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BEHAVIORAL SUPPRESSION FOLLOWING 3,4-METHYLENENEDIOXYMETHAMPHETAMINE

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Summary

Rotation in rats was employed as an assay of the central dopaminergic activity of 3,4-methylenedioxymethamphetamine (MDMA). This agent was observed to possess predominantly amphetamine-like actions at low doses. However, at higher doses it also appears to stimulate the dopamine receptor directly. Following a third dose of MDMA, a significant decrease in rotation was evident to this drug and to amphetamine, suggesting a neurotoxic or long-term suppressive action of MDMA.

The synthetic amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) is widely abused in the United States (1,2). On the street, this drug is known as "ecstasy," most probably because it has been reported by abusers to intensify emotional feelings. The pharmacological actions of this agent are reported to be mediated through a release of serotonin (5-HT) in the brain (3-5). However, the structurally-related drugs, methamphetamine and amphetamine, also have effects on dopamine (DA) systems (1).

Following the initial observation that rats with a unilateral lesion of the nigrostriatal pathway would rotate in a round chamber (6), it was demonstrated that unlesioned animals also would rotate in such an apparatus (7). While lesioned and unlesioned animals circled without drug treatment, amphetamine amplified rotation ipsilateral to the striatum with the lesser concentration of DA (7). Unlesioned animals have been observed to rotate ipsilateral, contralateral, or not at all after treatment with the direct DA agonist, apomorphine, although an individual animal's direction and magnitude of response is consistent (unpublished observations). A group of rats with a consistent response in direction and magnitude after administration of amphetamine or apomorphine was utilized to test the effects of MDMA on the direction and magnitude of their rotation.

Methods

A total of 12 male Sprague-Dawley rats were randomly assigned to one of 3 groups of 4 each. All animals were housed in individual home cages, which allowed the rat free access to food and water. The rats were kept on a 12-hour light-dark cycle (lights on at 0600) at 20-22^{o}C and constant humidity and were tested between 0900 and 1500 hours.

Each apparatus consisted of four 31 cm diameter by 42 cm high round polyethylene chambers (8). Animals were placed into a wire harness and lowered

into the chamber. The harness then was attached to a rod which penetrated the lid. As the animal circled, an arm on this rod activated one of four switches interfaced to a Commodore 64 computer. The turns were measured as quarter or full, left or right for each 5 min period and were summed for each of the daily sessions.

Animals initially were placed into a chamber and run for 15 min to determine baseline activity. Subsequently, each rat was picked up and received an i.p. injection either of 1.25 or 2.5 mg/kg d-amphetamine sulfate, 1 mg/kg apomorphine HCl (Sigma; both as the salt), or an equal volume (1 ml/kg) of physiological saline vehicle. Rotations were counted for one hour. Rats received these drugs in random order and received saline vehicle treatments every other day prior to the MDMA trials. No more than 3 drug injections were made in any one week period. The (+/-)-MDMA (supplied by Dr. Richard Hawks, NIDA) was dissolved in saline to a final concentration of 1.0, 1.5, or 2.5 mg/kg. Rats were tested for 15 min, then received MDMA by i.p. injection and were run for one hour. The order of drug administration was: 2.5 mg/kg MDMA, saline, apomorphine, saline, 1.0 mg/kg MDMA, saline, 1.5 mg/kg MDMA, and saline. Following this, rats were tested with d-amphetamine (0.625, 1.25 or 2.5 mg/kg) three times each over 3 weeks.

The data was analyzed by an analysis of variance with a Scheffe posthoc test. Significance was set at 0.05

Results

Prior to MDMA, six rats consistently turned right and six left after saline or amphetamine. The response of the animals as a group to 2.5 mg/kg d-amphetamine was lower that the response to 1.25 mg/kg d-amphetamine. MDMA was found to enhance rotational activity in these animals (Fig 1) as compared with saline.

On a molar basis, 2.5 mg/kg MDMA and 2.5 mg/kg d-amphetamine were equipotent in enhancing rotation. The overall dominant direction of circling following each dose of MDMA was the same as that induced by amphetamine. Interestingly, 1.5 mg/kg MDMA induced no more circling than the 1.0 mg/kg dose of this agent. Additionally, up to three weeks after the third MDMA treatment, 2.5 mg/kg d-amphetamine was significantly less effective in enhancing rotations than previous to MDMA treatment. While the overall response of all 12 rats to 1.25 mg/kg d-amphetamine was not significantly suppressed after MDMA treatment, the rotation of 8 of these animals was depressed. The lowest dose of d-amphetamine (0.625 mg/kg) produced circling comparable or slightly higher than 1.25 mg/kg of this agent.

In 4 of these animals, apomorphine caused rotation in the opposite direction to that seen after amphetamine, whereas 5 showed little rotation, and the remainder exhibited a similar direction of rotation. When 5-min periods were examined in the rats showing opposite rotation to these two drugs (Fig 2), 2.5 mg/kg MDMA was found to cause both amphetamine-direction and apomorphine-direction rotation for the first 30-35 min, followed by solely amphetamine-like effects for the remainder of the session. The two lower doses of MDMA did not cause any turning in the apomorphine direction. The time course of 2.5 mg/kg MDMA was similar to amphetamine, peaking at 40-50 min, whereas apomorphine peaked at 20 min and had no effects beyond 35 min. The lower doses of MDMA produced a constant rotation, which extended throughout the 60 min session.

Discussion

The animals utilized in this study showed a similar pattern of rotation to

apomorphine or amphetamine as other groups studied in this laboratory, except that they were less responsive to 2.5 mg/kg than to 1.25 mg/kg d-amphetamine. In previous studies from this laboratory, animals have been found to rotate most with doses of 2.5 to 5.0 mg/kg of amphetamine. Higher doses caused considerable stereotypic behaviors such as head bobbing, which decreased rotational behavior, whereas lower doses had little effect. The present animals showed more stereotypy at 2.5 than at 1.25 mg/kg amphetamine.

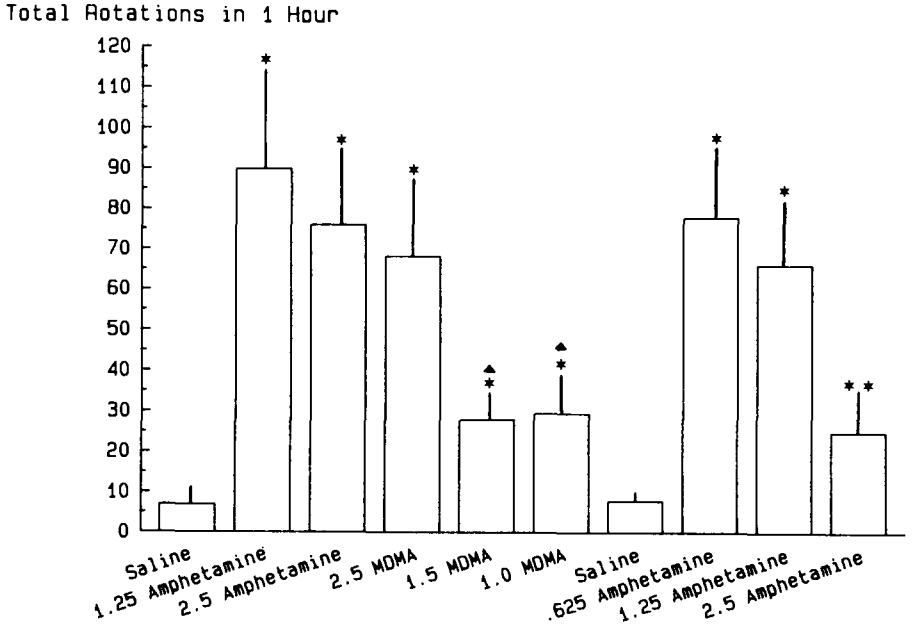

FIG. 1

The rotational response to saline or amphetamine prior to MDMA treatment (3 left bars) is compared with the response to MDMA and and the response after MDMA trials (4 right bars). * different from saline; ** different from same dose prior to MDMA; ▲ different from 1.25 or 2.5 mg/kg amphetamine, pre-MDMA ($p < 0.05$)

MDMA is structurally related both to amphetamine and mescaline and, thus, may have actions on both DA and 5-HT systems in the brain (1,3). In this study, rotational direction and quantification was employed as a behavioral assay of presumed dopaminergic neurotransmission to determine whether MDMA has amphetamine-like or apomorphine-like actions in the brain. While the initial response to this agent (2.5 and 1.0 mg/kg) was a dose-related increase in rotation, the long-term effect was behavioral suppression. This suppression first became apparent when the response to 1.5 mg/kg MDMA was no greater than to the lowest dose. MDMA has been shown to elevate striatal DA levels initially (4,9), but not after 5 days treatment (9). Thus, the decrease in rotation might reflect a decreased effect of MDMA in releasing DA. However, the rotational response of these rats to d-amphetamine also was observed to be suppressed. Five of these same animals were studied by in vivo voltammetry for striatal dopamine release in response to 1.25 mg/kg d-amphetamine (Dr. B. Yamamoto, personal communication). In these animals, the asymmetry in DA

release in the 2 striata paralleled the direction of rotation and the response to amphetamine was blunted by about 20% as compared with naive animals. A similar 20% decrease in rotation to 1.25 mg/kg amphetamine was noted herein.

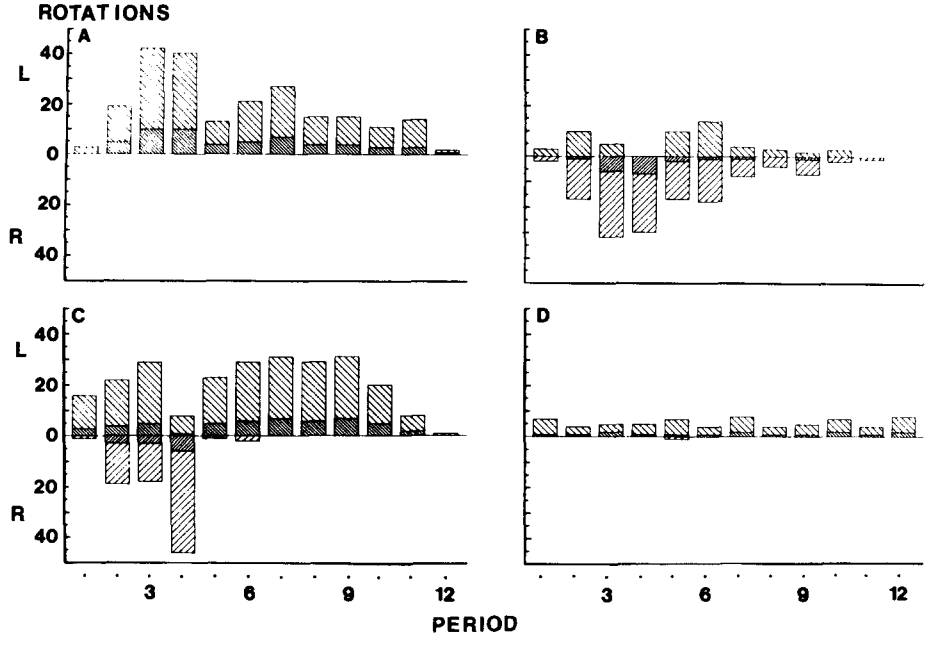

FIG. 2

Right (R) and left (L) quarter and full (small cross hatched) rotations per 5 min period in an animal that circled left (A) after 1.25 mg/kg amphetamine and right (B) after 1 mg/kg apomorphine. The circling after 2.5 mg/kg MDMA (C) has right and left components for 30 minutes followed by left rotations only, whereas 1.0 mg/kg MDMA (D) reveals no right rotations.

The direction in which animals rotated suggests that MDMA produced mostly amphetamine-like effects, probably mediated through a release of DA in the neostriatum. At higher doses, MDMA also had an effect on rotation consistent with a direct stimulation of DA receptors, comparable to apomorphine. It is unclear whether adverse actions on DA or 5-HT neurons or receptors are responsible for the long-term (3 weeks) behavioral suppression seen herein. A neurotoxic effect has been seen with MDMA (4,5,9) and MDA (1,9) on 5-HT neurons and methamphetamine on DA and 5-HT neurons (10), but only at higher doses than utilized in the present study. Thus, it is difficult to ascribe the behavioral suppression to a neurotoxic effect of MDMA on either 5-HT or DA neurons. However, while rotational activity is generally ascribed to be finally mediated through the dopaminergic nigrostriatal pathway, other neurotransmitter systems, including 5-HT (11), can influence rotation. While MDMA has effects on DA levels (4,9), no neurotoxic effects on DA systems, as judged by depletion of DA levels or tyrosine hydroxylase activity, have been demonstrated. MDMA does bind, albeit weakly, to the DA-2 receptor (12) and changes in DA receptors would affect rotational activity.

It is important to note that 1.0-1.5 mg/kg of MDA-HCl is the effective dose in humans, whereas 7.5 mg/kg may cause death (13). In mice, the lethal dose of MDMA is similar to MDA (14), so the doses of MDMA utilized herein were not excessively high. However, the animals did have a drug history having received amphetamine (1.25 or 2.5 mg/kg) or 1.0 mg/kg apomorphine several times prior to MDMA. It is possible that the behavioral suppression seen herein would not have occurred in naive animals given MDMA. The suppression is not due to amphetamine or apomorphine alone, however, as animals receiving these drugs for several weeks longer than in the present experiment show a consistent increase in rotation over time.

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