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BEHAVIORAL PROFILE OF 3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA) IN AGONISTIC ENCOUNTERS BETWEEN MALE MICE

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Abstract

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1. The effects of acute administration of 3,4-methylenedioxymethamphetamine (MDMA), a synthetic amphetamine derivative (0.5-20 mg/kg, i.p) on agonistic behavior elicited by isolation in male mice were examined.

2. Individually housed mice were exposed to anosmic "standard opponents" 30 min after MDMA injection, and the encounters were videotaped and evaluated using an ethologically based analysis.

3. MDMA (5-20 mg/kg) exhibited a behavioral profile characterized by a reduction of aggression (threat and attack) without a concomitant increase of immobility, accompanied by a decrease of social investigation and a increment of exploration from a distance, avoidance/flee and defense/submission behaviors.

4. This ethopharmacological profile might suggest an anxiogenic-like activity of MDMA in albino male mice.

Keywords: aggression, agonistic encounters, anxiogenic activity, mice, serotonin

Abbreviations: methylenedioxymethamphetamine (MDMA)

Introduction

3,4-methylenedioxymethamphetamine (MDMA), popularly known as "ecstasy", is a synthetic amphetamine derivative developed in 1914 as an appetite suppressant. This compound structurally similar to methamphetamine has become one of the most widely used illicit substances for its psychotropic effects. Likewise, affective and psychotic disturbances are being increasingly recognized in association with MDMA abuse (Cohen, 1995; Cohen and Cocores, 1997; McCann et al, 1996).

Pharmacological studies indicate that MDMA produces a mixture of central stimulant and psychedelic actions, many of which appear to be mediated by brain monoamines, especially serotonin and dopamine (Steele et al,1994). In fact, MDMA is a potent serotonin releaser. Moreover, it also facilitates dopamine release, being this action probably mediated through 5-HT2 receptor activation (Koch and Galloway, 1997).

Amphetamines generally disrupt social and aggressive behaviours in various animal species (Miczek, 1987). Although the effects of amphetamines on aggression are well known, the ethopharmacological profile of MDMA has not been investigated. Therefore, in the present study the authors examined the effects of a wide range of doses of MDMA (0.5-20 mg/kg, i.p) on agonistic behavior in male mice, using an animal model of isolation-induced aggression. The term "agonistic behaviour" comprises all elements of behavior present in situations of conflict, including attack, defense and flight. Although this model mainly represents offensive aspects of agonistic behaviors, defensive aspects are also present, which renders the model useful to measure more subtle activity of drugs as well (Krsiak, 1979).

Methods

Animals

192 albino male mice of the OF.1 strain weighing 25-30 g were used. Animals arrived in the laboratory at 42 days of age and were housed under standardised lighting conditions (white lights on: 20:00-8:00), a constant temperature (20±2°C)

and food and tap water available ad libitum, except during behavioral trials. Upon arrival in the laboratory, the subjects were allocated to two different categories. Half of the animals were individually housed in plastic cages (24 x 13.5 x 13 cm) as experimental animals. The remainder (n= 96) were housed in groups of five to be used as "standard opponents" and were temporally rendered anosmic by intranasal lavage with 4% zinc sulphate solution (Sigma Laboratories) on both 1 and 3 days before testing. Fighting in mice, as in most rodents, is closely related to olfaction. This type of opponent was employed because it elicits attack but never initiates such behavior (Brain et al, 1981). Such animals rarely direct spontaneous attacks toward the test animal. Therefore, fighting is always unidirectional, being easily quantified.

All the experimental animals underwent an isolation period of 30 days before the behavioral test (isolation-induced aggression model). Social isolation is one method of increasing the level of aggressiveness in different species of animals. This phenomenon is particularly well demonstrated in laboratory mice.

Drug Administration

Eight groups of mice were used. Individually housed animals were randomly allocated to one control group (N=12) receiving physiological saline and seven experimental groups (N=12 each) receiving acute MDMA injections. MDMA (Sigma Laboratories) was diluted in physiological saline to provide appropriate doses for injections and administered acutelly in seven doses: 0.5, 1.25, 2.5, 5, 10, 15 and 20 mg/kg. Drug or saline were injected intraperitoneally in a volume of 10 ml/kg. Aggression tests were performed 30 min after injections.

Experimental Procedure

After injections, an isolated animal and a "standard opponent" (marked with fur dye for identification) were confronted each other in a neutral arena for 10 min. This neutral cage consisted of an all-glass area, measuring 50 x 26 x 30 cm, with a fresh sawdust substrate. While separated by means of a plastic barrier, the animals were allowed 1 min of adaptation to the neutral cage before the encounter. The social encounters were videotaped using a Sony-V8 camera. All tests were carried out under white lighting between the second and sixth hours of the dark phase of the artificial cycle of the animals. After each encounter the neutral cage was washed out and the sawdust bedding replaced.

The tapes were analysed using a microprocessor and a custom-developed programme (Brain et al, 1989) which facilitated estimation of time allocated to ten broad behavioral categories. Each category included a collection of different behavioral postures and elements. The names of categories and their constituent elements are as follows: 1) Body care (abbreviated groom, self-groom, wash, shake, scratch); 2) Digging (dig, kick dig, push dig); 3) Non-social exploration (explore, rear, supported rear, scan); 4) Explore from a distance (approach, attend, circle, head orient, stretched attention); 5) Social investigation (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around); 6) Threat (aggressive groom, sideways offensive, upright offensive, tail rattle); 7) Attack (charge, lunge, attack, chase); 8) Avoidance/flee (evade, flinch, retreat, ricochet; wheel, startle, jump, leave, wall clutch); 9) Defense/submission (upright defensive, upright submissive, sideways defensive), and 10) Immobility (squat, cringe).

A detailed description of all elements can be found in Brain et al. (1989). This ethoexperimental procedure allows a complete quantification of the behavioral elements shown by the subject during the social encounters. Only the behavior of the isolated animals was assessed. This analysis was performed by a trained experimenter who was unaware of treatment of the groups.

Data Analysis

The medians for times allocaed to each broad behavioral category were determined. Nonparametrics 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 behavior in the different treatment groups. The analysis were performed using nonparametric statistics since the criteria for parametric statistics were not met by the data.

Results

Table 1 illustrates medians (with ranges) of accumulated times allocated to the broad categories of behavior described above. Kruskal-Wallis analysis showed that there was significant variance in the categories of threat, attack and social

investigation ($p<0.01$), body care, nonsocial exploration, avoidance/flee and defense/submission ($p<0.05$), digging and exploration from a distance ($p<0.02$).

Paired comparisons revelead that MDMA significantly reduced the time spent in threat and attack (5-20 mg/kg) behaviors, in comparison with the saline group ($p<0.002$). Exploration from a distance (2.5-20 mg/kg; $p<0.002$) and non social exploration (5-20 mg/kg; $p<0.02$) were significantly increased, whereas social investigation (5-20 mg/kg), digging (1.25-20 mg/kg) and body care (2.5 and 20 mg/kg) behaviors were decreased after acute treatment with the drug ($p<0.002$). Moreover, avoidance/flee and defense/submission behaviors were also augmented in mice treated with MDMA (5-20 mg/kg) ($p<0.05$). The mean values for the behavioral category of immobility were zero for all groups.

Discussion

Serotonergic drugs with different mechanisms of action are all capable of affecting aggressive behaviors in isolated male mice (Olivier and Mos, 1992; Cologer-Clifford et al, 1997). Table I shows, MDMA (5-20 mg/kg) significantly reduced offensive behaviors (threat and attack) in isolated male mice. This action was apparently selective since it was not accompanied by an increase of immobility.

These findings are in concordance with those described by Miczek and Hanley (1994), who had also examined the effects of MDMA (0.1-10 mg/kg) on aggressive behavior. In this work, MDMA potently decreased attack (3, 6 and 10 mg/kg) in adult male CFW mice using a resident-intruser animal model. However, no information was given in relation to the effects of the drug on defensive, social and motor behaviors.

In our study, MDMA (5-20 mg/kg) produced a reduction of aggression without a concomitant increase of immobility. However, other behavioral categories were markedly affected, suggesting that antiaggressive action of MDMA was clearly unselective. Thus, avoidance/flee and defense/submission behaviors were weak, but consistenly, enhanced after treatment with the drug. Likewise, the composite of reduced social investigation/increased exploration from a distance is consistent with an increase of anxiety. Furthermore, the enhancement of non-social

Table 1

Median Values (with ranges) for Times (in seconds) Allocated to Broad Behavioral Categories in Animals Receiving Acute Treatment with MDMA

Behavioural categories	Doses of MDMA (mg/kg)							
	Saline	0.5	1.25	2.5	5	10	15	20
Body care*	5.04 (0.2-16)	6 (0-21)	0.85 (0-10.5)	0.83### (0-4.2)	4.5 (0-10.9)	1.2 (0-26.3)	3.5 (0-18.3)	0### (0-1.75)
Digging**	10.54 (0.6-32)	6.32 (0-29)	1.68### (0.2-8)	0.85### (0-4.4)	0### (0-0.2)	0### (0-3.3)	0### (0-1.9)	0### (0-0)
Non social exploration*	378.7 (328-463)	405.8 (255-517)	369.3 (250-469)	401.9 (257-505)	454.9## (334-500)	451.1## (332-511)	499.8## (428-578)	477.8## (376-505)
Explore from a distance**	50.46 (32-97)	53.4 (20-99)	67.2 (45-112)	70.2## (54-141)	109.2### (66-213)	116.7### (48-183)	87.04### (55-212)	107.7### (0.3-198)
Social*** investigation	35.4 (1.2-153)	37.4 (9.5-203)	52.4 (5.8-277)	38.1 (0-187)	18## (0.7-26)	9.44### (0-56)	1.45### (0-22)	0.93### (0-128)
Threat***	76.9 (21-136)	52.9 (0-188)	68.09 (0-200)	57.77 (0-108)	0### (0-31)	0### (0-125)	0### (0-82)	0### (0-0.4)
Attack***	9.16 (0-40)	2.4 (0-43)	2.55 (0-88)	2.49 (0-24)	0### (0-4)	0### (0-6)	0### (0-0.6)	0### (0-0)
Avoidance/Flee*	0 (0-1.05)	0 (0-1.35)	0 (0-0.4)	0 (0-4.72)	0.48# (0-6.2)	0.25# (0-6.19)	0.953# (0-8.88)	0.63# (0-11.52)
Defense/ Submission*	0 (0-0)	0 (0-0.82)	0 (0-0.55)	0 (0-4.13)	0# (0-8.47)	0.55# (0-19.8)	0.72# (0-32)	3.46## (0-25.4)

Kruskal-Wallis test showed significant variance, *p<0.05; **p<0.02; ***p<0.01
Differs from controls on Mann-Whitney *U*-test, #p<0.05; ##p<0.02; ###p<0.002

exploration may reflect attempts to escape from the test arena. Overall, this ethological pattern might suggest the existence of a anxiogenic-like activity of this compound in mice, especially from 5 mg/kg. In fact, such elements (social investigation, exploration, avoidance/flee and defense/submission) have been commonly used to assess the anxiety-changing properties of drugs (Brain et al., 1991; Rodgers, 1997). This behavioral profile is similar to that described with d-amphetamine. This substance is considered predominantly antiaggressive enhancing flight behaviour and decreasing offensive behaviors (Moro et al., 1997).

Conclusion

MDMA (5-20 mg/kg) administration produced a marked reduction of offensive behaviors (threat and attack) with no affectation of immobilty. However, this compound also provoked a decrease of social investigation accompanied by an increase of exploration from a distance, avoidance/flee and defense/submission behaviors. This ethopharmacological profile suggests an anxiogenic-like activity of MDMA in male mice.

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