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Progress in Neuro-Psychopharmacology & Biological Psychiatry 26 (2002) 1151–1154

Progress In Neuro-Psychopharmacology & Biological Psychiatry

Acute and subchronic effects of MDMA (“ecstasy”) on anxiety in male mice tested in the elevated plus-maze

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Accepted 1 May 2002

Abstract

3,4-Methylenedioxymethamphetamine (MDMA) is a compound structurally similar to methamphetamine, which has become one of the most widely used illicit substances. Animal studies investigating acute effects of MDMA on anxiety are unclear since, although an anxiogenic-like action of MDMA in different animal models of anxiety has been mainly described, there is also evidence supporting an anxiolytic-like effect for this drug. An attempt was made to clarify the possible anxiogenic-like profile of MDMA (1, 8 and 15 mg/kg ip) by analyzing its effect on behavior of male mice in the elevated plus-maze test. Moreover, the possible development of tolerance to the effects of MDMA on anxiety after its subchronic administration for 5 consecutive days was examined. The parameters evaluated included: (1) total time in open arms, (2) total time in closed arms, (3) total time in central area, (4) number of open arm entries, (5) number of closed arm entries and (6) number of central area entries. Acute treatment with MDMA (8 mg/kg) significantly reduced the time spent in the open arms, as well as markedly increasing the number of entries in the closed arms and in the central area, as compared with the control group, suggesting that MDMA, at this dose, has an anxiogenic-like activity. Mice subchronically treated with the drug (1 and 8 mg/kg) displayed a notable reduction in the time spent in the open arms, accompanied by an increase in the time spent in the closed arms and in the central platform. These results indicate that the anxiogenic-like effect found after acute treatment is not only maintained but also more marked after subchronic treatment. In contrast, mice treated subchronically with the highest dose of MDMA (15 mg/kg) exhibited a significant increase in the time spent in the open arms as well as a marked reduction in the time spent in the closed arms, supporting an anxiolytic-like activity of the drug. A possible dual pharmacological property of MDMA on anxiety is suggested.

Keywords: Anxiety; Elevated plus-maze test; 3,4-Methylenedioxymethamphetamine; Mice

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), a synthetic amphetamine derivative popularly known as “ecstasy,” acts as an indirect monoaminergic agonist, stimulating the release and inhibiting the reuptake of serotonin and, to a lesser extent, other neurotransmitters (Steele et al., 1994; Koch and Galloway, 1997). Pharmacological studies indicate that MDMA produces a mixture of central stimulant and psychedelic actions. Their psychological effects largely depend on carrier-mediated 5-HT release, while the mild hallucinogen-like perceptual effects of this drug appear due to serotonergic 5-HT2 receptor stimulation (Liechti and Vollenweider, 2001).

Currently, MDMA 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. It is readily available as an illicit drug at many clubs and recreational venues (Parrot and Lasky, 1998). Recent studies suggest that heavy MDMA users can suffer from a number of psychiatric sequelae, including anxiety (Soar et al., 2001). In fact, numerous authors suggest, on the basis of their studies on recreational users of MDMA, that users demonstrate higher levels of anxiety (Gamma et al., 2000; Parrot et al., 2000). Moreover, several case studies report anxiogenic and panicogenic actions of the drug in human users (McCann and Ricaurte, 1992; Solowij et al., 1992; Cohen and Cocores, 1997; Windhaber et al., 1998). In these studies, however, it was not possible to control the purity of the MDMA consumed. It is therefore likely that

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine.

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0278-5846/02/$ – see front matter © 2002 Published by Elsevier Science Inc.
PII: S0278-5846(02)00250-6

preclinical studies will be useful to shed light on the effects of this compound on anxiety.

Results from animal studies investigating acute effects of MDMA on anxiety are unclear. Recent experimental findings indicate that acute treatment with this drug may provoke both anxiogenic and anxiolytic effects depending upon the test situation (Morley and McGregor, 2000) or the dose range employed (Lin et al., 1999). Although an anxiogenic-like action of MDMA in different animal models of anxiety has been described (Bhattacharya et al., 1998; Lin et al., 1999; Navarro and Maldonado, 1999, in press; Maldonado and Navarro, 2000, 2001; Morley and McGregor, 2000), there is also evidence supporting an anxiolytic-like effect of this drug (Winslow and Insel, 1990; Lin et al., 1999; Morley and McGregor, 2000).

In this study, an attempt was made to clarify the possible anxiogenic-like profile of MDMA (1, 8 and 15 mg/kg ip) by examining its effects on behavior of male mice in the elevated plus-maze. Likewise, the possible development of tolerance to the effects of MDMA on anxiety after its subchronic administration for 5 consecutive days was analyzed. The elevated plus-maze test is routinely used to study anxiety-related behavior in mouse (Lister, 1987; Rodgers et al., 1999). In this situation, mice will show a pattern of behavior characterized by open-arm avoidance. This tendency is suppressed by anxiolytics and potentiated by anxiogenic agents (Belzung and Griebel, 2001).

2. Materials and methods

2.1. Animals

Seventy-three albino male OF.1 strain mice weighing 25–30 g were used. Animals were housed in groups of five in plastic cages (24×13.5×13 cm) under standardized lighting conditions (white lights on 20:00–08:00 h), 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 10 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).

2.2. Drug administration

Seven groups of mice were used. Animals were randomly allocated to one control group (n=13) receiving physiological saline and six 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 and subchronically (for 5 consecutive days) at one of three doses: 1, 8 and 15 mg/kg. These doses were selected on the basis of the results obtained previously in several studies carried out in our laboratory. Drug or saline was injected intraperitoneally in a volume of 10 ml/kg. Tests were performed 30 min after injections.

2.3. Apparatus and behavioral test

The plus-maze apparatus consists of two open arms (30×5 cm, surrounded by a 0.25-cm high border) and two closed arms (30×5 cm, surrounded by 15-cm high walls), with the two pairs of identical platform, which emerged from a central platform (5×5 cm), positioned opposite each other (Lister, 1987; Manzaneque et al., 2002). The apparatus was elevated 40 cm above the floor.

Mice were tested on the maze in randomized order. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Each session lasted 5 min, being recorded by a videocamera. All tests were carried out under dim red lighting between the second and seventh hour of the dark phase. After each test, the maze was thoroughly cleaned. Behavioral analysis was performed by a trained experimenter who was unaware of treatment of the groups.

A number of classical parameters were collected during the session, including: (1) open arm duration: the total amount of time the mouse spent in the open arms; (2) closed arm duration: the total amount of time the mouse spent in the closed arms; (3) central platform duration: the total amount of time the mouse spent in the central platform; (4) open arm frequency: the frequency of mouse entry with all four paws into the open, unprotected arms; (5) closed arm frequency: the frequency of mouse entry with all four paws into the closed, protected arms; and (6) central platform frequency: the frequency of mouse entry with all four paws into the central platform.

2.4. Data analyses

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 analyses were carried out using nonparametric statistics since the criteria for parametric statistics were not met by the data. A value of $P<.05$ was considered to be statistically significant.

3. Results

Table 1 illustrates medians (with ranges) of duration and number of entries in the all different areas of the maze. Kruskal–Wallis analysis showed that there were significant ($P<.05$) differences in the measures of duration (time spent in the open arms, closed arms and central area) and

Table 1
Median values with ranges of behavioral measures in the elevated plus maze after acute and subchronic MDMA treatment

Treatment	Dose	Duration (s)			Frequency (number of entries)		
		Open^{+}	Closed^{+}	Center^{+}	Open	Closed^{+}	Center^{+}
	Vehicle	119.84 (19.8–226)	82.44 (0.8–129)	95.78 (57–159)	6 (1–12)	9 (1–19)	17 (6–27)
Acute	1 mg/kg	99.13 (20.6–120)	71.6 (8.5–120)	132.17**–(96.7–255)	5 (1–10)	9 (2–22)	15 (6–26)
	8 mg/kg	76.61** (13.8–186)	96.03 (53–231)	119.97 (53–231)	6 (1–18)	20** (9–31)	26** (19–39)
	15 mg/kg	108.24 (1.9–299)	79.71 (0–154)	119.65 (0.8–195)	11 (1–17)	23 (0–37)	31 (1–40)
Subchronic	1 mg/kg	37.91**,$^{\#}$ (10–107)	101.66 (54.6–143)	143.57** (121–211)	5 (1–9)	15 (6–19)	19 (7–27)
	8 mg/kg	11.51$^{\#}$,* (0–129)	134.22** (38.3–201)	127.74** (91.5–209)	2 (0–15)	18 (13–37)	27 (17–40)
	15 mg/kg	190.2**,$^{\#}$ (0–299)	27.42** (0–171)	77.13 (0.5–129)	11 (0–37)	8$^{\#}$ (0–34)	27 (1–55)

* Mann–Whitney *U* test, $P<.01$, as compared to control group.
** Mann–Whitney *U* test, $P<.05$, as compared to control group.
$^{\#}$ Mann–Whitney *U* test, $P<.05$, as compared to acute treatment.
$^{+}$ Kruskal–Wallis test, $P<.05$.

frequency (number of entries in the closed arms and in the central area).

Paired comparisons using Mann–Whitney *U* tests revealed that mice acutely treated with MDMA showed a significant increase in the time spent in the central area (1 mg/kg, $P<.05$), as well as a decrease in the time spent in the open arms (8 mg/kg, $P<.05$), in comparison with the control group. Likewise, the intermediate dose of the drug produced a significant increase in the number of entries in the central area and in the closed arms, as compared with the control group ($P<.05$).

Mice treated subchronically with MDMA exhibited a significant reduction in the time spent in open arms (1 and 8 mg/kg, $P<.05$ and $P<.01$, respectively), whereas this measure was significantly increased with the highest dose used, as compared with the control group ($P<.05$). Moreover, MDMA provoked a significant increase in the time spent in the closed arms (8 mg/kg, $P<.05$) and in the central area (1 and 8 mg/kg, $P<.05$). Time spent in closed arms was significantly reduced in mice treated with 15 mg/kg of MDMA, as compared with the control group ($P<.05$).

Finally, there were significant differences in the time spent in the open arms (all doses) as well in the number of entries in the closed arms (15 mg/kg), when acutely and subchronically MDMA-treated groups were compared ($P<.05$).

4. Discussion

The elevated plus-maze test is based on the natural aversion of rodents to heights and open spaces. Thus, subjects prefer to spend time in the closed arms rather than in the open arms of a maze. Lister (1987) has validated the model in mice with different classes of substances and demonstrated that it is effective for examining anxiogenic-like and anxiolytic-like effects of drugs.

Acute treatment with MDMA (8 mg/kg) significantly decreased the time spent in the open arms, as well as markedly increasing in the number of entries in the closed arms and in the central area, as compared with the control group. These results suggest that MDMA, at this dose, exhibits an anxiogenic-like activity, and they extend previous findings described with other animal models of anxiety. It has been reported that acute administration of MDMA elicits anxiogenic-like behaviors in social encounters between mice (Maldonado and Navarro, 2001; Navarro and Maldonado, 1999, in press) and in the light–dark box (Maldonado and Navarro, 2000). In the elevated plus maze, MDMA also shows an anxiogenic-like profile in both rats (Bhattacharya et al., 1998; Morley and McGregor, 2000) and, at low doses, in mice (Lin et al., 1999). There are, however, conflicting reports in the social interaction test since the drug has been described as having both anxiogenic-like (Bhattacharya et al., 1998; Maldonado et al., 2000) and anxiolytic-like effects (Morley and McGregor, 2000). The discrepancy found among the different studies might be related, at least in part, to the fact that MDMA has a very different interaction with monoaminergic systems in the mouse CNS compared to most other species, including rats and primates (Stone et al., 1987; Mechan et al., 2002).

An important objective of this study was to examine the possible development of tolerance to the effects of MDMA on anxiety after its subchronic administration for 5 consecutive days. Mice subchronically treated with the drug (1 and 8 mg/kg) spent significantly less time in the open arms, accompanied by an increase in the time spent in the closed arms and in the central area, compared to the control group. These results suggest an anxiogenic-like response to the prolonged administration of MDMA. They further show that the anxiogenic-like effect found after acute treatment is not only maintained but more marked after subchronic treatment. In fact, this anxiogenic-like behavior is observed even with the lowest dose (1 mg/kg) of MDMA used. An absence of tolerance to the anxiogenic-like effects has been also described in agonistic encounters between male mice after subchronic administration of MDMA (2.5 and 5 mg/kg) (Navarro and Maldonado, in press).

In contrast, mice treated subchronically with the highest dose of MDMA (15 mg/kg) exhibited significantly more time spent in the open arms as well as a notable decrease in the time spent in the closed arms, in accord with an

anxiolytic-like activity of the drug (which was not observed after acute treatment). This possible dual pharmacological property of MDMA has been suggested by Lin et al. (1999), who found that single administration of this compound had anxiogenic-like effects at lower doses (4 mg/kg) and anxiolytic-like at higher doses (20 mg/kg) in mice tested in the elevated plus-maze. The precise mechanism by which MDMA could provoke this biphasic action on anxiety remains unknown. MDMA is an active releaser of serotonin, dopamine and noradrenaline, and all these transmitter systems are related to different behavioral effects of the drug. It is reasonable, therefore, to question whether these three neurotransmitters play a role or interact with each other in mediating anxiogenic-like and anxiolytic-like effects of MDMA. Further studies are needed to clarify the neurobiological mechanisms involved in this biphasic action on anxiety.

5. Conclusion

Acute administration of MDMA (8 mg/kg) produced an anxiogenic-like response in male mice tested in the elevated plus maze, in concordance with other studies using different animal models of anxiety. No tolerance to this effect of MDMA is developed after its subchronic administration. In fact, this anxiogenic-like action was even more marked after subchronic treatment with the drug (1 and 8 mg/kg). When the dose of MDMA was increased to 15 mg/kg, a behavioral profile suggestive of an anxiolytic-like activity was found. This effect was only observed when the drug was injected subchronically for 5 consecutive days. It is suggested that MDMA may exhibit dual pharmacological properties on anxiety.

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