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Acute and Subchronic Effects of Methylenedioxymethamphetamine [(±)MDMA] on Locomotion and Serotonin Syndrome Behavior in the Rat

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SPANOS, L. J. AND B. K. YAMAMOTO. *Acute and subchronic effects of methylenedioxymethamphetamine [(±)MDMA] on locomotion and serotonin syndrome behavior in the rat.* PHARMACOL BIOCHEM BEHAV **32**(4) 835–840, 1989.—Specific behaviors comprising the serotonin syndrome (low body posture, forepaw treading, headweaving) and the autonomic signs of piloerection and salivation were determined and analyzed with locomotor activity in response to MDMA at three doses (2.5, 5.0, and 7.5 mg/kg). All behaviors were dose-responsive. Serotonin syndrome behaviors increased in both intensity and duration of response with increasing doses. In contrast, locomotion varied only in intensity. Subchronic injections, in the same group of animals, permitted an analysis of acute vs. subchronic effects on these same behaviors. Both the serotonin syndrome and locomotor behaviors were augmented on subsequent testing, indicating that, (±)MDMA, like amphetamine, is capable of producing behavioral sensitization.

3,4-Methylenedioxymethamphetamine (MDMA) Serotonin syndrome Hyperlocomotion

THE increased street use of the amphetamine analog, 3,4-methylenedioxymethamphetamine [(±)MDMA], has prompted an intensified effort to determine both the acute and subchronic neurochemical effects of this drug. Acute and chronic high doses of MDMA release [^{3}H]serotonin (5-HT) (10,23), decrease tryptophan hydroxylase activity (28), and decrease 5-HT and 5HIAA tissue content in the striatum and limbic brain regions (21,28). The drug is also neurotoxic to serotonergic nerve terminals (1, 2, 16, 18, 21, 22, 28, 29). Overall, these acute and long-term effects of (±)MDMA on the serotonergic system are similar to those reported for parachloroamphetamine (13).

The effects of MDMA on the dopaminergic system are less pronounced (10,28). However, in vitro (10) and in vivo studies (31) have shown that the drug stimulates release and elevates striatal tissue content of dopamine (23,28). Behaviorally, (±)MDMA appears similar to amphetamine and can produce amphetamine-like stimulant effects using a drug discrimination paradigm (4,5). Both (±)MDMA and d-amphetamine maintain self-injection behavior in baboons (8,14) and produce discriminative stimulus effects that are stereoselective in such a manner that the (+)isomer is more potent than the (−)isomer (20). These behavioral effects have led Lyon *et al.* (15) to propose that MDMA's mechanism of action is more similar to that of dopaminergically mediated amphetamine than to that of the serotonergically mediated hallucinogens. However, it is possible that the drug has a complex mechanism that involves both neurotransmitter systems.

Activation of the serotonergic and dopaminergic systems is differentially associated with specific behaviors. The serotonin syndrome, consisting of the low body posture, side-to-side headweaving, lateral forepaw treading and the autonomic signs of piloerection and salivation, is a valid and reliable model of 5-HT mechanisms (9, 13, 26). In contrast, activation of the dopaminergic system has been associated with increased locomotion and stereotypical gnawing (19).

The present study utilized these models of the serotonin syndrome and hyperlocomotion to determine if the behavioral profile of MDMA action was consistent with serotonergic and/or dopaminergic activation. To date, there have been no published reports quantifying the motor effects of (±)MDMA. Therefore, the purpose of this study was to quantify, in a dose and time-dependent manner, the serotonin syndrome and locomotor activity following acute and subchronic low doses of (±)MDMA.

METHOD

Animals

Male Sprague-Dawley rats weighing 200–300 grams were used for this study. Rats were housed 4/cage in a temperature- (23 ± 1°C)

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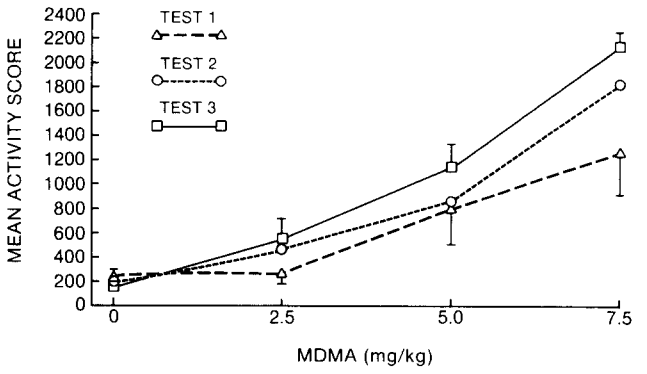

FIG. 1. Time course of locomotor behavior in response to acute 2.5, 5.0 or 7.5 mg/kg MDMA (n = 6). Ordinate refers to mean photocell counts at each 5-min time interval of the 120-min test. Insert refers to the mean total activity for the 120-min test (±SEM).

and humidity- (55±5%) controlled animal colony and were maintained on a 12-hour light-dark cycle (6 a.m.–6 p.m.) with free access to food and water.

Drugs

(±)MDMA was generously provided by Dr. Richard Hawks, National Institute of Drug Abuse, and was dissolved in 0.9% saline. All doses of the drug were administered IP in a volume of 1 ml/kg.

Activity

Four activity cages of the same dimensions (16×20″) and plastic construction as the animals' home cages were placed in a sound-attenuated chamber. Eight photocell beams were located along the sides of each cage at a height 2 inches above the floor and spaced 4 inches apart. These photocells were connected to a Commodore-64 computer programmed to monitor the pattern of beam interruption (Boja *et al.*, in preparation). Consecutive interruption of two beams registered one unit of activity. The computer was programmed to collect data in five-minute periods for the duration of the 120-minute test.

Serotonin Syndrome Behavior

The criteria for determining the presence of the serotonin syndrome were based on the following definitions: Forepaw treading—A pivoting movement resulting from lateral side-to-side stepping of the forepaws while the hindlimbs remain relatively stationary. Headweaving—Lateral side-to-side movements of the head with no net locomotion. Low body posture—Movement with the stomach nearly touching the floor of the cage and the hindlimbs abducted.

Syndrome behavior was observed through a Plexiglas panel mounted in the wall of the sound-attenuated chamber. Behaviors were rated according to a scale adapted from Kutscher and Yamamoto (13): 0 = absent; 1 = occasional (the behavior was present at some time during the one-minute observation period); 2 = frequent (the behavior was present for >30 seconds during the one-minute observation period); and 3 = constant (the behavior was present for the entire one-minute observation period). Piloerection and salivation were rated as 0 = absent or 1 = present. Scores for each behavior were summed for each time period to produce a total serotonin syndrome score.

Procedure

Acute study. Twenty-four hours before testing, rats were moved from the animal colony to a quiet room and placed in activity cages. Four animals were tested per session. Each test was conducted between the hours of 10 a.m.–12 p.m. On the day of testing, animals were injected with saline or with 2.5, 5.0, or 7.5 mg/kg of (±)MDMA and monitored for locomotor activity for a

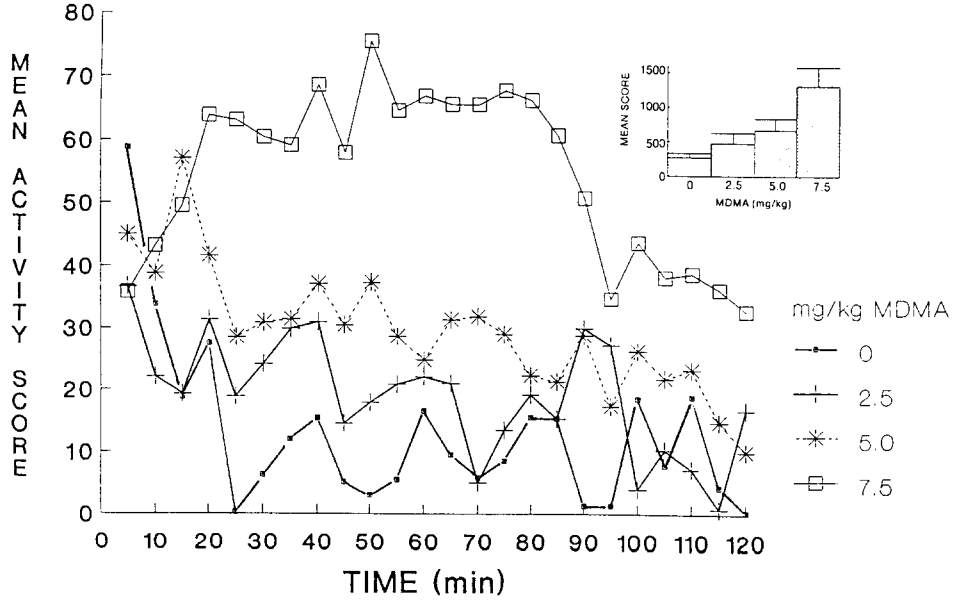

FIG. 2. Comparison of locomotor responses with acute (TEST 1) and subchronic (TEST 2 and TEST 3) 2.5, 5.0, and 7.5 mg/kg MDMA (n = 6). Ordinate refers to mean total activity score (±SEM).

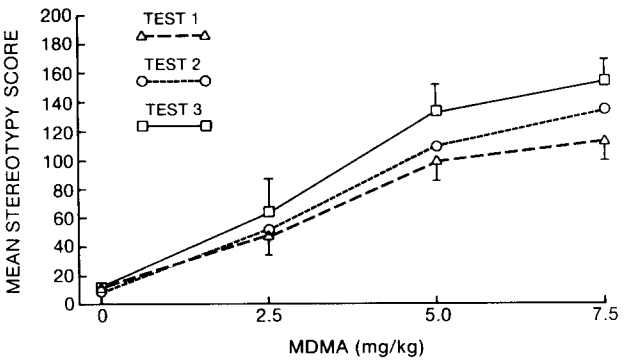

FIG. 3. Comparison of 5-HT syndrome responses using acute (TEST 1) or subchronic (TEST 2 and TEST 3) 2.5, 5.0 or 7.5 mg/kg MDMA (n = 6). Ordinate refers to mean serotonin syndrome score (± SEM).

period of 120 minutes. Serotonin syndrome behavior was simultaneously rated by an observer who was blind to the treatment condition. Animals were rated for a 1-minute period every 5 minutes for the duration of the 120-minute test. After testing was completed, the animals were returned to their home cage in the animal colony.

Chronic study. The same group of animals were weighed and injected every other day with the test dose of (±)MDMA or saline vehicle. Forty-eight hours after the sixth and twelfth injection (TEST 2 and TEST 3, respectively), animals were again tested as described above. To determine the effects of stress due to subchronic injections, separate groups of animals (n = 6) received saline injections and were placed in activity cages according to this same schedule but received MDMA (2.5, 5.0 or 7.5 mg/kg) forty-eight hours after the twelfth injection (TEST 3).

RESULTS

Locomotor Behavior

Acute effects. The dose-dependence of the locomotor response to an acute injection of MDMA was analyzed with a 1-way ANOVA using total mean activity scores. Figure 1 shows a significant dose effect for TEST 1 ($F = 6.24$, $p < 0.01$) and Fig. 2 illustrates the time course of locomotor activity. Between 5 and 25 minutes after injection, all animals showed an elevated activity score due to the stress of injection. From 25 minutes until the conclusion of the experiment (120 minutes), MDMA produced a dose-dependent hyperlocomotor response when compared to saline controls.

Subchronic effects. The effects of subchronic treatment on the locomotor response were analyzed with a 2-way ANOVA with repeated measures (Dose vs. Test) using the mean total activity scores for each animal. There was a significant dose-dependent enhancement of locomotor activity ($F = 4.55$, $p < 0.05$) from TEST 1 to TEST 3 (Fig. 1). Activity scores of animals subchronically injected with saline and administered MDMA on TEST 3 did not differ significantly from animals given a single acute injection of MDMA.

5-HT Syndrome Behavior

Acute effects. Analysis using a 1-way ANOVA with repeated measures and the mean total scores of all behaviors comprising the serotonin syndrome (forepaw treading, headweaving, low body posture, piloerection and salivation), showed that acute MDMA administration increased syndrome behavior (Fig. 3) in a dose-dependent manner ($F = 7.39$, $p < 0.01$). Saline controls did not have a measurable response and therefore the data are not illustrated. Figure 4A, B, and C show a significant dose-dependent increase for forepaw treading ($F = 4.76$, $p < 0.05$) and low body posture ($F = 6.71$, $p < 0.05$), but not for headweaving. Time course analysis revealed that, of the separate behaviors comprising the syndrome, low body posture was the most frequently and intensely displayed component. A post hoc Dunnetts test showed that this behavior contributed significantly to the total syndrome score ($p < 0.05$).

TABLE 1

THE EFFECTS OF MDMA ON SALIVATION AND PILOERECTION

	Saline	2.5	5.0	7.5
Salivation				
TEST 1	0	0.8 ± 0.6	4.2 ± 1.4	7.2 ± 2.4
TEST 2	0	0.5 ± 0.5	5.0 ± 2.0	11.0 ± 4.5
TEST 3	0	0.3 ± 0.2	10.2 ± 2.9	13.3 ± 0.5
Piloerection				
TEST 1	7.4 ± 3.4	19.8 ± 3.9	22.7 ± 0.7	24.0 ± 0
TEST 2	6.0 ± 2.2	23.2 ± 0.5	23.8 ± 0.2	24.0 ± 0
TEST 3	9.2 ± 3.8	22.0 ± 1.6	24.0 ± 0	24.0 ± 0

Subchronic effects. Subchronic treatment with MDMA increased the mean total score for serotonin behavior (Fig. 3) as a function of dose ($F = 36.86$, $p < 0.01$) and length of treatment ($F = 4.81$, $p < 0.01$). Alterations in the response for separate syndrome behaviors are shown in Fig. 4D, E, and F. For clarity, only the results from TEST 3 are illustrated, although TEST 2 was conducted in a similar detailed manner. Analysis of the separate syndrome behaviors revealed that the subchronic treatment paradigm does not affect each component of the syndrome equally. Low body posture was the only component that was potentiated by the lowest dose (2.5 mg/kg) in TEST 3 when compared to TEST 1. Subchronic treatment increased the intensity of all behaviors in a dose-dependent manner and differentially affected the time course of the individual behaviors (Test vs. time interaction, forepaw treading, $F = 15.79$, $p < 0.01$; headweaving, $F = 16.84$, $p < 0.01$; low body posture, $F = 26.58$, $p < 0.01$).

Table 1 presents the results from the autonomic components of salivation and piloerection. Although there were dose-dependent increases in both salivation ($F = 53.83$, $p < 0.001$) and piloerection ($F = 42.61$, $p < 0.001$) these increases were not dose-responsive with subchronic treatment.

To determine the possibility that the stress of repeated injections alone contributed to the enhanced response seen with subchronic MDMA treatment, a separate group of controls received saline injections according to the same injection schedule as the MDMA-treated animals and then received MDMA for TEST 3 of the study. For both the serotonin syndrome and hyperlocomotion, there were no significant differences in the intensity or duration of response between this group (receiving saline subchronically and MDMA on TEST 3) and that of the acutely injected MDMA animals.

DISCUSSION

The present study illustrates that (±)MDMA produces amphetamine-like hyperlocomotion as well as a "serotonin syndrome" consisting of forepaw treading, headweaving and low body pos-

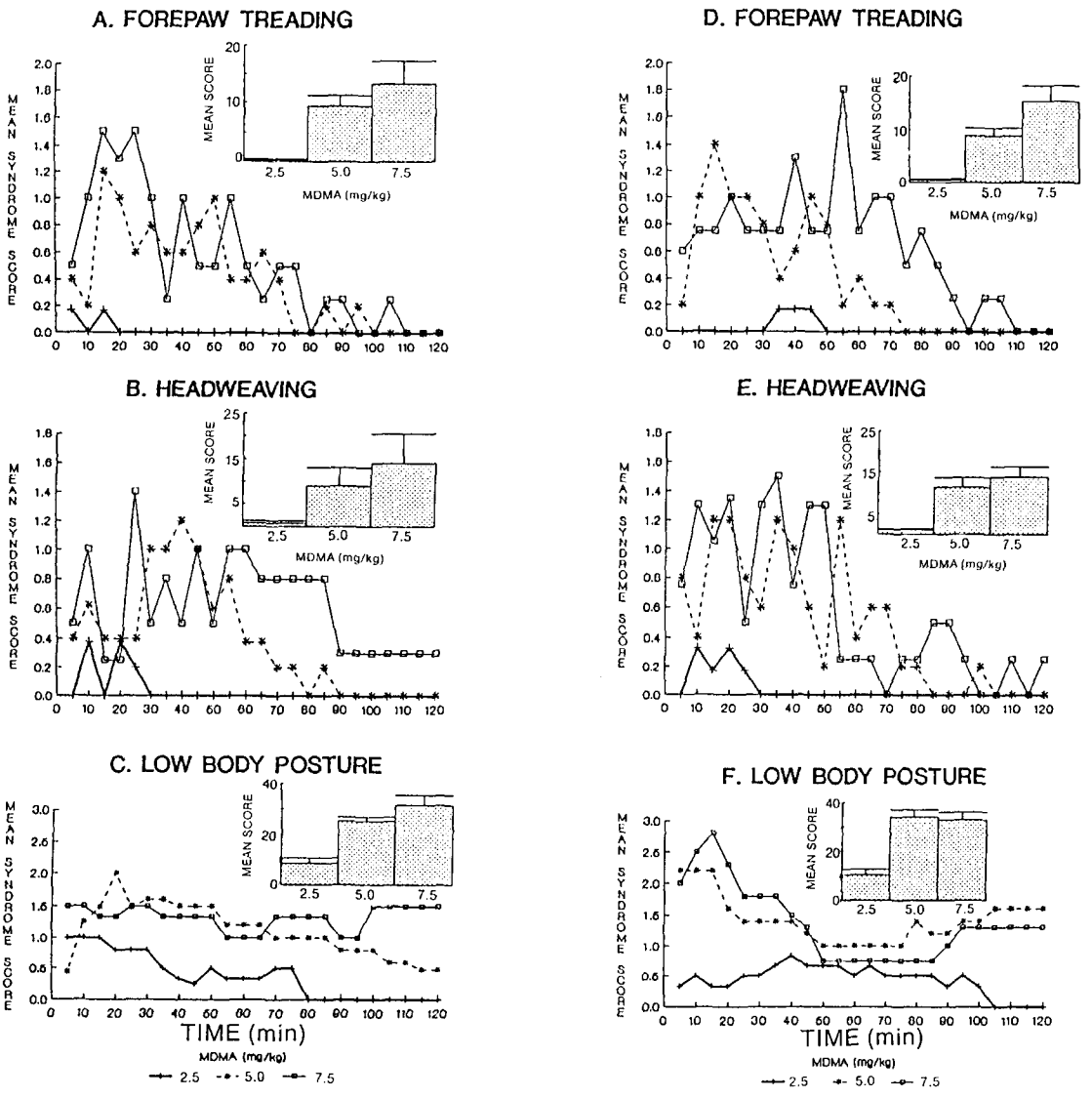

FIG. 4. Time course of separate 5-HT syndrome behaviors after acute (A, B, and C) and subchronic (D, E, and F) MDMA (n = 6). Ordinate refers to the mean score for forepaw treading (A, D), headweaving (B, E), and low body posture (C, F) for each 5-min time period of the 120-min test using 2.5, 5.0 and 7.5 mg/kg of MDMA. Insert refers to the mean total score at these doses.

ture and the autonomic signs of salivation and piloerection. These behaviors are concurrently displayed in a time- and dose-dependent manner and are augmented with subchronic treatment.

Analysis of the behavioral components contributing to the serotonin syndrome reveals that low body posture is the most reliable, and enduring component displayed in response to all doses of MDMA and that this behavior parallels the time course of the locomotor response. Additionally, subchronic MDMA treatment results in a differential enhancement of these specific behaviors. Likewise, examination of the autonomic components contributing to the syndrome reveals that subchronic treatment does not increase the frequency of piloerection and salivation in a dose-dependent manner. In fact, subchronic MDMA treatment produces a ceiling effect for piloerection that may indicate the insensitivity of our scoring method for this component.

Previous studies have examined the relative contribution of the separate components comprising the serotonin syndrome and have found slight differences in the behavioral profile (6,13). Kutscher and Yamamoto (13) found forepaw treading to be the most reliable indicator for 5-HT activation following p-chloroamphetamine (PCA), and Goodwin and Green (6) found low body posture to be the most robust component of the syndrome in response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Although both drugs are known to release serotonin, these results suggest that there are subtle differences in their mechanisms of action. (±)MDMA has been shown to release serotonin in vitro. However, to date, no studies have been done with intact animals to determine the time course of this effect. Future studies are needed to determine if there is a correlation between the syndrome behavior displayed with MDMA and its effect of serotonin release.

Serotonin release is known to produce hyperactivity in rodents but recent studies indicate that this effect is produced via catecholaminergic mechanisms (7). Although MDMA binds with low affinity to dopaminergic receptors, this drug releases dopamine both in vitro (28), and in vivo (31). Interestingly, the time course of MDMA-induced dopamine release seen with in vivo voltam-

metry (31) parallels the time course of hyperlocomotion seen in the present study. Additionally, evidence that the hyperactivity produced by amphetamine is mediated by dopaminergic systems and the finding that MDMA is similar to amphetamine in various behavioral paradigms (4, 5, 8, 14, 15, 20) indicates a dopaminergic mechanism for MDMA-induced hyperlocomotion.

The present study provides evidence for another possible similarity between MDMA and amphetamine. Like amphetamine (17), repeated intermittent administration of (±)MDMA results in augmented hyperlocomotion. Previous studies have indicated that the augmented amphetamine response (sensitization) is accompanied by increased dopamine turnover due to enhanced dopamine release in both the mesolimbic and striatal areas in the rat (24). It is also possible that the augmented behavioral response is due to altered postsynaptic mechanisms.

Another hypothesis to explain amphetamine sensitization, and perhaps the augmented responses to MDMA, relates to the pharmacokinetic properties of these drugs and/or their metabolites. Intermittent exposure to MDMA may result in accumulation of long-lasting metabolites. Also, MDMA itself may have a long half-life that could result in a cumulative effect with multiple injections. Although this explanation for amphetamine sensitization has been discounted by numerous investigators (3, 12, 24), MDMA metabolism has not been characterized sufficiently to exclude this possibility. Nevertheless, subchronic treatment with MDMA not only potentiates locomotor activity but also serotonin syndrome behavior. This is a major finding of this study and may indicate that the mechanism underlying the augmented response seen with subchronic MDMA is not exclusive to dopaminergic systems.

Some studies suggest that stress enhances the behavioral response to many of the stimulant drugs. To determine the contribution of this component to the present study, a control group was subchronically injected with saline. After the 4-week injection period, this group's response to MDMA was no different than that of acutely injected animals. This suggests that the stress of multiple injections was not a significant factor in the present findings.

The present study has described the behavioral profile and time course of action in response to acute and subchronic doses of (±)MDMA in the rat. Serotonin syndrome behavior and hyperlocomotion, indicative of serotonergic and dopaminergic activation respectively, are excellent behavioral models that parallel the acute neurochemical effects of the drug. Additionally, the augmented response produced with subchronic treatment provides a behavioral parameter for studying long-term MDMA effects. Utilization of these models in conjunction with in vivo neurochemical studies may result in a better understanding of the mechanism of action of MDMA and the consequences of chronic abuse of this drug.

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