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Reversal of chlorpromazine-induced avoidance depression by the *N*-methyl-D-aspartate antagonist, dizocilpine, in mice

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Abstract—The non-competitive *N*-methyl-D-aspartate (NMDA) antagonist MK-801 (dizocilpine) was tested, alone or in combination with chlorpromazine, in mice previously trained in the shuttle-box. The lowest doses of dizocilpine (0·02 and 0·04 mg kg^{-1}) attenuated the disrupting action of the neuroleptic (1·5 mg kg^{-1}) on avoidance-performance, while avoidance depression induced by 1·5 and 2 mg kg^{-1} chlorpromazine was completely or almost completely reversed by 0·08 mg kg^{-1} NMDA antagonist. The highest dose (0·16 mg kg^{-1}) of dizocilpine did not ameliorate avoidance-performance of mice receiving 2 mg kg^{-1} chlorpromazine, perhaps because of ataxic effects produced by the drug combination, at these doses. The results support suggestions for a potential use of NMDA antagonists in the treatment of extrapyramidal side-effects of neuroleptics.

Interactions between glutamatergic and monaminergic systems may have important clinical implications (Carlsson & Carlsson 1990). In particular, the dopamine-glutamate interaction within the striatum has prompted a discussion on a potential use of glutamatergic antagonists in the treatment of Parkinson's disease and of neuroleptic-induced parkinsonian symptoms (Klockgether & Turski 1989; Carlsson & Carlsson 1990; Marrow et al 1992). In animal studies, the non-competitive *N*-methyl-D-aspartate (NMDA) antagonist, MK-801 (dizocilpine), exerted a protective action on some behavioural disturbances induced by neuroleptics. In particular, dizocilpine prevented haloperidol-induced catalepsy (Schmidt & Busber 1989) and reversed movement-initiation deficits induced by the neuroleptic in rats trained in a rewarded reaction-time task (Hauber & Schmidt 1990). Moreover, the antagonist ameliorated some behavioural deficits induced by chlorpromazine in a conditioned-reaction time task (Marrow et al 1992). The present study demonstrated a protective effect of dizocilpine against the disrupting action exerted by chlorpromazine on avoidance behaviour of mice previously trained in the shuttle-box. The selective inhibition of active avoidance behaviour has been considered a typical action of neuroleptic agents (Cook & Davidson 1978), predictive of the antipsychotic efficacy and, perhaps, of the extrapyramidal side-effects of these drugs (Cook & Sepinwall 1975).

Materials and methods

Animals. The subjects were naive male mice, 8–9 weeks, 30–35 g, of the randomly-bred CD-1 strain (Charles River, Italy). The mice were housed in standard transparent plastic cages (8 per cage) under standard animal-room conditions (free access to food and water, 12-h light/dark cycle, ambient temperature of 23°C). The experiments were carried out between 0900 and 1400 h.

Drugs. Saline (0·9% NaCl), dizocilpine (RBI) and chlorpromazine hydrochloride (Farmitalia), dissolved in distilled water, were injected intraperitoneally in a volume of 10 mL kg^{-1}. Combinations of drugs were given as mixed solutions, in a single injection.

Active avoidance. The apparatus consisted of eight automated shuttle-boxes, each one divided into two 20 × 10 cm compartments, connected by a 3 × 3 cm opening. A light (10 W) was switched on alternately in the two compartments and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s and overlapped it for 25 s. The US was an electric shock (0·2 mA) applied continuously to the grid floor. The inter-trial interval was 30 s. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 5 s after the onset of the CS. If animals failed to avoid the shock they could escape it by crossing during the US. Spontaneous crossings from the dark to the light compartment were punished and recorded as inter-trial responses.

The mice were subjected to 13 daily 100-trial training sessions. The last three training sessions were preceded (15 min) by injection of saline. Only animals reaching a criterion of 70% avoidance responses were used to test drug effects, 24 h after the last training session. Chlorpromazine (1·5 or 2 mg kg^{-1}) and dizocilpine (0·02, 0·04, 0·08 and 0·16 mg kg^{-1}), were given alone or combined, 15 min before the avoidance session, in groups of eight mice. Doses of chlorpromazine were chosen on the basis of previous experience (Sansone & Messeri 1974).

Statistical analysis. The two-tailed Student's *t*-test, for related samples, was used to compare, for each treatment, avoidance responses of the drug session with those of the previous control session. In addition, the effects of drug combinations were evaluated by a one-way analysis of variance for each dose of

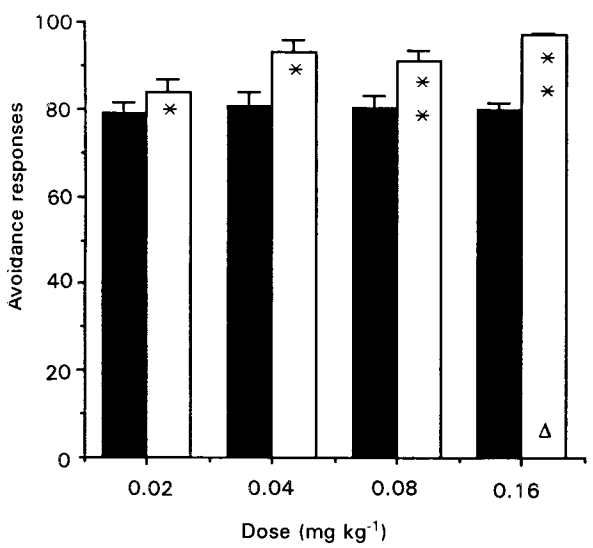

FIG. 1. Effect of dizocilpine on shuttle-box avoidance performance of trained mice. Columns represent, for each dose, the mean number of avoidance responses in the last 100-trial training session (control session; black column) and in the drug session (white columns). Vertical lines indicate s.e.m. Asterisks denote a significant difference between control and drug session (* $P < 0·05$; ** $P < 0·001$; Student's *t*-test, for related samples). The triangle (within the column) represents the mean number of trials in which inter-trial responses occurred.

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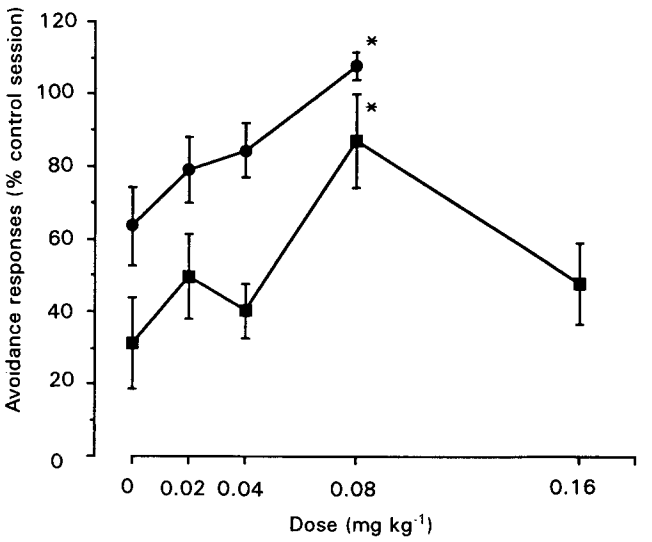

FIG. 2. Effect of dizocilpine on the depressant action of chlorpromazine, given at the dose of 1·5 (●) or 2 mg kg^{-1} (■). The results are presented as percentages of the performance in the control session (means ± s.e.). The asterisk denotes a significant difference ($P < 0.01$; Duncan's test) vs dose 0 of dizocilpine (chlorpromazine alone).

chlorpromazine combined with various doses of dizocilpine. Individual between-group comparisons were made using Duncan's multiple-range test.

Results

Dizocilpine alone. In spite of the good performance exhibited by the trained animals selected for the experiment, dizocilpine increased avoidance responses at all tested doses (Fig. 1). When put in the shuttle-box, 15 min after the injection of the highest dose (0·16 mg kg^{-1}) of the drug, mice displayed behavioural stimulation, without signs of ataxia. The animals were not observed during the avoidance test, but mice treated with 0·16 mg kg^{-1} dizocilpine still showed signs of excitement, revealed by the occurrence of inter-trial responses, a type of response (punished by electric shock) usually absent in trained animals.

Chlorpromazine alone. Given 15 min before the avoidance test in doses of 1·5 and 2 mg kg^{-1}, chlorpromazine alone (dose 0 of dizocilpine in Fig. 2) significantly depressed avoidance performance, by 36·4 and 68·6%, respectively, in comparison with the previous control session ($P < 0.01$ and $P < 0.001$, respectively). Failure of escape responses was never observed.

Chlorpromazine and dizocilpine combined. Fig. 2 shows the effects of various doses of dizocilpine on avoidance depression induced by 1·5 or 2 mg kg^{-1} chlorpromazine. For each treatment, the performance of the mice in the drug session was expressed as a percentage of the performance in the preceding non-drug (control) session. Analysis of variance showed a significant main effect of dizocilpine either in mice receiving 1·5 mg kg^{-1} chlorpromazine ($F(3,28): 4{\cdot}90$, $P < 0.01$) or 2 mg kg^{-1} of the neuroleptic ($F(4,35): 3{\cdot}55$, $P < 0.05$). Duncan's test indicated a significant effect of dizocilpine at the dose of 0·08 mg kg^{-1}, which completely, or almost completely, reversed the avoidance depressant action induced by chlorpromazine at the doses of 1·5 and 2 mg kg^{-1}, respectively. However, it must be noted that the lowest doses of dizocilpine (0·02 and 0·04 mg kg^{-1}) were not quite ineffective, but partially reversed the inhibitory action of the lowest dose of chlorpromazine. In fact, chlorpromazine alone (1·5 mg kg^{-1}, dose 0 of dizocilpine) significantly reduced avoidance responses, in comparison with the control session (*t*-test), while performance impairment did not reach significant levels in mice receiving this dose of neuroleptic combined with every tested dose of dizocilpine. On the other hand, no further improvement was obtained by increasing the dosage of dizocilpine, as demonstrated by failure of the dose of 0·16 mg kg^{-1} to reverse the depressant action of 2 mg kg^{-1} chlorpromazine. Mice receiving 0·16 mg kg^{-1} dizocilpine combined with chlorpromazine displayed an avoidance behaviour disturbed by inter-trial responses, as did animals treated with the same dose of the NMDA antagonist alone.

Discussion

In the present study, shuttle-box avoidance depression induced by 1·5 mg kg^{-1} chlorpromazine was attenuated by 0·02 and 0·04 mg kg^{-1} and completely reversed by 0·08 mg kg^{-1} of dizocilpine, an *N*-methyl-D-aspartate (NMDA) antagonist. This dose of dizocilpine almost completely reversed even the strong avoidance-disrupting action of 2 mg kg^{-1} chlorpromazine, whilst lower doses were ineffective. No performance improvement was produced by 0·16 mg kg^{-1} dizocilpine in mice receiving 2 mg kg^{-1} chlorpromazine.

The locomotor stimulatory action of dizocilpine (Carlsson & Carlsson 1990) might have contributed to increased active-avoidance responses, but it is unlikely that performance improvements produced by the drug, alone or combined with chlorpromazine, were merely due to hyperactivity. In fact, doses of dizocilpine lower than 0·1 mg kg^{-1} did not stimulate locomotor activity in mice (Mele et al 1992), but increased avoidance responses, if given alone, and partially or completely reversed chlorpromazine-induced avoidance depression. On the other hand, 0·16 mg kg^{-1} dizocilpine, a dose which strongly stimulated locomotion in mice (Liljequist 1991), did not antagonize the inhibitory action of chlorpromazine on avoidance behaviour.

Failure of 0·16 mg kg^{-1} dizocilpine to improve avoidance behaviour in mice receiving chlorpromazine, might be due to the occurrence of ataxic effects, usually induced by higher doses of the NMDA antagonist alone (Venable & Kelly 1990; Liljequist 1991). Performance impairment, due to myo-relaxant effects, was also observed, in a reaction-time task, in rats receiving 0·16

mg kg^{-1} dizocilpine combined with 2 mg kg^{-1} chlorpromazine (Marrow et al 1992). Even phencyclidine, another NMDA antagonist, at doses producing a locomotor stimulation of an ataxic, compulsive nature, impaired performance of previously learned avoidance response (Pryor et al 1977). Conversely, attenuation or reversal of chlorpromazine-induced avoidance depression, by lower doses of dizocilpine, might be due to interactive effects of the two drugs on motor mechanisms, similar to those observed in reaction time tasks. In these tasks, dizocilpine ameliorated the deficits in movement initiation induced by haloperidol (Hauber & Schmidt 1990) and, to a lesser extent, those induced by chlorpromazine (Marrow et al 1992). The NMDA antagonist might act in the same way in mice previously trained in the shuttle-box and receiving chlorpromazine, since a deficit in locomotor initiation seems responsible for chlorpromazine-induced suppression of avoidance response (Posluns 1962).

Reversal of haloperidol-induced movement-initiation deficits by dizocilpine was considered indicative of a potential therapeutic utility of NMDA antagonists in the treatment of Parkinson's disease (Hauber & Schmidt 1990). Instead, in this respect, caution was suggested by Marrow et al (1992), who observed that various aspects of performance, in a reaction time task, were similarly disrupted by chlorpromazine, but neuroleptic-induced deficits were not equally ameliorated by dizocilpine. In comparison with these last results, the present findings, showing reversal of chlorpromazine-induced avoidance depression by dizocilpine, may appear even less predictive of a clinical efficacy of the antagonist. In fact, contrary to what happened in the reaction-time task, the avoidance improving effects of dizocilpine, in mice treated with chlorpromazine, did not seem due to a specific reversal of the depressant action of the neuroleptic, since the NMDA antagonist improved performance even in mice which did not receive chlorpromazine. However, in view of a clear, even if not specific, antagonism of the avoidance disrupting action of chlorpromazine, it cannot be excluded that NMDA antagonists may counteract the extrapyramidal side-effects of neuroleptic drugs.

For the neurochemical mechanisms involved in the effects of chlorpromazine, dizocilpine and their combinations on avoidance behaviour, it seems important to note that chlorpromazine is considered primarily a dopamine antagonist (Baldessarini 1985) and that such antagonistic action is probably responsible for its disrupting action on avoidance behaviour, in which centrally acting catecholamines are strongly implicated (Oei & King 1980). A dopamine-glutamate interaction at the post-synaptic level (Carlsson & Carlsson 1990) might be involved in the ameliorating action of the NMDA antagonist on the avoidance response, in the presence of a dopamine antagonist, such as chlorpromazine. Moreover, dizocilpine, which acts as a non-competitive NMDA antagonist, blocking the voltage-independent channel associated with the NMDA receptor (Wong & Kemp 1991), has also been reported to increase synthesis and release of dopamine (Krebs et al 1991; Löscher et al 1991). The glutamate-dopamine interaction is the basis of the proposal for a therapeutic use of NMDA antagonists in the treatment of neuroleptic and idiopathic Parkinson's disease.

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