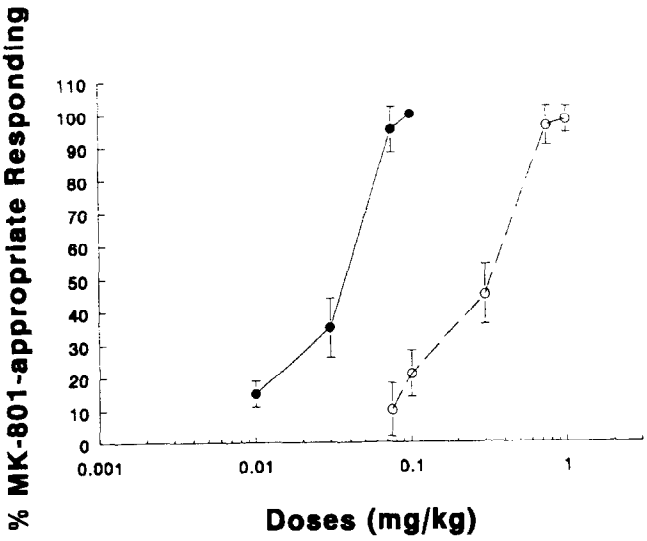

FIG. 1. MK-801 (-●-) and PCP (-○-) dose dependently produced MK-801-appropriate responding in rats trained to discriminate MK-801 (0.075 mg/kg) from saline. MK-801 and PCP were administered intraperitoneally 30 min before testing (n = 6/dose).

Rats administered haloperidol (0.03-0.3 mg/kg) failed to antagonize the discriminative stimulus cue produced by 0.075 mg/kg of MK-801, with all subjects choosing the MK-801-appropriate choice lever (Table 1). Higher doses of haloperidol (1.0 mg/kg) produced significant sedation such that the rats were unable to complete all of the trials. On the other hand, clozapine dose dependently antagonized the discriminative stimulus cue produced by MK-801 (0.075 mg/kg). Clozapine at 3.0 mg/kg completely antagonized the 0.075 mg/kg MK-801 discriminative stimulus cue, with all rats choosing the saline-appropriate choice lever.

DISCUSSION

The present results show that MK-801 can produce a discriminative stimulus cue and that PCP generalizes to this cue. This is in agreement with previous studies showing that the noncompetitive NMDA antagonists PCP and MK-801 completely generalized to each other (19,33), and that their discriminative stimulus cues are primarly mediated through the noncompetitive NMDA site on the NMDA-glycine receptor complex (20). In addition, clozapine antagonized this MK-801 discriminative stimulus cue, suggesting that clozapine can attenuate the interoceptive cue produced by noncompetitive NMDA antagonists. However, haloperidol failed to antagonize the discriminative stimulus cue produced by MK-801. Other investigators have also shown that haloperidol failed to antagonize a PCP discriminative stimulus cue in rats trained to discriminate PCP from saline (1,2,23). Therefore, clozapine's antagonism of the MK-801 effects appears to be novel among antipsychotic agents. In addition, haloperidol does not substitute for clozapine in rats trained to discriminate clozapine from saline (12,32).

Discrimination studies using amphetamine and PCP demonstrated that the effects of these two compounds are mediated through different mechanisms as amphetamine does not generalize to PCP in rats trained to discriminate PCP from

TABLE 1

MEAN RESPONDING ON MK-801 APPROPRIATE LEVER BY RATS TRAINED TO DISCRIMINATE MK-801 (0.075 mg/kg) FROM SALINE

Treatment	Dose (mg/kg)	No. responses on MK-801 lever	No. responses on saline lever
Saline	–	0 ± 0	20.0 ± 0
MK-801 (0.075 mg/kg)	–	19.0 ± 1	1.0 ± 1
PCP (0.75 mg/kg)	–	19.5 ± 0.5	0.5 ± 0.5
MK-801 +			
Haloperidol	0.03	18.0 ± 1.0	2.0 ± 1
Haloperidol	0.10	18.6 ± 0.4	1.3 ± 0.4
Haloperidol	0.30	17.7 ± 0.7	2.3 ± 0.8
Haloperidol	1.0	ND	ND
MK-801 +			
Clozapine	0.1	18.6 ± 0.7	1.3 ± 0.4
Clozapine	0.3	12.8 ± 3.4	7.2 ± 3.3
Clozapine	1.0	10.8 ± 3.1*	9.1 ± 3.1
Clozapine	3.0	2.0 ± 1.4*	18.0 ± 1.4

Rats were administered haloperidol or clozapine 30 min before MK-801 and tested 30 min later for antagonism of the MK-801 interoceptive cue.

*$p > 0.05$ significantly different from MK-801 appropriate lever, Dunnett's test. $n = 6$ rats per group.

ND, Not determined because of sedation.

saline (23). Amphetamine discriminative stimulus cue is primarly mediated through the dopamine D_1 and D_2 receptor subtypes as SCH 23390, raclopride, and the typical antipsychotic agents haloperidol and chlorpromazine all antagonized this stimulus cue, whereas clozapine failed to antagonize this amphetamine cue (3,11,24). These results suggest that clozapine's mode of action in antagonizing the MK-801 discriminative stimulus cue is not primarly mediated through the dopamine D_1 or D_2 receptor subtypes.

Although clozapine does not bind directly to the MK-801 binding site on the NMDA–glycine receptor complex (unpublished observations), other explanations for clozapine's antagonist effect on MK-801 discriminative stimulus cue cannot be excluded. In receptor binding studies, clozapine has an affinity for serotonin, α-adrenergic, cholinergic, and, to a lesser extent, dopamine receptor subtypes (7). However, only the 5-HT_{1c} antagonist mesurgiline was shown partially to discriminate to a clozapine cue (14), suggesting that the 5-HT_{1c} antagonist properties of clozapine are at least partially responsible for its discriminative cue and may contibute to its antagonism of the MK-801 discriminative stimulus cue. In addition, molecular cloning techniques have revealed a number of dopamine receptor subtypes including a dopamine D_4 receptor subtype (6), to which clozapine binds with high affinity (30). It has been postulated that at physiologic concentrations that would be efficacious for schizophrenia, typical antipsychotic agents bind to the dopamine D_2 receptor subtype, whereas clozapine binds to the dopamine D_4 receptor subtype (25). Future studies may reveal that the unique profile of clozapine may be due to selective interactions at this site.

In summary, pharmacologic agents such as PCP induce psychoses similar to schizophrenia in normal human subjects (16). PCP induces a psychotomimetic state similar to both the positive and negative symptomatology of schizophrenia. To date, neuroleptic agents that block dopamine D_2 receptors have been effective in alleviating mainly the positive symptoms (18). However, some schizophrenic patients fail to respond to these classical agents and have been termed neuroleptic treatment-resistent schizophrenic patients. Because clozapine has been shown to be effective in this group of patients (22), the discriminative stimulus cue produced by the noncompetitive NMDA antagonists may be useful as an animal model for selecting novel drugs to treat this treatment-resistent population.

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