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Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy) : an experimental study

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Abstract. Methylenedioxymethamphetamine (MDMA), commonly known as Ecstasy, is widely abused as a recreational agent. Reports of death following MDMA intake has aroused serious concern. Although some of the clinical symptoms of MDMA toxicity include severe anxiety, panic, excitation and agitation, behavioural studies on the drug are sparse and incomplete. The present study investigated the anxiogenic activity of MDMA in rats, using yohimbine (2 mg/kg, i.p.) as the standard anxiogenic agent for comparison. The experimental methods used were the open-field, elevated plus-maze, social interaction and novelty-suppressed feeding latency tests, all the tests being experimentally validated as rodent models of clinical anxiety. In addition, the effect of MDMA was assessed on rat brain tribulin activity in terms of endogenous monoamine oxidase (MAO) A and B inhibition. Tribulin has been postulated to function as an endogenous marker of anxiety. MDMA (5 and 10 mg/kg, i.p.) reduced ambulation and rears, and increased immobility and defaecation, in the open-field test. These doses of MDMA produced a dose-related decrease in the number of entries and time spent on the open arms of the elevated plus maze, reduced social interaction in paired rats and increased the feeding latency time in an unfamiliar environment in food deprived rats. A qualitatively similar response was induced by yohimbine in all these test parameters. Both MDMA (5 and 10 mg/kg, i.p.) and yohimbine (2 mg/kg, i.p.) increased rat brain tribulin activity, the increase in the MAO A inhibitor component being more than that on the MAO B inhibitor component. Several anxiogenic agents induce a similar response, as does anxiety associated with addictive drug

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withdrawal. The anxiogenic effects of MDMA and yohimbine on the elevated plus maze were inhibited by the benzodiazepine anxiolytic lorazepam (0.25 mg/kg, i.p.). However, the 5-HT_{1A} receptor agonist-antagonist, buspirone (2.5 mg/kg, i.p.), selectively inhibited the anxiogenic effect of MDMA. Earlier studies have indicated that MDMA has significant effect on rat brain 5-HT_{1A} receptors and induces an increase in serotonergic activity in this species. The results of the present study indicate that MDMA induces significant anxiogenic response in rats which may involve the serotonergic neurotransmitter system.

Key words : methylenedioxymethamphetamine; Ecstasy; anxiety; tribulin, yohimbine; lorazepam; buspirone

INTRODUCTION

Methylenedioxymethamphetamine (MDMA) is a widely used recreational drug of abuse. This illegal designer drug, related to amphetamine, is also known as 'ecstasy', 'XTC', 'E' and 'love drug' in abuser circles (Duxbury, 1993). MDMA was patented as an appetite suppressant and investigated as a mood-modifying agent as early as 1914 (Duxbury, 1993). By the 1970s the drug appeared in the illicit drug market as a safe and non-toxic means to produce 'warm loving relaxation' (Duxbury, 1993). As its abuse increased, making it the most popular recreational drug after cannabis, LSD and amphetamines, it became evident that MDMA was not the ideal safe non-toxic recreational agent, as was claimed earlier and concerns have been raised about MDMA's addictive potential and possible neurotoxicity (Steele, *et al.*, 1994). The drug was found to induce severe psychological effects, including marked mood swings, mental confusion, anxiety, panic attacks, excitation and agitation, accompanied by raised blood pressure, shivering, hyperreflexia, rigidity, loss of appetite, dehydration and severe thirst (Bodner *et al.*, 1995). What was more alarming was the incidence of MDMA-related deaths (Randall, 1992a,b) which is reported to exceed 50 in Britain alone since 1990. Although MDMA abuse is on the increase there is little information on its behavioural effects in experimental situations. The clinical profile of behavioural

activity induced by MDMA is unlike those induced by hallucinogens or psychomotor stimulants, including amphetamine. The discriminative stimulus properties of MDMA in rats supports the hypothesis that the primary behavioural activity of MDMA is unlike that of hallucinogens, like LSD, or stimulants, like amphetamine, and represents a new class of psychoactive agents, the enactogens (Oberlender and Nichols, 1990). However, using conditioned locomotor paradigms, drug discrimination procedures and human subjective questionnaires, it has been concluded that behavioural effects of MDMA resemble those of classical psychostimulants such as amphetamine and cocaine (Gold and Koob, 1989). Since the clinical features of MDMA abuse toxicity suggest that the drug induces marked anxiety, we investigated the anxiety-inducing potential of MDMA on experimentally validated rat models of clinical anxiety. Yohimbine, which is known to induce anxiety in animals (Lal *et al.*, 1983; Johnston, *et al.*, 1988) and man (Charney, *et al.*, 1983), was used as the standard anxiogenic agent for comparison. Lorazepam, a well known benzodiazepine (BDZ) anxiolytic, was utilized to validate the anxiogenic action of MDMA on the elevated plus maze.

Accumulated evidence has shown a central role for the neurotransmitter serotonin (5-HT) in mediating the behavioural and discriminative stimulus properties of MDMA. In drug discrimination studies, MDMA generalises to serotonergically active agents like fenfluramine and 1-(3-trifluoromethylphenyl) piperazine (TFMPP) (Schechter, 1988; 1991). In startle reflex tests, the excitatory effects of MDMA are prevented by 5-HT uptake inhibitors and by depletion of central 5-HT with 5,7-dihydroxytryptamine, a serotonergic neurotoxin (Kehne *et al.*, 1992). Similarly, MDMA-induced hyperactivity is antagonised by 5-HT reuptake inhibitors and by prior depletion of 5-HT with p-chlorophenylalanine (Callaway, *et al.*, 1990). Furthermore, a range of MDMA effects, such as those on drug discrimination (Schechter, 1988; 1991), operant responding (Rosecrans and Glennon,

1987), conditioned place preference (Bilsky and Reid, 1991), locomotor activity (Gold and Koob, 1988) and analgesia (Crisp *et al.*, 1989) are modified by various serotonergic antagonists.

There is evidence that presynaptic serotonergic, but not dopaminergic, mechanisms are critical in the enactogen-like discriminative stimulus properties of MDMA (Oberlender and Nichols, 1990). MDMA increases the number of rat brain 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors (Aguirre *et al.*, 1995), which may induce increased release of 5-HT from presynaptic terminals, especially those from the dorsal raphe (Kaskey, 1992). The involvement of 5-HT_{1A} receptors in anxiety disorders has been reviewed (Coplan, *et al.*, 1995) and are consonant with the serotonergic hypothesis of anxiety (Graeff, 1993; Jacobs and Fornal, 1995). Buspirone, an anxiolytic with agonist-antagonist action on 5-HT_{1A} receptors (Coplan, *et al.*, 1995), was used to investigate the role of these receptors in the anxiogenic action of MDMA on the elevated plus maze.

MATERIALS AND METHODS

Animals

Adult male Charles Foster rats (180-220 g), procured from the Institute Central Animal House, were used. The rats were housed in groups of 4-5 in colony cages, unless otherwise mentioned, at ambient temperature of 25±1° C and 45-55% relative humidity, with a 12-h light/12-h dark cycle (lighting up time 08:00 h). Unless otherwise specified, the animals had free access to pellet chow (Lipton-Brook Bond) and water. The experiments were conducted between 09:00 and 14:00 h.

Drugs

The following drugs were used : MDMA (synthesized), yohimbine hydrochloride (Sigma, UK), lorazepam (Cipla, India) and buspirone hydrochloride (Torrent, India). MDMA was synthesized by one of us (SG) from 3,4-methylenedioxyphenyl propanoid intermediate by condensing with the appropriate alkylamine. The identity and homogeneity of the compound was established by electron impact mass spectrometry (EIMS) and HPTLC, using silica gel 60 F-254 as adsorbent and chloroform-methanol, 95:5, as the developer. The synthesis was done on a laboratory scale yielding material sufficient for the present investigation. All the drugs were dissolved in 0.9% saline and administered i.p. in a volume of 0.25 ml/100 g. Control animals received equivalent volume of the vehicle. The pretreatment time for MDMA and yohimbine was 30 min, whereas lorazepam and buspirone were administered 15 min prior to MDMA or yohimbine.

Methods

The following experimental methods were used.

Open-field test

The apparatus consisted of a dimly-lit green area (96x96 cm) divided into 16 squares. Naive vehicle- or drug-treated rats were individually placed on one corner and observed for the next 15 min by a 'blind' observer recording the number of squares crossed, rears and faecal pellets, and the total period of immobility (Bhattacharya, *et al.*, 1995).

Elevated plus-maze test

The maze consisted of two opposite open arms (50x10 cm), crossed with two closed arms of the same dimension but with walls 50 cm high. The arms were connected with a central square (10x10 cm) giving the maze the shape of a plus-sign. The maze was placed in a dimly-lit room and elevated 50 cm above the floor. Naive rats, pretreated with the vehicle or the test drugs, were placed individually on the maze centre facing an enclosed arm. A 'blind observer' recorded the number of entries and the time spent during the next 5 min on the open and closed arms by the rat (Johnston *et al.*, 1988).

Social interaction test

Rats were housed individually for 5 days prior to the test. The social interaction arena was a dimly-lit wooden box (60x60x35 cm) with a solid floor. The rats were paired on the basis of weight and each rat received two 7.5 min familiarisation sessions, individually, in the box at a 2-h interval. 24 h later, the rat pairs were treated with the vehicle or the test drugs and placed in the test box. A 'blind' observer recorded, during the next 7.5 min, the time spent by the rat pair in active socialization, characterized by sniffing, following, grooming, kicking, boxing, biting, crawling under or over the partner (Johnston *et al.*, 1988). In another experimental group, rats treated as above, were placed individually in an eight-beam photocell motor activity chamber. Locomotor activity, indicated by photobeam breaks, was recorded for 7.5 min (Bhattacharya, 1995).

Novelty-suppressed feeding

The test apparatus was the same as was used for the social interaction test. The floor was covered with a 2 cm layer of wooden chips and 15 laboratory chow pellets were evenly placed on the floor. A similar arrangement was made in the home cage, which had similar dimensions but was made polypropylene. Food was removed from the home cage 48 h prior to the test but water was provided. The rats were pretreated with the vehicle or the test drugs and placed individually in the test chamber. The latency to begin eating, defined as chewing the food pellet and not merely sniffing or playing with it, was recorded by a 'blind' observer. If the rat had not eaten within 360 s, the test was terminated and a latency score of 360 s was assigned to the animal. The results were compared with another group of rats where the latency to feed was recorded under identical conditions in the home cage (Bodnoff *et al.*, 1988).

Rotarod test

Rats were pre-treated with the vehicle or test drugs and placed individually on a rotarod apparatus rotating at a speed of 16 r.p.m. and were required to remain on the rod for 30s. Each animal was allowed up to three trials to reach criterion, and the percentage of animals not reaching criterion was recorded (Bennett *et al.*, 1985).

Rat brain tribulin activity

Tribulin activity was assessed in terms of endogenous monoamine oxidase (MAO) A and MAO B inhibitory activity (Medvedev *et al.*, 1992). Rats, pre-treated with vehicle or test drugs, were sacrificed by decapitation. The brains were removed and the left half of the brain was weighed and used for tribulin estimation. Extraction of the brain and the assay procedure for tribulin activity was essentially the same as described by Medvedev *et al.* (1992). The enzyme sources and the substrates for estimating MAO A and MAO B inhibitory activity were human placental homogenate and human platelets, and [^{14}C]5-hydroxytryptamine and [^{14}C] phenylethylamine, respectively.

Statistical analysis

The Mann-Whitney U-test was used for statistical evaluation of the data. A probability level of 0.05 was accepted as being statistically significant.

RESULTS

Open-field test

MDMA (5 and 10 mg/kg, i.p.) produced a dose-related decrease in the number of squares crossed and rears, with concomitant increase in immobility and defaecation, effects

qualitatively similar to that induced by yohimbine (2 mg/kg, i.p.) (Table 1).

Table 1.
Effects of MDMA and yohimbine on the open-field test in rats

Groups (mg/kg, i.p.)	*n*	Squares crossed (*N*)	Immobility (s)	Rears (*N*)	Faecal pellets (*N*)
Vehicle	12	138.6±9.8	42.4±7.5	24.2±5.4	4.4±0.9
MDMA (5)	8	109.2±7.6[a]	62.8±6.4[a]	14.4±3.8[a]	6.2±0.7[a]
MDMA (10)	8	82.4±7.9[a]	84.6±9.2[a]	10.6±2.0[a]	7.9±1.2[a]
Yohimbine (2)	8	92.3±6.6[a]	74.2±5.9[a]	12.6±3.1[a]	7.2±0.8[a]

Data represent means±SEM
[a] $p < 0.05$ different from the vehicle-treated control group.

Elevated plus-maze test

MDMA (5 and 10 mg/kg, i.p.) induced a dose-related decrease in the number of entries and time spent on the open arms of the maze, with a concomitant increase in these indices on the closed arms. A similar effect was induced by yohimbine (2 mg/kg, i.p.) (Table 2).

Table 2.
Effects of MDMA and yohimbine on the elevated plus-maze test in rats

Groups (mg/kg, i.p.)	*n*	% Time spent on open arms	% Entries on open arms
Vehicle	12	32.6±6.1 (226±25.3)	24.4±6.9 (32.5±6.3)
MDMA (5)	8	21.8±3.9[a] (199±32.1)	16.6±3.7[a] (30.2±7.9)
MDMA (10)	8	13.7±3.0[a] (182±24.5)	12.2±2.6[a] (26.4±8.2)
Yohimbine (2)	8	16.4±5.1[a] (212±21.4)	13.7±3.6[a] (9.3±7.2)

Data represent means ± SEM
Figures in parenthesis indicate total time spent(s) and entries (*N*) on both open and closed arms
[a] $p < 0.05$ different from vehicle-treated control group.

Social interaction test

MDMA (5 and 10 mg/kg, i.p.) reduced the time spent by the rat pairs in active social interaction, as did yohimbine (2 mg/kg, i.p.) without affecting locomotor activity (Table 3).

Table 3.
Effects of MDMA and yohimbine on social interaction in paired rats

Groups (mg/kg, I.p.)	*n* (pairs)	% Time spent in interaction	Locomotor activity *n*	Locomotor activity Counts
Vehicle	12	48.4±6.9	24	168.9±43.5
MDMA (5)	8	29.4±4.7[a]	16	196.8±35.2
MDMA (10)	8	16.9±3.4[a]	16	179.6±26.8
Yohimbine (2)	8	19.7±5.9[a]	16	188.9±24.6

Data represent means ± SEM
[a] $p < 0.05$ different from vehicle-treated control group.

Novelty-suppressed feeding

MDMA (5 and 10 mg/kg, i.p.) and yohimbine (2 mg/kg, i.p.) produced an increase in the feeding latency in the unfamiliar test cage. Although a similar increase was also noted in the home cage, the drug effects were not statistically significant (Table 4).

Table 4.
Effects of MDMA and yohimbine on latency to feed in 48-h food-deprived rats in familiar and novel environments

Groups (mg/kg, i.p.)	Home cage *n*	Home cage Feed latency (s)	Test cage *n*	Test cage Feed latency (s)
Vehicle	10	54.4±8.9	12	122.4±12.6
MDMA (5)	8	64.9±7.7	8	154.2±16.1[a]
MDMA (10)	8	70.4±9.9	8	197.4±18.9[a]
Yohimbine (2)	8	69.3±8.6	8	204.4±15.8[a]

Data represent means±SEM.
[a] $p < 0.05$ different from vehicle-treated control group.

Rotarod test

MDMA, yohimbine, lorazepam and buspirone had marginal and statistically non-significant (X^2 test) effects on this test in the doses used in this study. The performance deficit ($\underline{n}$ = 10) with MDMA (5 and 10 mg/kg, i.p.), yohimbine (2 mg/kg, i.p.), lorazepam (0.25 mg/kg, i.p.) and buspirone (2.5 mg/kg, i.p.) was 10, 10, 0, 20 and 10%, respectively.

Rat brain tribulin activity

The MAO B inhibitory component of tribulin was 45.7% higher than the MAO A inhibitory component in the vehicle treated group. MDMA (5 and 10 mg/kg, i.p.) produced 75.7 and 125.2% increase in MAO A inhibition, respectively, and 25.8 and 43.1% increase in MAO B inhibition, respectively. The increase in MAO A and MAO B inhibition induced by yohimbine (2 mg/kg, i.p.) was 113.8 and 50.0, respectively (Table 5).

Table 5.
Effects of MDMA and yohimbine on rat brain tribulin activity

Groups (mg/kg,i.p.)	*n*	Brain weight (g)	Tribulin activity (% MAO inhibition/g wet weight) MAO A	MAO B
Vehicle	16	1.69±0.09	21.0±2.6	30.6±1.9
MDMA (5)	8	1.78±0.06	36.9±2.9[a]	38.5±2.2[a]
MDMA (10)	8	1.82±0.09	47.3±1.6[a]	43.8±1.9[a]
Yohimbine (2)	8	1.74±0.08	44.9±2.0[a]	45.9±2.3[a]

Data represent means±SEM
[a] p <0.05 different from the vehicle-treated control group.

Effects of lorazepam and buspirone on MDMA and yohimbine actions on the elevated plus-maze

Lorazepam (0.25 mg/kg, i.p.) inhibited the anxiogenic action of both MDMA (5 and 10 mg/kg, i.p.) and yohimbine (2 mg/kg, i.p.). On the contrary, buspirone (2.5 mg/kg, i.p.) selectively inhibited the anxiogenic action of only MDMA (Table 6).

Table 6.
Effects of lorazepam (0.25 mg/kg, i.p.) and buspirone (2.5 mg/kg, i.p.) on MDMA and yohimbine actions on the elevated plus-maze in rats

Groups (mg/kg, i.p.)	*n*	% Time spent on[a] open arms	% Entries on[b] open arms
Vehicle	8	29.9±4.6	26.4±5.2
MDMA (5)	6	18.3±3.9[c]	14.2±3.8[c]
MDMA (10)	6	12.4±2.8[c]	10.1±1.9[c]
Yohimbine (2)	6	13.7±2.8[c]	11.6±3.0[c]
Lorazepam (LZP)	6	41.3±5.5[c]	44.0±6.8[c]
Buspirone (BSP)	6	38.4±3.3[c]	39.3±4.9[c]
LZP + MDMA (5)	6	25.4±2.8[d]	22.3±2.8[d]
LZP + MDMA (10)	6	21.3±3.9[d]	19.6±3.3[d]
LZP + Yohimbine	6	22.7±3.9[d]	20.0±2.6[d]
BSP + MDMA (5)	6	26.6±2.7[d]	23.9±3.0[d]
BSP + MDMA (10)	6	22.0±3.3[d]	20.3±3.9[d]
BSP + Yohimbine	6	17.4±3.3	14.9±2.7

Data represent means±SEM
[a] Total time spent (s) on both open and closed arms in the vehicle treated group = 246±25.1
[b] Total number of entries on both open and closed arms in the vehicle-treated group = 30.4±5.5
[c] p <0.05 different from vehicle-treated control group
[d] p < 0.05 different from respective MDMA or yohimbine treated groups.

DISCUSSION

The animal models of anxiety used in this study are essentially based on the premise that, when animals are confronted with a novel environment, they exhibit fear-conflict induced behavioural perturbations similar to the clinical state of anxiety (Treit, 1985; Lister, 1990; Geyer and Markou, 1995; Bhattacharya and Satyan, 1997). These models have been subjected to extensive critical appraisal and have predictive and face validity, and are capable of identifying anxiogenic and

anxiolytic agents under identical experimental conditions (Treit, 1985; Lister, 1990; Bhattacharya and Satyan, 1997).

The present study indicates that MDMA has a qualitatively similar profile of behavioural activity as that of the anxiogenic agent yohimbine (Charney *et al.*, 1983). Thus, both the drugs reduced ambulation and rears, while augmenting immobility and defaecation, in the open-field test, and increased the number of entries and the duration of stay on the closed arms of the elevated plus-maze, effects indicative of anxiety (Treit, 1985; Lister, 1990; Bhattacharya and Satyan, 1997). Likewise, both the drugs reduced social interaction in previously isolated rat pairs and increased the latency to feed in an unfamiliar environment, effects associated with anxiogenic agents (Treit, 1985; Bodnoff *et al.*, 1988). The behavioural effects induced by MDMA and yohimbine, are not likely to be due to any motor deficit produced by these agents since neither of them had any significant adverse effect on locomotor activity in an activity cage or on the ability to stay on a rotating drum in the rotarod test. The fact that lorazepam, a well established benzodiazepine anxiolytic, could significantly attenuate the anxiogenic effects of MDMA and yohimbine on the elevated plus-maze, provides further evidence for the validity of the observations noted in the present study.

Tribulin is a low molecular weight endogenous inhibitor of MAO and benzodiazepine receptor binding, widely distributed in mammalian tissues and biological fluids (Glover and Sandler, 1993). Tribulin has been postulated to function as an endogenous anxiogenic factor, based on extensive experimental and clinical evidence (Sandler *et al.*, 1988; Glover and Sandler, 1993). Rat brain tribulin activity is increased by anxiogenic agents, including yohimbine (Bhattacharya *et al.*, 1991; Bhattacharya, 1995; Bhattacharya *et al.*, 1996) and this increase is attenuated by anxiolytic drugs (Bhattacharya *et al.*, 1987; Bhattacharya and Mitra, 1991). Tribulin has now been shown to have at least two components, one inhibiting MAO B and having little affinity for benzodiazepine receptors, identified as isatin (2,3-dioxoindole), and the other, not yet

identified, inhibiting MAO A and benzodiazepine receptor binding (Glover and Sandler, 1993). Although isatin is known to induce an anxiogenic response in rodents and primates (Bhattacharya *et al.*, 1991; Glover *et al.*, 1991; Bhattacharya and Acharya, 1993; Palit *et al.*, 1997), it does not appear to account for the whole of tribulin activity. The MAO A inhibitory component is likely to be a major factor in tribulin function (Medvedev *et al.*, 1992; Glover and Sandler, 1993). Several anxiogenic agents, including yohimbine, were shown to increase the MAO A inhibitor component of tribulin (Bhattacharya *et al.*, 1996). A similar feature was noted during addictive drug withdrawal anxiety (Bhattacharya *et al.*, 1995). Since both MDMA and yohimbine were found to induce more marked increases in the MAO A inhibitory component, rather than the MAO B inhibitory component, the findings are consonant with the proposed function of the former in anxiety (Bhattacharya *et al.*, 1995; 1996).

Despite the widespread abuse of MDMA and the marked psychological and autonomic perturbations noted clinically (Randall, 1992 a, b; Duxbury, 1993; Steele *et al.*, 1994), there is, surprisingly, scant information on its behavioural effects in experimenal situations (Aguirre *et al.*, 1995). However, there is sufficient experimental data to indicate that MDMA is neurotoxic to central serotonergic neurones in rats and monkeys (Scanzello *et al.*, 1993; Series *et al.*, 1994; Colado and Green, 1995). A single dose (10 mg/kg, i.p.) of MDMA can induce marked neuronal release of 5-hydroxytryptamine (5-HT), leading to 40% loss of the amine and its metabolite, 5-hydroxyindole acetic acid (5-HIAA), in rat hippocampus and cortex (Colado and Green, 1995). The damage to 5-HT neurones, induced by this dose of MDMA, is permanent in monkeys but neuronal recovery is evident in rats (Scanzello *et al.*, 1993). MDMA has also been shown to inhibit ATP-dependent 5-HT accumulation in neurones and to promote its efflux from presynaptic terminals (Rudnick and Wall, 1992). The increase in presynaptic release of 5-HT, and the resultant augmentation in the postsynaptic availability of the amine, may be due to the increase in 5-HT_{1A} receptors present in

serotonergic presynaptic and postsynaptic membranes (Coplan *et al.*, 1995). These receptors function as autoreceptors regulating neuronal release of 5-HT (Glennon and Dukat, 1995).

MDMA has been reported to induce a syndrome, designated as the serotonin syndrome, either alone or when combined with MAO inhibitors (Kaskey, 1992). This syndrome, is induced by a variety of serotomimetic agents, including fluoxamine, a selective serotonin reuptake inhibitor antidepressant, in toxic doses (Lenzi *et al.*, 1993). Bodner *et al.*, (1995) have reviewed 81 cases of this syndrome reported in literature. Although the clinical signs and symptoms vary, they include symptoms of extreme mental agitation, restlessness, anxiety, panic attacks, disorientation, mental confusion and autonomic instability (Bodner *et al.*, 1995). It has been suggested (Bodner *et al.*, 1995) that, like the other agents inducing the syndrome, MDMA promotes the release of 5-HT from serotonergic neurones via its effect on the 5-HT_{1A} receptors. Serotonergic modulation of MDMA-induced reduction in self-stimulation response has been reported recently (Lin *et al.*, 1997). As mentioned earlier, a number of central actions of MDMA are reported to be 5HT-mediated responses.

The elevated plus-maze test was utilized for the interaction study between MDMA and the anxiolytics, lorazepam and buspirone, because this test has been extensively validated as an animal model of anxiety, using both behavioural and physiological measures (Pellow *et al.*, 1985; Geyer and Markou, 1995; Bhattacharya and Satyan, 1997; Bhattacharya et al., 1997).

The anxiogenic action of MDMA on the elevated plus-maze was inhibited by the BDZ, lorazepam, and by the 5-HT_{1A} partial agonist, buspirone. However, the latter failed to attenuate the action of yohimbine. Lorazepam-induced inhibition of MDMA may represent physiological antagonism, since earlier studies have indicated that BDZs can inhibit the anxiogenic effects of a variety of agents acting via diverse mechanisms, including anxiogenic beta-carbolines, isatin, quinine, indolealkylamines, caffeine, yohimbine, pentylenetetrazol, cholecystokinin, bradykinin and scorpion venom (Lydiard, 1994; Bhattacharya and Acharya, 1994; Bhattacharya, 1995; Bhattacharya *et al.*, 1995; Bhattacharya et al., 1997).

There is now considerable evidence to implicate the serotonergic system with anxiety. Increased brain 5-HT activity has been linked to anxiety (Iversen, 1984; Soderpalm and Engel, 1990; Graeff, 1993). On the contrary, depletion of 5-HT by synthesis inhibitors or selective neurotoxins, blockade of 5-HT_2 and 5-HT_3 receptors, and inhibition of neuronal release of 5-HT by 5-HT_{1A} receptor agonists, have anxiolytic effects (Marsden, 1989; Costall *et al.*, 1990; Soderpalm and Engel, 1990; Handley and McBlane, 1993; Coplan *et al.*, 1995). BDZs also reduce central 5-HT activity (Stein *et al.*, 1975). The emergence of a new non-BDZ generation of anxiolytics represented by 5-HT_{1A} receptor partial agonists like buspirone, gepirone and ipsapirone (Coplan *et al.*, 1995), and the 5-HT_3 receptor antagonist ondansetron (Costall *et al.*, 1990), represent attempts to introduce anti-anxiety agents devoid of the disadvantage of BDZs, including tolerance, dependence and amnesia (Costall *et al.*, 1990; Coplan *et al.*, 1995).

The present investigation indicates that MDMA exerts significant anxiogenic activity in rats which may explain some of the symptoms of MDMA toxicity. The behavioural studies with MDMA have now been extended to rhesus monkeys (*Macaca mulatta)* and preliminary findings indicate that the drug may exert behavioural effects associated with anxiogenic agents in this non-human primate species (Palit *et al.*, 1997) as well (unpublished data). The reported effect of MDMA on 5-HT_{1A} receptors and neuronal release of the amine may explain the inhibition of MDMA anxiety by buspirone.

However, further investigations are required to elucidate the putative involvement of the 5-HT neurotransmitter system and the receptor subtype associated with the anxiogenic activity of MDMA. These studies are now in progress.

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