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EFFECTS OF 3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA) ON ANXIETY IN MICE TESTED IN THE LIGHT-DARK BOX

ENRIQUE MALDONADO and JOSE FRANCISCO NAVARRO

Area de Psicobiología, Facultad de Psicología, Universidad de Málaga, Málaga,
Spain

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Abstract

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1. The effects of acute administration of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") on anxiety tested in the light/dark box were examined in albino male mice of the OF.1 strain.
2. Animals were evaluated in the light/dark test 30 min after injection of MDMA (1, 8, and 15 mg/kg, i.p) or saline. The following parameters were recorded (for 5 min): (a) number of exploratory rearings in the light and dark sections; (b) number of transitions between the lit and dark areas; (c) time spent in the light and dark areas; (d) latency of the initial movement from the light to the dark area, and (e) locomotor activity in light area.
3. MDMA (8 and 15 mg/kg) produced a significant reduction in exploratory activity (rearings and transitions), without decreasing motility, in comparison with saline-treated mice. However, time spent in lit/dark compartments was not significantly affected by the drug, which could be a consequence of the anti-exploratory properties of MDMA.
4. Overall, the behavioral profile found in the light/dark test indicates an anxiogenic-like activity of MDMA in mice. It is suggested, however, that animal models of anxiety which emphasize a social interaction could be more sensitive to the effects of this substance.

Keywords: anxiety, "ecstasy", light-dark box test, mice, serotonin

Abbreviations: methylenedioxymethamphetamine (MDMA)

Introduction

3,4-methylenedioxy-methamphetamine (MDMA), a synthetic amphetamine derivative popularly known as "ecstasy", was initially developed in 1914 as an appetite suppressant. Currently it is used as a recreational substance because of its ability to induce a novel state of consciousness comprised of altered mood and enhanced perception of emotions, being readily available as an illicit drug at many clubs and recreational venues (Parrot and Lasky, 1998). However, as the popularity of MDMA has increased, psychiatric alterations have also emerged associated with repeated drug use. The main symptoms described include paranoia, depression, hallucinations, mental confusion and, especially, persistent anxiety and panic attacks (McCann and Ricaurte, 1992; Cohen, 1995; McCann et al, 1996; Cohen and Cocores, 1997). Moreover, MDMA has been consistently shown to produce long-term neurotoxic degeneration of serotonin nerve terminals in several brain regions in laboratory animals (Steele et al, 1994; Green et al, 1995). Its possible neurotoxicity in humans led to the assignment of MDMA as a Schedule I compound by the U.S. Drug Enforcement Agency in 1985. Therefore, the increasing use and abuse of this drug has become a serious public health problem (Grob and Poland, 1997).

Like many drugs of abuse, MDMA acts at several neural targets. It shares close structural similarities to both stimulant amphetamines and hallucinogenic phenethylamines, being also a potent indirect monoaminergic agonist producing both release and reuptake inhibition of serotonin and, to a lesser extent, of dopamine (Steele et al, 1994; Green et al, 1995). This action probably is mediated through 5-HT₂ receptor activation (Koch and Galloway, 1997).

Recently, Navarro and Maldonado (1999) have reported that MDMA could exhibit an anxiogenic-like activity in agonistic encounters between male mice. In this study, it was found that animals acutely treated with MDMA (5-20 mg/kg) showed a behavioral profile characterized by a reduction of aggression, accompanied by a decrease of social investigation and a increment of exploration from a distance, avoidance/flee and defense/submission behaviors. This ethopharmacological pattern seems to indicate the existence of an anxiogenic activity of the drug in mice. Furthermore, Searce-Levie et al (1999) have described a decrease of exploratory behaviors (rearings and nose pokes) in a dose range from 3.3 to 30 mg/kg of

MDMA in knockout mice lacking the 5-HT_{1B} receptor. This diminished exploratory activity has been usually considered as a measure of anxiety (Rodgers, 1997; Takeda et al, 1998).

These preliminary observations suggest that MDMA could display anxiogenic properties in mice. However, to date no study has been carried out to analyze the effects of MDMA using specific animal models of anxiety. Therefore, the aim of this experiment was to examine the action of an acute treatment with MDMA (1, 8 and 15 mg/kg, i.p) on anxiety evaluated by means of the light-dark preference test. This test is based in the fact that rodents have a natural tendency to explore a novel environment which is opposed to the aversive nature of the brightly illuminated area of the light/dark box test (Crawley and Goodwin, 1980).

Methods

Animals

40 albino male mice of the OF.1 strain weighing 25-30 g were used. Animals were housed in groups of five in plastic cages (24x13.5x13 cm) under standardized lighting conditions (white lights on 20:00-8:00), a constant temperature (20°C) and food and tap water available ad libitum, except during behavioral tests. Cage maintenance was undertaken twice weekly, but never on the day of testing. Mice were housed 7 days before the experiment.

This experiment was carried out in accordance with the guiding principles for care and use of Laboratory Animals approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Drug Administration

Four groups of mice were used. Animals were randomly allocated to one control group (N=10) receiving physiological saline and three experimental groups (N=10 each) receiving MDMA injections. MDMA (Sigma Laboratories) was diluted in physiological saline to provide appropriate doses for injections and administered acutely in three doses: 1, 8 and 15 mg/kg. These doses were selected on the basis of the results obtained previously in a pilot study carried out in our laboratory. Drug or saline were injected intraperitoneally in a volume of 10 ml/kg. Tests were

performed 30 min after injections.

Apparatus

The light-dark aversion test was used according to Belzung et al. (1987) procedure. The apparatus consisted of two glass boxes (27x21x24 cm) with an interconnecting grey plastic tunnel (7x10 cm). One of these boxes was painted in black, being weakly lit by a red 25-W bulb (0 lux). The other box was lit by a 60-W desk lamp (400 lux) placed 30 cm above the box, which provided the only laboratory illumination. The floor was lined into 9 cm squares. The apparatus was positioned on a bench 70 cm above the floor.

Experimental Procedure

The test was performed in a quiet, darkened room. After injection (saline or treatment) mice were placed in their home cage. At the beginning of the test, naive mice were placed individually in the middle of the light area facing away from the opening, and were videotaped during 5 min using a Sony V8 camera. The following parameters were recorded: (a) number of exploratory rearings in the light and dark sections; (b) number of transitions between the lit and dark areas; (c) time spent in the light and dark areas; (d) latency of the initial movement from the light to the dark area, and (e) locomotor activity in light area (measured as the number of squares visited for 5 min). A mouse was considered to have entered the new area when all four legs were in this area. None of these animals were used on more than one occasion. The group order was counterbalanced according to a Latin square design. At the conclusion of the test period, mice were returned to their cages, and another animal was placed into the box. The floor of each box was cleaned between sessions. Tests were carried out between 10 a.m and 4 p.m. Behavioral analysis was performed by a trained experimenter who was unaware of treatment of the groups.

Data Analysis

Nonparametric Kruskal-Wallis tests were used to assess the variance of the behavioral measures over different treatment groups. Subsequently, appropriate paired comparisons were performed using Mann-Whitney *U*-tests to contrast the parameters in the different treatment groups. The analysis was performed using nonparametric statistics since the criteria for parametric statistics were not met by the data. Differences were considered significant if the probability of error was less

than 5%.

Results

Table 1 illustrates medians (with ranges) of the parameters used. Kruskal-Wallis analysis showed that there was significant variance in the parameters of latency ($p < 0.01$) and rearings in light and dark compartments ($p < 0.05$).

Paired comparisons revealed that MDMA (15 mg/kg) significantly increased the latency of the initial movement from the light to the dark area, in comparison with the control group ($p < 0.05$). Likewise, transitions between the lit and dark compartments and number total of rearings were significantly reduced in mice treated with MDMA (8 and 15 mg/kg), as compared with the saline group ($p < 0.05$). Time spent in lit and dark compartments as well as locomotor activity were not significantly affected by the drug.

Discussion

As Table 1 shows, MDMA produced a significant decrease in exploratory activity, in concordance with an anxiogenic-like profile of the drug. Thus, the number of rearings was markedly reduced in mice treated with MDMA (8 and 15 mg/kg), in comparison with saline-treated mice. Likewise, transitions between compartments were also diminished after MDMA administration (8 mg/kg). Similar results have been recently described by Searce-Levie et al (1999), who also found a decrease of exploratory activity in mice treated with MDMA, virtually eliminating rearings and nose pokes behaviors in an open field test. This effect appears to be completely independent of the 5-HT_{1B} receptor.

The latency time for the first passage from the light compartment to the dark one is a controversial parameter which is not commonly discussed in studies on experimental anxiety. An increased latency could be the result of an anxiolytic-like action of the drug (like that occurs with benzodiazepines; Bourin et al, 1992). In the present study, a significant increase of latency (without decreasing motility) was observed in mice treated with the highest dose of MDMA (15 mg/kg), apparently

Table 1

Effects of MDMA on Behavioral Parameters in the Light/dark Test in Mice (Median Values with Ranges)

Parameters	Doses of MDMA (mg/kg)			
	Saline	1	8	15
Latency (in sec) **	8 (2-25)	10 (3-22)	16 (4-106)	51# (1-173)
Number of transitions	10 (1-32)	9 (1-31)	1# (1-19)	6 ## (1-21)
Rearings (lit area) *	0 (0-22)	0 (0-24)	0# (0-1)	0# (0-9)
Rearings (dark area) *	4 (0-14)	4.5 (0-26)	0# (0-8)	0# (0-3)
Total number of rearings *	6 (0-33)	6.5 (0-33)	0# (0-10)	0# (0-9)
Time in lit area (in sec)	49 (4-133)	33.5 (7-138)	23 (5-163)	69 (30-193)
Time in dark area (in sec)	251 (167-296)	266.5 (162-293)	277 (137-295)	231 (107-270)
Motor activity (number of squares visited in lit area)	13 (3-35)	14.5 (5-44)	11 (2-55)	21 (6-56)

Kruskal-Wallis test showed significant variance, * $p < 0.05$, ** $p < 0.01$

Differs from controls on Mann-Whitney *U*-test, # $p < 0.05$, ## $p < 0.1$

indicating the existence of an anxiolytic activity. However, for interpreting this effect of the drug another explanation may be suggested. In our experiment, the increased latency could be a reflect of the reduction of exploratory activity. In fact, rats treated with MDMA (30 mg/kg) and exposed to a novel ambient (open field) show an evident avoidance to the center of the test. Moreover, it has been demonstrated that MDMA initially reduces locomotor activity when animals are positioned in the open field, in comparison with saline-treated animals which actively explore the new ambient (Gold et al, 1988).

In a two compartment box, rodents will prefer the dark area. Drugs with anxiogenic properties should increase the time spent in the dark compartment whereas anxiolytics should increase the time spent in the lit compartment (Hascoët and Bourin, 1998; Timothy et al, 1999). In our study, no significant differences between experimental and control groups were found in this parameter. It might be also related to the anti-exploratory effect of MDMA. On the other hand, these findings are partially in concordance with those described with d-amphetamine in the light-dark test by Merlo and Samanin (1989) and Young and Johnson (1991), who found that the time spent in both compartments was not significantly modified by the drug. Nevertheless, other authors have reported contradictory results with the same compound (Onaivi and Martin, 1989; Häscoet and Bouring, 1998), being therefore the evidence available in this respect clearly controversial.

In sum, although the decrease of the exploratory behaviors observed in the light/dark test indicates that MDMA exhibits an anxiogenic-like activity, the results obtained with other parameters (e.g., time spent in both areas) might not support above conclusion. Considering the different results obtained with various parameters, it would seem reasonable to suggest that some tests may be more appropriate than other for the detection of anxiogenic-like properties of a given drug (Rodgers, 1997). In this sense, animal models of anxiety which stress a social interaction could be more sensitive to the effects of MDMA.

Conclusion

MDMA administration (8 and 15 mg/kg) produced a reduction of exploratory behaviors (especially rearings) in the light/dark test without depressing motility. This behavioral profile could be an indicator of an anxiogenic-like activity of the drug in mice. However, the time spent in the lit area (a classical parameter to assess changes on anxiety in this test) was not significantly affected, probably as a consequence of the marked anti-exploratory action of MDMA. It is suggested that animal models of anxiety which emphasize a social interaction could be more sensitive to the effects of this substance.

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Inquiries and reprint requests should be addressed to:

Dr. José Francisco Navarro
Area de Psicobiología. Facultad de Psicología.
Campus de Teatinos, Universidad de Málaga, 29071 Málaga, Spain.
E-mail: navahuma@uma.es