Prepulse inhibition of the acoustic startle response is disrupted by N-ethyl-3,4-methylenedioxyamphetamine (MDEA) in the rat

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N-Ethyl-3,4-methylenedioxyamphetamine (MDEA) is a derivative of methylenedioxyamphetamine (MDA), a substituted amphetamine with demonstrated abuse liability. MDA, MDEA and a third substituted amphetamine, methylenedioxymethamphetamine (MDMA), all produce a destructive action on central serotonin neurons and appear to induce some similar behavioral effects. The present study investigated the effects of racemic MDEA and its stereoisomers on prepulse inhibition of the acoustic startle response, a behavioral model of sensorimotor gating that is sensitive to psychostimulant drugs. Rats were subjected to 122 dB[A] acoustic noises, some of which were preceded by a weak 80 dB[A] prepulse noise. In vehicle-injected control rats, the prepulse induced a significant decrease in startle amplitude when compared to trials in which startle stimuli were not preceded by prepulses. Administration of racemic MDEA (0.3-10.0 mg/kg) and (+) MDEA (0.1-3.0 mg/kg) induced a significant attenuation in prepulse inhibition, while (−) MDEA (0.3-10.0 mg/kg) did not. Racemic MDMA (0.3-10.0 mg/kg) produced similar though not significant effects. These results confirm a stimulant-like behavioral effect of MDEA despite its relatively modest effects on dopamine markers, and support findings that the (+) stereoisomers of substituted amphetamines are more potent than the (−) stereoisomers in producing psychostimulant-like biochemical and behavioral effects.

MDEA (N-ethyl-3,4-methylenedioxyamphetamine); MDMA (methylenedioxymethamphetamine); Startle; Prepulse inhibition; (Rat)

1. Introduction

Much of the recent research on substituted amphetamines has focused their abuse liability and destructive action on central monoaminergic systems (Nichols, 1986). Behavioral studies have revealed that the amphetamine derivative methylenedioxyamphetamine (MDA) loses its hallucinogenic properties when N-methylated to methylenedioxymethamphetamine (MDMA; 'ecstasy'), though substantial reinforcing properties (albeit qualitatively different) are retained (Nichols, 1986). Both MDA (Ricaurte et al., 1985) and MDMA (Stone et al., 1986; Schmidt et al., 1987) produce severe long-term decreases in central serotonin levels, which have been linked to decreases in serotonin-specific uptake sites and cell degeneration (Commins et al., 1987). Isomeric studies of MDMA indicate that while the (−)-R stereoisomer possesses higher affinity for serotonergic binding sites in the CNS (Lyon et al., 1986), the (+)-S isomer appears more potent in depleting serotonin and in producing behavioral effects (Glennon et al., 1987; Johnson et al., 1988).

N-Ethyl-methylenedioxyamphetamine (MDEA; 'eve') is the N ethyl derivative of MDA. Though subjective reports indicate MDEA to be less potent than MDMA in producing euphoria (Dowling et al., 1987), this compound may be at risk for abuse

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since it is not currently subject to legal restrictions. Biochemical studies indicate that MDEA induces a significant, though lesser and shorter-lived, decrement in central serotonin content than do MDA and MDMA, as well as inducing smaller changes in dopaminergic markers (Johnson et al., 1987; Ricaurte et al., 1987; Stone et al., 1986). In addition, both MDEA and MDMA produce increases in locomotor activity similar in some respects to those induced by amphetamine (Gold and Koob, 1988; Gold et al., 1988).

Drug discrimination studies have also suggested a similarity between the behavioral effects of d-amphetamine, MDA, MDMA and MDEA (Kamien et al., 1986; Evans and Johanson, 1986; Boja and Schechter, 1987). These amphetamine-like stimulus properties are attributed to the (+)-S stereoisomers (Glennon et al., 1988). The (−)-R stereoisomer of MDA, but not of MDMA, however, appears to produce stimulus effects similar to those of classical hallucinogens (Glennon et al., 1982; Glennon, 1986), and may be in part responsible for MDA's more pronounced hallucinogenic actions. The (+)-S isomers of substituted amphetamines are more behaviorally active and more potent in releasing serotonin and dopamine, while the (−)-R isomers are more active in binding to serotonergic receptors and in producing hallucinogenic stimulus properties. However, the actions of these compounds which are responsible for their unique reinforcing properties remain unclear.

In the present study the effects of MDEA, its stereoisomers, and racemic MDMA were examined on the prepulse inhibition model of sensorimotor gating (Graham, 1975). In this model, startle responses are produced by presenting the subjects with intense, yet discrete, sensory events (in this case a loud noise). Preceding this stimulus with a weak, non-startling stimulus results in a greatly reduced startle response. This inhibited, or gated, response has been shown to be sensitive to disruption by amphetamine and apomorphine (Mansbach et al., 1988). Disruption of prepulse inhibition by MDEA would indicate, as in studies of locomotor activity (Gold et al., 1988), that this compound shares some common behavioral effects with dopaminergic stimulants, despite evidence implicating a relatively weak dopaminergic influence in its biochemical effects (Johnson et al., 1987). Moreover, a stereoselective blockade of prepulse inhibition by the (+)-S isomer of MDEA would support other behavioral reports that the dextro stereoisomers of substituted amphetamines exert amphetamine-like effects. In addition, racemic MDEA was administered to rats in a prepulse inhibition procedure using a low-intensity startle stimulus. This manipulation was made to determine whether a possible ceiling effect, in which all startle responses were raised by the drug to their maximum, was responsible for the blockade of startle inhibition, rather than a disruption of sensorimotor gating.

The effects of MDMA were also assessed using the prepulse inhibition model. Since MDMA appears to share some common behavioral effects with MDEA (Boja and Schechter, 1987; Gold et al., 1988), it was expected that MDMA would induce similar effects on startle as well.

2. Materials and methods

The apparatus used to detect and record startle responses has been described in detail elsewhere (Mansbach et al., 1988). Briefly, rats were placed in non-restrictive Plexiglas cylinders, which were suspended within a rigid frame and housed inside a ventilated enclosure. Movements by the rats (whole-body startle responses) caused slight vibrations in the cylinder, which were transduced by a phonographic cartridge, the tonearm of which was mounted on the rigid frame. These analog signals were digitized and stored on a microcomputer and interface assembly, which also controlled delivery of acoustic stimuli (San Diego Instruments, San Diego, CA, U.S.A.).

2.1. Procedure

Adult male (300-350 g) rats (Harlan Sprague-Dawley, Indianapolis, IN, U.S.A.) were tested using a procedure similar to that described in Mansbach et al. (1988). After a pretreatment interval, animals were placed into the startle apparatus and presented with 5 min of 70 dB[A]

background white noise. The startle stimulus consisted of a 122 dB[A] acoustic burst 40 ms in duration. The prepulse, when applied, consisted of a 80 dB[A] noise, 20 ms in duration, presented with its onset 100 ms prior to the onset of the startle stimulus. The main comparison of interest was between startle responses elicited by the startle pulse alone (pulse-alone) and responses produced by an identical pulse preceded by the weak prepulse (prepulse + pulse). Additional trials of either the prepulse alone (prepulse-alone) or no stimulus (nostim) were also presented. The session consisted of 61 trials: an initial pulse-alone followed by 15 presentations of the four aforementioned trial types (pulse-alone, prepulse + pulse, prepulse-alone, and nostim) in an irregular order. The average intertrial interval was 15 s. An identical session was used in the low-intensity study of MDEA (see fig. 4), except that the startle stimulus was set at 108 dB[A]; the prepulse intensity was not changed. Group sizes for all experiments ranged from 10 to 12, with each animal being tested only once.

2.2. Data analysis

Excluding the initial trial, startle response amplitudes for each trial type were averaged for each animal across the entire test session, as per Mansbach et al. (1988). For each experiment, a two-way analysis of variance (ANOVA) was conducted on the pulse-alone and prepulse + pulse trials with drug treatment as a between subjects factor and trial type as a repeated measure. Subsequent one-way ANOVAs and post hoc Tukey tests (Winer, 1971) were also conducted on startle amplitudes for pulse-alone and prepulse + pulse trial types. The arithmetic difference between mean startle responses for pulse-alone and prepulse + pulse was also calculated for each subject. Since the results of ANOVA conducted on these figures are equivalent to the F ratio in the two-way ANOVA described above, post hoc Tukey or Dunnett's tests were performed on difference scores across groups in cases where the two-way interaction term was found to be significant.

2.3. Drugs

(±), (+)-S and (−)-R MDEA HCl and (±) MDMA HCl (provided by the National Institute on Drug Abuse) were dissolved in saline and administered subcutaneously, 10 min prior to placing animals in the test chambers, in a volume of 1.0 ml/kg.

3. Results

3.1. Effects of racemic MDEA

Figure 1 illustrates the effects of racemic MDEA on startle responses in the rat. An analysis of variance revealed that the drug had no overall effect on startle, $F(4,55) = 1.3$, n.s., but that there was a significant effect of trials, $F(1,55) = 83.3$ and a significant drug-by-trial interaction, $F(4,55) = 5.1$, $P < 0.002$, indicating a blockade of prepulse inhibition. However, there was no significant effect of the drug on either pulse-alone ($F(4,55) = 1.5$, n.s.) or prepulse + pulse ($F(4,55) = 2.1$, n.s.) in one-way analyses. When scores were transformed to the difference between pulse-alone and prepulse

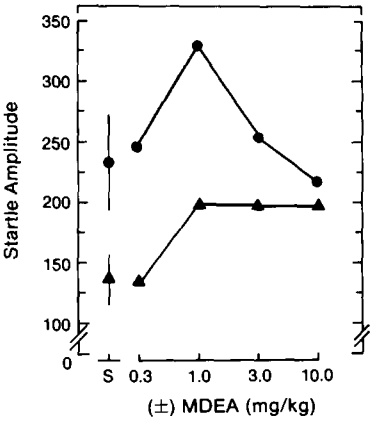

Fig. 1. Effects of racemic N-ethyl-3,4-methylenedioxyamphetamine (MDEA) on prepulse inhibition of the startle response in rats. Circles represent startle amplitudes following presentation of the startle stimulus alone (pulse-alone); triangles represent responses when the startle stimulus was preceded by a weak prepulse (prepulse + pulse). Unconnected symbols to the left illustrate startle responses following saline ± S.E. (n = 10-12).

+ pulse scores, individual Tukey comparisons indicated significant differences between the MDEA 10.0 mg/kg dose and both the 0.3 and 1.0 mg/kg doses ($P < 0.05$). Stabilimeter readings on the nostim and prepulse-alone trial presentations remained uniformly low and were not significantly affected by drug presentation in this or any of the following experiments (data not shown).

3.2. Effects of (+)-S and (−)-R stereoisomers of MDEA

As with the racemate, (+)-S MDEA induced a blockade of prepulse inhibition, as indicated by a significant drug-by-trial interaction, $F(4,55) = 3.8$, $P < 0.01$ (fig. 2, left panel). There was no significant overall effect of the drug, $F(4,55) < 1.0$, but a significant effect of trials was evident, $F(1,55) = 70.9$, $P < 0.001$. One-way ANOVAs on the individual trial types revealed no significant effects of drug on either pulse-alone or prepulse + pulse, $F(4,55) < 1.0$. Tukey comparisons confirmed a significant change in the calculated pulse-alone minus prepulse + pulse difference scores between the 3.0 mg/kg group and both the saline and 0.3 mg/kg groups ($P < 0.05$).

Administration of (−)-R MDEA induced no significant overall effect on startle, $F(4,55) < 1.0$, n.s., nor was there a disruption of prepulse inhibition, as reflected by the lack of a significant drug × trials interaction, $F(4,55) = 1.1$, n.s. (fig. 2, right panel). A trials effect did remain, however, $F(1,55) = 92.3$, $P < 0.001$, confirming the preservation of prepulse inhibition. Analysis of pulse-alone and prepulse + pulse scores in one-way ANOVAs yielded no significant effects on the drug factor.

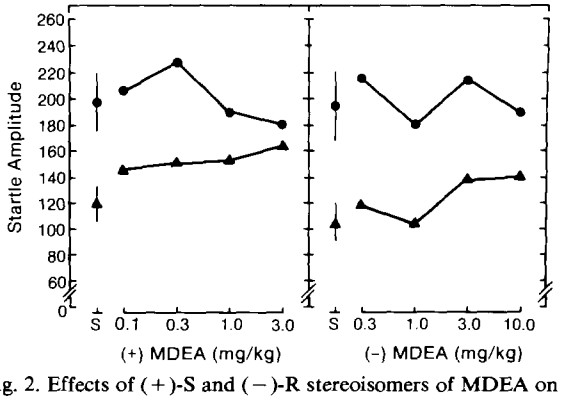

Fig. 2. Effects of (+)-S and (−)-R stereoisomers of MDEA on prepulse inhibition in the rat. See Fig. 1 for details (n = 10-12).

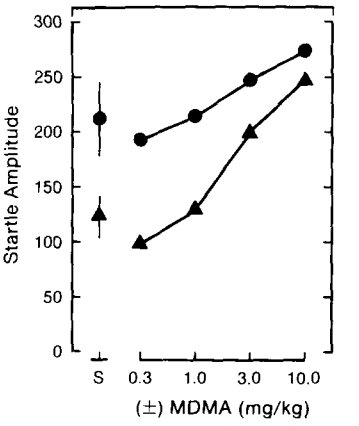

Fig. 3. Effects of racemic 3,4-methylenedioxymethamphetamine (MDMA) on prepulse inhibition in the rat. See fig. 1 for other details (n = 10-12).

3.3. Effects of racemic MDMA

Racemic MDMA (fig. 3) induced a significant overall increase in startle magnitude, $F(4,50) = 2.8$, $P < 0.05$, and a significant trials effect, $F(1,50) = 64.9$, $P < 0.001$. There was also a strong trend toward a drug-by-trial interaction which fell just short of significance, $F(4,50) = 2.5$, $P = 0.056$. The increases in pulse-alone startle were not found to be significant. $F(4,50) < 1.0$, n.s., but the drug-induced increases in prepulse + pulse were, $F(4,50) = 6.4$, $P < 0.001$.

3.4. Effects of MDEA (low intensity study)

When 10 mg/kg MDEA was administered to rats responding to a 108 dB[A] startle stimulus, there was no overall effect of drug, $F(1,22) < 1.0$, n.s. An effect of trials was evident, however, $F(1,22) = 138.2$, $P < 0.001$, and also a drug × trials interaction, $F(1,22) = 24.8$, $P < 0.001$, confirming a blockade of prepulse inhibition. The 108 dB stimulus produced startle responses clearly lower than those at 122 dB (fig. 4). There was no significant effect of drug on either the pulse-alone or

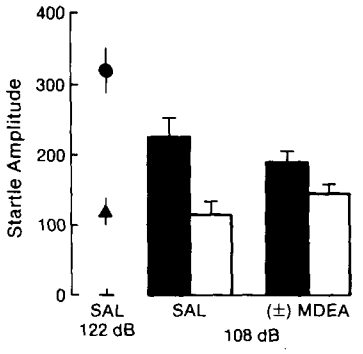

Fig. 4. Effects of MDEA (10.0 mg/kg s.c.) on prepulse inhibition at a lower noise intensity. The circle and triangle at the left of the figure represent startle responses ± S.E. induced by the 122 dB[A] acoustic startle stimulus alone and when preceded by the prepulse, respectively. Filled bars illustrate startle responses (± S.E.) induced by the 108 dB[A] startle stimulus alone; open bars show responses (± S.E.) when the prepulse was also presented. Analysis of variance on the 108 dB[A] groups revealed a significant drug-by-trial interaction, confirming a blockade of prepulse inhibition. Separate groups of animals (n = 12) were used for each condition; the intensity of the prepulse stimulus was the same as in the other studies.

prepulse-pulse trial type, $F(1,22) = 1.8$ and 1.6, respectively.

4. Discussion

The present results indicate that MDEA decreases prepulse inhibition in a manner similar to the dopaminergic stimulants d-amphetamine and apomorphine (Mansbach et al., 1988). At low doses, racemic MDEA (fig. 1) increased startle responses to both pulse-alone and prepulse inhibited stimuli, while at higher doses only responses on the prepulse-plus-pulse trials were elevated. The (+)-S stereoisomer of MDEA (fig. 2) also significantly decreased prepulse inhibition, though its startle-increasing effects were less apparent than with the racemate. In contrast, the (−)-R isomer of MDEA (fig. 2) did not block prepulse inhibition in the same dose range, as reflected by the lack of a significant drug-by-trials interaction. These findings are reminiscent of other reports of substantial behavioral and biochemical effects with the racemate and (+) isomer of MDMA, and lesser effects with the (−) isomer (Nichols, 1986; Schmidt et al., 1987). These separate, dissociable actions of the stereoisomers may underlie the unique behavioral effects of MDMA. The dopaminergic effects of MDMA help to explain its similarity to amphetamine in drug discrimination and locomotor studies, as well as its effectiveness in maintaining self-administration in animals (Beardsley et al., 1986), a property uncommon among traditional hallucinogens.

MDMA's effect on startle also parallels that of d-amphetamine. Like amphetamine, MDMA produced an overall increase in acoustic startle (fig. 3). Though MDMA did not convincingly attenuate prepulse inhibition in study study, the results having barely missed statistical significance ($P = 0.056$), there was a strong indication that this drug possesses stimulant-like effects on sensorimotor gating. The lack of a significant interaction between drug and trial type was likely due to the main effect on startle magnitude, which tends to negate interaction trends and produce more parallel dose-effect curves.

Administration of 10 mg/kg MDEA in the low-intensity experiment resulted in a significant attenuation of prepulse inhibition at a noise level of 108 dB[A] (fig. 4). These results confirm that the blockade of prepulse inhibition was not simply due to the overall increases in startle magnitude induced by MDEA, since the effect occurred even when startle responses were clearly not at their maximum. Since all mean startle scores at this intensity were lower than those at the 122 dB level, it is unlikely that the blockade of prepulse inhibition could merely by an artifact of a ceiling effect (cf. Mansbach et al., 1988).

The parallel, though not identical, effects of MDEA and MDMA on prepulse inhibition are partially corroborated by studies of schedule-controlled responding. The parent compound, MDA, appears to exert both hallucinogenic and psychostimulant discriminative stimulus properties, with the (+) isomer more amphetamine-like and the (−) isomer more hallucinogen-like (Glennon et al., 1982; Glennon and Young, 1984). Transformation to MDMA diminishes hallucinogenic effects, and, accordingly, administration of either isomer of MDMA does not produce response generalization in animals trained to discriminate the

$5HT_2$ hallucinogen, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), from vehicle (Glennon et al., 1988). In a study of the disruptive effects of MDA and MDMA on operant behavior, Rosecrans and Glennon (1987) found that the response-rate decreasing actions of (−) MDA, but not (+) MDA or either stereoisomer of MDMA was reversed by the 5-HT_2 antagonist pirenpirone, confirming MDMA's lack of classical hallucinogenic effects. The psychostimulant actions of MDA do, however, appear to be preserved in the transformation to MDMA and MDEA; responding of animals trained to discriminate amphetamine from vehicle generalized to MDA and MDMA (Evans and Johanson, 1986; Kamien et al., 1986), and administration of MDMA to MDEA-trained rats also produced generalization (Boja and Schechter, 1987). This latter report is in contrast with a recent paper in which neither isomer of MDEA produced drug-appropriate responding in MDMA-trained rats (Glennon et al., 1988). Several studies have emphasized the dual nature of substituted amphetamines in producing both hallucinogen-like and stimulant-like behavioral effects; these combined effects most likely account for the unique threat of this drug class for abuse. Further, the loss of prepulse inhibition induced by MDEA does parallel deficits in prepulse inhibition associated with schizophrenic disorders (Braff et al., 1978). It is possible that, along with the stimulant actions of these compounds, there is a risk of the induction of psychosis.

While MDEA does not induce as great or as long-lasting a decrement in central serotonin as MDMA, and probably produces a less intense euphoric experience (Dowling et al., 1987), the present results and other recent studies suggest that MDEA exerts substantial stimulant-like effects on several behavioral indices, and may pose a liability for abuse similar to that of its better-known analogues. Despite MDEA's stimulant-like behavioral profile, its lackluster effects on dopamine and dopamine metabolites in the CNS (Johnson et al., 1987) pose some questions as to its mechanism of action in producing such behaviors. Further studies of MDEA in conjunction with dopamine and serotonin site-specific antagonists may help to clarify these issues.

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