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BRIEF COMMUNICATION

Different Capability of *N*-Methyl-D-Aspartate Antagonists to Affect Locomotor/Exploratory Activity of Mice in a Computerized On-Line Open Field Test

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DIANA, G. AND S. SAGRATELLA. *Different capability of N-methyl-D-aspartate antagonists to affect locomotor/exploratory activity of mice in a computerized on-line open field test.* PHARMACOL BIOCHEM BEHAV 48(1) 291–295, 1994. – The effects of the competitive *N*-methyl-D-aspartate (NMDA) antagonists CGS 19755 and CPP, and of the noncompetitive NMDA antagonists PCP, MK 801, and dextromethorphan (DM) have been studied on the locomotor/exploratory activity of mice in a computerized on-line open field test. CGS 19755 (12.5–25 mg/kg, IP) induced a dose-dependent decrease in the locomotor/exploratory activity of mice; CPP (25–50 mg/kg, IP) did not present such an effect. PCP (1.25–10 mg/kg, IP) induced a dose-dependent increase/decrease in the locomotor/exploratory activity of mice, and DM (25–50 mg/kg, IP) and MK 801 (0.125–0.250 mg/kg, IP) increased it. The data show that NMDA antagonists affect locomotor/exploratory activity of mice in different ways, inducing both potentiating and inhibitory effects.

NMDA Exploration Locomotor activity Mice

THE role of the excitatory amino acid receptors, especially the *N*-methyl-D-aspartate (NMDA) subtype, has been recently emphasized in the control of synaptic transmission at the level of basal ganglia (15). In fact, various NMDA antagonists induce a behavioural pattern in rodents consisting of both barbiturate-like effects, such as ataxia, and amphetamine-like effects, such as hyperactivity (1,12,13). Furthermore, these drugs have been reported to antagonize the catalepsy induced by haloperidol (23,24) and to potentiate the antiparkinson effects of L-DOPA (11) in rats. In particular, the motor hyperactivity induced by NMDA antagonists in rats was characterized by the appearance of stereotypies such as circling or body- and head-weaving (1,12,13,18–20). Recently, it has been shown that the noncompetitive NMDA antagonists phencyclidine (PCP) and MK 801 increase the basal locomotor activity in rats (3,25). Conversely, the effects of competitive NMDA antagonists on locomotor activity appear controversial in literature. In fact, the competitive NMDA antagonists CPP and CGP 39551 failed to affect basal locomotor activity in rats (3,11), but potentiated the increase of the locomotor activity induced by L-DOPA (11). In addition, it has been shown that NMDA antagonists also influence locomotor/exploratory activity in the four-plate test and in the open arms of an elevated plus maze (21). NMDA antagonists have been shown to differently affect the locomotor/exploratory activity in the four-plate test under nonshocked conditions. In particular, the noncompetitive NMDA antagonists phencyclidine, MK 801, and dextromethorphan (DM) increased exploration during 1 min in the four-plate test, while the competitive NMDA antagonists AP7 and CGS 19755 reduced it (21).

In the present study, we examined the influence of various competitive (CGS 19755 and CPP) and noncompetitive (PCP,

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MK 801, and DM) NMDA antagonists on the locomotor activity of mice in an on-line computerized open field test to further characterize the effect of NMDA antagonists on locomotor/exploratory activity and on basal locomotor activity to establish: 1) the involvement of NMDA transmission in the two types of locomotion; 2) the relationship between these two types of behaviour.

METHOD

The subjects were adult Swiss male mice, weighing 25–30 g. They were housed 50 per cage on a 12 L : 12 D schedule in a temperature-controlled (23 ± 1°C) colony room. The animals were given free access to standard pellet food and water and were habituated for 6 h to laboratory conditions before the test. Each animal was tested once in the behavioural test. Animals were studied individually, during locomotion in a 39.5 × 34.5 × 31 cm box, where they were put 30 s before starting the experiment. Two types of automated measurements were used. In the first, the animals were monitored with a videocamera during two consecutive monitoring sessions. The images were digitalized and tracked by means of a contrast-sensitive, automated video tracker (Videomex-V, Columbus Instruments) (26). A printout for each session showed the pattern of the ambulatory movements of the animals in the open field box. The distance travelled in cm by the animals in the horizontal locomotor activity was analyzed. Data were collected and analyzed between 0900 and 1700 h in two consecutive sampling periods, each lasting 2 min. For the second type of measurement, the box was placed on an activity meter apparatus (Automex-II, Columbus Instruments) set at a standard level for horizontal activity. Data were collected and analyzed between 0900 and 1700 h in three sampling periods, each lasting 8 min at 30, 45, and 60 min after the administration of the drugs. In addition, the total locomotor counts, 30 min after the injection of the drugs, over a period of 40 min (basal locomotor activity), were analyzed. The results in each experimental group were expressed as mean ± SEM. Each group was composed of a minimum of eight mice. Statistical analysis of the results was performed using the one-way ANOVA followed by Newman–Keuls test for multiple comparisons.

All drugs were injected IP in a volume of 10 ml/kg 30 min before testing. Phencyclidine hydrocloride (PCP) was kindly supplied by NIDA. CGS 19755 (*cis*-4-phosphonomethyl-2-piperidine-carboxylic acid), CPP (+3-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid), and MK 801 (dizocilpine) were obtained by Tocris. Dextromethorphan hydrobromide was obtained by Sigma.

RESULTS

Compared with saline-treated mice, CGS 19755 (12.5–25 mg/kg) induced a significant ($p < 0.01$) decrease in both locomotor/exploratory activity and in the basal locomotor activity (Table 1); the dose of 6.25 mg/kg was ineffective. The time course of the exploration during the three 8-min monitor-

TABLE 1

INFLUENCE OF NMDA ANTAGONISTS ON THE LOCOMOTOR ACTIVITY OF MICE IN AN ON-LINE OPEN FIELD TEST

Drug	Dose (mg/kg, IP)	Exploration		Basal Activity	
		N	cm/4 Min (mean ± SEM)	*N*	Counts/40 Min (mean ± SEM)
Saline	–	26	1321 ± 75	16	1921 ± 124
CGS 19755	6.25	8	1215 ± 135	–	–
CGS 19755	12.50	8	865 ± 156*	8	1021 ± 110*
CGS 19755	25.00	8	397 ± 130†	8	560 ± 130†
CPP	25.00	8	1367 ± 149	–	–
CPP	50.00	8	1534 ± 252	–	–
PCP	1.25	11	1692 ± 127	–	–
PCP	2.50	11	2551 ± 161†	8	1934 ± 221
PCP	5.00	8	2048 ± 178*	8	2620 ± 129†
PCP	10.00	8	789 ± 220*	8	1964 ± 197
PCP	15.00	–	–	8	1310 ± 120†
MK 801	0.062	13	1621 ± 108	–	–
MK 801	0.125	13	2465 ± 205†	8	3099 ± 235†
MK 801	0.250	12	2375 ± 127†	8	2882 ± 248*
MK 801	0.500	11	1449 ± 240	8	1856 ± 281
MK 801	1.000	–	–	8	1323 ± 167*
DM	6.25	8	1287 ± 245	–	–
DM	12.50	8	1628 ± 125	–	–
DM	25.00	8	2312 ± 336†	8	1798 ± 174
DM	50.00	8	2756 ± 198†	8	2358 ± 294

The influence of NMDA antagonists on the horizontal distance travelled by the mouse in the 4-min monitoring period and on the locomotor counts in the 40-min monitoring period, 30 min after the injection of the drugs. *N* = number of animals; DM = dextromethorphan.

*Significantly different from saline ($p < 0.05$ according to ANOVA, Newman–Keuls test).

†Significantly different from saline ($p < 0.01$ according to ANOVA, Newman–Keuls test).

TABLE 2

INFLUENCE OF NMDA ANTAGONISTS ON THE TIME COURSE OF LOCOMOTOR ACTIVITY OF MICE IN AN ON-LINE OPEN FIELD TEST

Drug	Dose (mg/kg, IP)	N	Locomotion (counts ± SEM/8 min) 30 Min	45 Min	60 Min
Saline	–	16	505 ± 34	443 ± 38	362 ± 49*
CGS 19755	12.50	8	210 ± 23†	190 ± 36†	205 ± 39†
CGS 19755	25.00	8	92 ± 21†	100 ± 24†	112 ± 35†
PCP	2.50	8	621 ± 40‡	341 ± 57*	282 ± 59*
PCP	5.00	8	701 ± 49†	513 ± 55*	401 ± 57*
PCP	10.00	8	303 ± 59†	209 ± 45‡	292 ± 66
MK 801	0.125	8	770 ± 27†	541 ± 62*	437 ± 98*
MK 801	0.250	8	578 ± 37	368 ± 71*	286 ± 49*
MK 801	0.500	8	547 ± 56	485 ± 76	390 ± 57
DM	25.00	8	557 ± 38	337 ± 42*	272 ± 50*
DM	50.00	8	705 ± 58†	490 ± 72*	384 ± 68*

The influence of NMDA antagonists on the locomotor counts exerted by the mouse in 8-min monitoring periods, 30, 45, and 60 min after the injection of the drugs. N = number of animals; DM = dextromethorphan.

*Significantly different from 30 min according to ANOVA, Newman-Keuls test.

†Significantly different from saline ($p < 0.01$ according to ANOVA, Newman-Keuls test. ‡Significantly different from saline $p < 0.05$ according to ANOVA, Newman-Keuls test).

ing sessions revealed an increased habituation with respect to saline-treated mice (Table 2).

CPP up to the dose of 50 mg/kg did not significantly affect the locomotor/exploratory activity (Table 1).

PCP (1.25–10 mg/kg) elicited a dose-dependent increase/decrease of the locomotor/exploratory activity of mice: animals injected with low-moderate doses of PCP (2.5–5 mg/kg) exhibited a significant ($p < 0.01$) increase in exploration (Table 1, Fig. 1), but the highest dose of PCP (10 mg/kg) caused a significant ($p < 0.05$) decrease in the exploration. An intermediate dose of PCP (5 mg/kg) induced a significant ($p < 0.01$) increase in the basal locomotor activity as well,

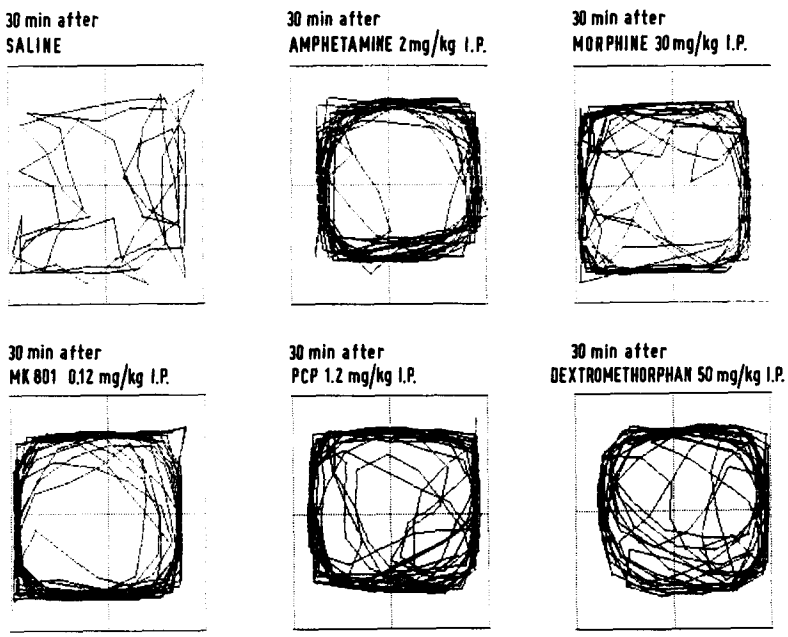

FIG. 1. Locomotor/exploratory pattern elicited by some psychotropic drugs and noncompetitive NMDA antagonists. The tracings show the locomotor pattern induced in mice (30 min after the injection of the drugs) in a 2-min monitoring period in an on-line open field test. Note the peculiar increase in the locomotor activity induced by the drugs.

and the highest dose of 15 mg/kg also reduced basal locomotor activity significantly ($p < 0.01$). Conversely, the low dose of PCP (2.5 mg/kg) selectively increased locomotor/exploratory activity only (Table 1). The time course of exploration during the three 8-min monitoring sessions revealed an increased habituation with respect to saline-treated mice in animals treated with the highest dose of PCP (10 mg/kg) and a decreased habituation in animals treated with the lowest dose of PCP (2.5 mg/kg) (Table 2).

DM (25–50 mg/kg) induced a significant ($p < 0.01$) increase in locomotor/exploratory activity with respect to saline-treated mice, but it did not significantly affect basal locomotor activity (Table 1, Fig. 1). The time course of exploration during the three 8-min monitoring sessions revealed a decreased habituation with respect to saline-treated mice (Table 2). Higher doses of DM (100 mg/kg) induced a significant ($p < 0.01$) decrease ($-45 \pm 10\%$) in the locomotor activity. In 50% of the experiments, however, this effect was accompanied by the appearance of behavioural convulsions, often followed by death.

MK 801 (0.125–0.250 mg/kg) induced a significant ($p < 0.01$) increase in both locomotor/exploratory activity and basal locomotor activity with respect to saline-treated mice (Table 1, Fig. 1). A higher dose of MK 801 (0.5 mg/kg) did not affect exploratory and basal locomotor activity with respect to saline-treated mice, and the highest dose of MK 801 (1 mg/kg) significantly ($p < 0.01$) reduced basal locomotor activity (Table 1).

The excitatory motor-stimulant drugs, morphine (30 mg/kg) and amphetamine (2 mg/kg), tested for comparison, elicited a significant ($p < 0.01$) increase in exploratory ($+70 \pm 15\%$ and $+85 \pm 9\%$, respectively) and basal ($+42 \pm 5\%$ and $+35 \pm 7\%$, respectively) locomotor activity (Fig. 1).

DISCUSSION

In the present study, the influence of various NMDA antagonists on locomotor activity in rodents has been studied. It has been shown that these drugs can strongly affect both locomotor/exploratory activity and basal locomotor activity in an on-line open field test on mice. The present data confirm previous reports in literature (1,3,21) showing that some NMDA antagonists, such as PCP and MK 801, increased exploratory and basal locomotor activity. In addition, the results demonstrate that all NMDA antagonists, with the exception of CPP, reduced the basal and exploratory locomotor activity at higher doses. The inability of CPP to affect locomotor activity is probably due to its poor penetration into the brain. The inhibitory effect of high doses of NMDA antagonists on locomotion can be ascribed to drug-induced marked ataxia and hypotonia, which mask or prevent locomotor or stereotypic activities. In line with this hypothesis, both competitive and noncompetitive NMDA antagonists elicit similar EEG cortical changes that accompany motor-depressive effects in rats (18–20). However, some data in literature indicate that the inhibitory effects of NMDA antagonists on locomotion might depend on a specific influence in some brain areas rather than on a diffuse nonspecific depressive effect within all the brain. In fact, the injection of the competitive NMDA antagonist AP5 into the nucleus accumbens decreased the locomotor activity induced by cocaine, heroin, and dopamine (17). In addition, the other competitive NMDA antagonist CGP 39551 decreased dopamine metabolism in the prefrontal cortex (3).

Some noncompetitive NMDA antagonists were able to strongly affect locomotor/exploratory activity only. The modifications of this activity in mice, like those occurring after administration of psychotropic drugs (6,7,10,14), are thought to depend on the interactive exposure of the animals to the drug and to external environmental stimuli (6,10,14). Exploration, in particular, is a behavioural response or reaction to novelty and environmental changes depending on the integrity of hippocampal spatial learning capacity and memory (2, 8,22). In fact, animals with hippocampal lesions frequently show poor habituation of locomotor/exploratory activity to environmental changes (2,8,22). NMDA antagonists have been shown to be critical for both the induction of a number of forms of hippocampal synaptic plasticity, such as long-term potentiation (5), and for the acquisition of tasks that are sensitive to hippocampal damage (16). Under our experimental conditions, the locomotor/exploratory activity in mice was selectively and dose-dependently increased by some noncompetitive NMDA antagonists such as PCP and DM. At certain doses these drugs selectively increased exploration and decreased habituation without affecting basal locomotor activity. Increased exploration by PCP and DM might depend on a selective functional depression of hippocampal circuitries. In disagreement with this hypothesis is the inability of MK 801 and CGS 19775 to selectively affect exploration in mice. Thus, the decreased habituation caused by PCP and DM is more likely to depend also on non-NMDA activity. As a matter of fact, PCP and DM, in addition to NMDA antagonism, exhibit the ability to affect sodium and/or calcium conductances (4,9). The increased exploration induced by these drugs might be due to a blockage of non-NMDA hippocampal circuitries such as those occurring between CA3 pyramidal cells and granule dentate cells, which have been postulated to be involved in the reaction to novelty (2,8).

On the whole, the present data show that NMDA antagonists strongly affect locomotor activity in mice, inducing both potentiating and inhibitory effects. The inhibitory effects are elicited by competitive and noncompetitive NMDA antagonists, and the excitatory effects are induced only by noncompetitive NMDA antagonists.

The results are nevertheless consistent with a considerable body of research demonstrating that although NMDA antagonists acting at different sites share some pharmacological effects (i.e., anticonvulsant and EEG effects), they show very different behavioural profiles. This may be of considerable importance for the eventual therapeutic use of such compounds.

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