ELSEVIER

Drug and Alcohol Dependence 36 (1994) 161–166

DRUG and ALCOHOL DEPENDENCE

Behavioral and developmental effects of two 3,4-methylenedioxymethamphetamine (MDMA) derivatives

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Received 8 April 1994; accepted 24 July 1994

Abstract

The effects of 3,4-methylenedioxymethamphetamine (MDMA or 'ecstacy') and two structurally related compounds, *N*-methyl-1-(3,4-methylenedioxyphenyl)-1-ethanamine (MDM1EA) and *N*-methyl-1-(3,4-methylenedioxyphenyl)-3-butanamine (HMDMA) were examined in two preparations: (i) a drug discrimination procedure in MDMA-trained rats and (ii) the chicken embryo, for determination of the direct effects of these compounds on the developing organism. The highest doses of MDM1EA and HMDMA partially substituted for MDMA, whereas higher (30–60 mg/kg) doses of HMDMA evoked clonic seizures in a separate group of rats. In chicken embryos MDMA had no effect on body, brain or liver weight, while the highest dose of MDM1EA decreased body weight and the 2 lowest doses of HMDMA increased body weight. All doses of HMDMA decreased liver weight (expressed as % body weight) when compared with contemporaneous water-treated controls. Taken together, the results of these experiments suggest that structurally related compounds share some stimulus properties with MDMA and may therefore share abuse liability. Furthermore, both MDMA-related compounds produced adverse effects on the developing organism, whereas MDMA did not.

Keywords: MDMA; Designer drugs; Substance abuse; Developmental toxicity; Drug discrimination

1. Introduction

MDMA (also known as 'ecstasy') is an N-substituted 3,4-methylenedioxyamphetamine (MDA) derivative that rapidly became a popular drug of abuse (Peroutka, 1987; Henry et al., 1992). Since MDMA's classification in 1985 as a Schedule I drug under the Controlled Substances Act (Lawn, 1988), there has been an increase in clandestine synthesis and illicit use of other N-substituted MDA derivatives, some of which have been shown to possess psychotomimetic activity similar to MDMA (Noggle et al., 1988).

N-substituted MDA derivatives are obtained via reductive amination of 1-(3,4-methylenedioxyphenyl)-2-propanone with the appropriate amine. In 1989, the Drug Enforcement Administration passed the chemical Diversion and Trafficking Act that controlled the availability of 1-(3,4-methylenedioxyphenyl)-2-propanone. The restricted availability of this starting reagent prompted clandestine chemists to explore alternative synthetic methods for the production of MDMA. For example, Noggle et al. (1989a) reported that some clandestine laboratories have mistakenly used the commercially available ketone 1-(3,4-methylenedioxyphenyl)-3-butanone as a starting reagent in the hopes of synthesizing MDMA. The resulting product, however, is HMDMA, a homologue of MDMA. Other ketones such as 3,4-methylenedioxyacetophenone, are also commercially available, and if used as the starting reagent, would yield the 2 carbon side chain homologue of MDMA, MDM1EA (Noggle et al., 1989b). As shown in Fig. 1, HMDMA represents an increase, and MDM1EA a decrease, of one methylene group between the aromatic ring and the *N*-methylamine moiety when compared to MDMA.

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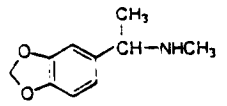
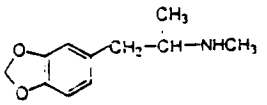
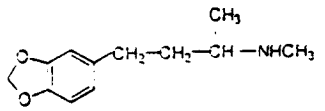
0376-8716/94/$07.00
SDI 0376-8716(94)01045-M



N-Methyl-(3,4-methylenedioxyphenyl)-1-ethanamine (MDM1EA)



N-Methyl-(3,4-methylenedioxyphenyl)-2-propanamine (MDMA)
(3,4-Methylenedioxymethamphetamine)



N-Methyl-(3,4-methylenedioxyphenyl)-3-butanamine (HMDMA)
(Homo-3,4-methylenedioxymethamphetamine)

Fig. 1. Structures of MDMA, MDM1EA and HMDMA.

One purpose of the present study was to examine the discriminative stimulus properties of HMDMA and MDM1EA in rats trained to discriminate MDMA from saline. The drug discrimination procedure has been used extensively in drug abuse research because it approximates the subjective effects of drugs in humans (Schuster and Johanson, 1988) and can be a reliable predictor of the abuse liability of a drug (Foltin and Fischman, 1991; Preston and Jasinski, 1991). In such studies, an animal is trained to discriminate the presence of a particular drug from presence of vehicle by reinforcing presses on one lever after administration of drug and on a different lever after administration of vehicle. Tests are then conducted with other drugs to determine their degree of similarity to the test drug, as measured by the proportion of lever presses on the drug-appropriate lever. Thus, it is possible to determine whether the discriminative stimulus (i.e., 'subjective effects') provided by the test drug are similar to those produced by the training drug. Such studies can be particularly important in the case of homologues and analogues of MDMA, because these compounds may be sold on the streets as MDMA, and if they share subjective effects with MDMA, then they may have similar abuse liability.

Increased drug abuse during pregnancy suggests that some pregnant women will be exposed to MDMA and its homologues and/or analogues. A further aim of the present study was to examine the effects of MDMA, HMDMA and MDM1EA on the developing organism. The chick embryo model was used for these studies because it allows for detection of direct drug effects, in the absence of potentially complicating maternal factors. In such studies, a single dose of a drug can be administered at one or more time points during embryogenesis, and the resulting effects on physical and/or biochemical parameters can then be measured quantitatively. More importantly, the effects of certain drugs such as opioids and alcohol are similar in the human and chicken embryo (Gillogley et al., 1990; Jakubovic et al., 1978; Stockard, 1914). For example, long before fetal alcohol syndrome was described in human infants, Stockard (1914) reported that exposure of the chick embryo to ethanol vapor resulted in developmental retardation and vascular, CNS and visual abnormalities.

2. Methods

2.1. Drug synthesis

Synthesis of the compounds was accomplished by reductive amination of 3,4-methylenedioxyphenylacetone (for MDMA), 3,4-methylenedioxybenzylacetone (for HMDMA) or 3,4-methylenedioxyacetophenone (for MDM1EA) with methylamine using sodium cyanoborohydride as the reducing agent (Braun, 1980). The structure of each compound was established by standard analytical and spectroscopic (IR, NMR) techniques and the purity established by elemental analysis (Noggle, et al. 1989a,b).

2.2. Drug discrimination studies

Subjects. Subjects were 7 male Long-Evans rats weighing 290–310 g. They were individually housed in hanging cages over corn-cob bedding and had free access to tap water except during the experimental sessions. Weights were maintained at approximately 300 g by restricted feeding of Purina commercial rat chow.

Behavioral apparatus. Two standard operant chambers (LHV, Model 1417C, Unit C-Lehigh Electronics), each containing 2 levers and a pellet dispenser located between the 2 levers, were enclosed in sound-attenuating booths equipped with exhaust fans. Electromechanical programming equipment located in an adjacent room was used to control and record the behavioral sessions.

Discrimination training. Rats were trained to lever-press during overnight autoshaping sessions. Once rats pressed each lever at least 100 times during a 30-min session, daily 15-min sessions were begun. The number of lever presses required for pellet delivery was increased each day by 1 until a total of 10 lever presses/reinforcer (fixed ratio 10, FR10) was in effect. During the FR10 training period, an injection of either 1.5 mg/kg MDMA or its vehicle, 0.9% sterile saline was administered 20 min before each session, and lever-pressing was only reinforced with food following the requisite number of responses on the injection-appropriate lever. The injection changed daily, i.e., saline-drug-saline-drug, etc. until the FR10 was in effect. For 3 rats, responses on the right lever were reinforced after administration of

MDMA, and responses on the left lever were reinforced after a saline injection. For the remaining 4 rats, the injection-appropriate levers were reversed. To eliminate odors left by previous rats, the levers were wiped with ethanol before each session. Once the FR10 was in effect, injections were made in a double alternation sequence, e.g., drug-drug-saline-saline-drug-drug, etc. When at least 80% of the responses before the first reinforcer were made on the injection-appropriate lever over a period of 10 consecutive days, a subject was considered to have acquired the discrimination.

Dose effect curves. Dose effect curves were determined for MDMA (0.09–6 mg/kg), followed by MDM1EA (1–30 mg/kg) and HMDMA (0.1–10 mg/kg). These doses were chosen based on the behavior observed when various doses of the compounds were administered to non-trained rats: doses used in the drug discrimination procedure were lower than those that increased locomotor activity, produced head weaving or were seizurogenic in non-trained rats of the same species. During each dose-effect curve, the order of injections was mixed, although the first dose tested was one that would not be likely to be behaviorally active. All drugs were dissolved in sterile 0.9% saline and were administered i.p., 20 min before the test session, in a volume of 1 ml/kg. All doses are expressed as the hydrochloride salt. The highest dose of each compound was one that decreased responding by at least 50% of saline control values. Each dose was given at least twice, once after a saline training session and once after an MDMA training session. Tests were conducted on Tuesday and Friday and were carried out in extinction, i.e., the session ended with no reinforcer after 10 responses had been made on either lever. i.p. injections were administered 20 min before the session as during training. Training continued on Monday, Wednesday and Thursday during all dose-effect determinations.

Data analysis. During tests, the total number of responses on the MDMA lever was divided by the total number of responses on both levers and multiplied by 100 to give percent-MDMA-appropriate responding. A drug was considered to have substituted for the training dose of MDMA if at least one dose produced ≥ 80% responding on the MDMA-appropriate lever during extinction tests. ED_{50} values were calculated by Probit analysis (Finney, 1952).

2.3. Chick embryo studies

Subjects. Fertile specific pathogen free eggs were obtained from the Poultry Sciences Department at Auburn University. The eggs were kept in a forced air incubator maintained at 37°C and 58% relative humidity. On day 14 of embryogenesis, eggs were removed from the incubator and candled to determine viability. This time point approximates the beginning of the third trimester in humans. After determining viability, distilled water or a single dose of MDMA (8, 16 or 32 mg/kg egg), MDM1EA (8, 16 or 32 mg/kg egg) or HMDMA (4, 8, 16 or 32 mg/kg egg) was injected directly into the chorioallantois of the egg close to the vitellene vessels, via a small hole drilled in the shell. The hole was then covered with transparent tape and the eggs were returned to the incubator. The doses used in the embryo were chosen because they encompass the range of doses (10–20 mg/kg) of MDMA that have been used to produce neurotoxicity in rats. It should be noted that for each drug there was a contemporaneous control group, i.e., control eggs were set at the same time as those that would receive drug. For example, in the case of MDMA, if 100 eggs were set on a particular day, 25 would be injected with water, 25 would receive the 8 mg/kg dose of MDMA, another 25 would receive the 16 mg/kg dose, and the remaining 25 would be injected with the 32 mg/kg dose. This was done because there is seasonal variability in hatchability, weight at hatch, etc. MDMA, MDM1EA and HMDMA were dissolved in distilled water and the injection volume was 0.05 ml/egg. Doses are expressed as the salt. On the 19th day of incubation, eggs were transferred to a forced air hatcher (maintained at 38°C and 58–60% relative humidity).

Body, brain and liver weight. Chickens were weighed at hatch, euthanized with CO_2 and their brains and livers were removed and weighed.

Data analysis. Body, brain and liver weight were analyzed by one way ANOVA and Fishers LSD post-hoc tests with a significance level set at $P < 0.05$.

3. Results

3.1. Drug discrimination

The results for MDMA, MDM1EA and HMDMA are shown in Fig. 2. MDMA dose-dependently substituted for the training dose of MDMA, with an ED_{50} of 0.54 ± 0.2 mg/kg, as calculated by Probit analysis. In contrast, MDM1EA did not fully substitute for MDMA. At 30 mg/kg, lever-pressing occurred at a very low rate so higher doses were not tested. It should be noted that 2/7 rats did select the MDMA lever, one at the 10 mg/kg dose of MDM1EA, and the other at 30 mg/kg; in both cases, the substituting dose was also one that significantly decreased responding in those particular rats. HMDMA appeared to produce partial substitution for the training dose of MDMA when the mean of all animals was examined, with maximum MDMA-appropriate responding at 10 mg/kg. When individual animals were examined, however, HMDMA substituted completely for MDMA in 5/7 animals and not at all in 2 animals. When HMDMA substituted, it did so with an ED_{50} of 2.93 ± 1.5 mg/kg. Higher doses of HMDMA were not tested in the drug discrimination procedure because doses of 30–60 mg/kg HMDMA resulted in clonic seizures in a separate group of untrained rats.

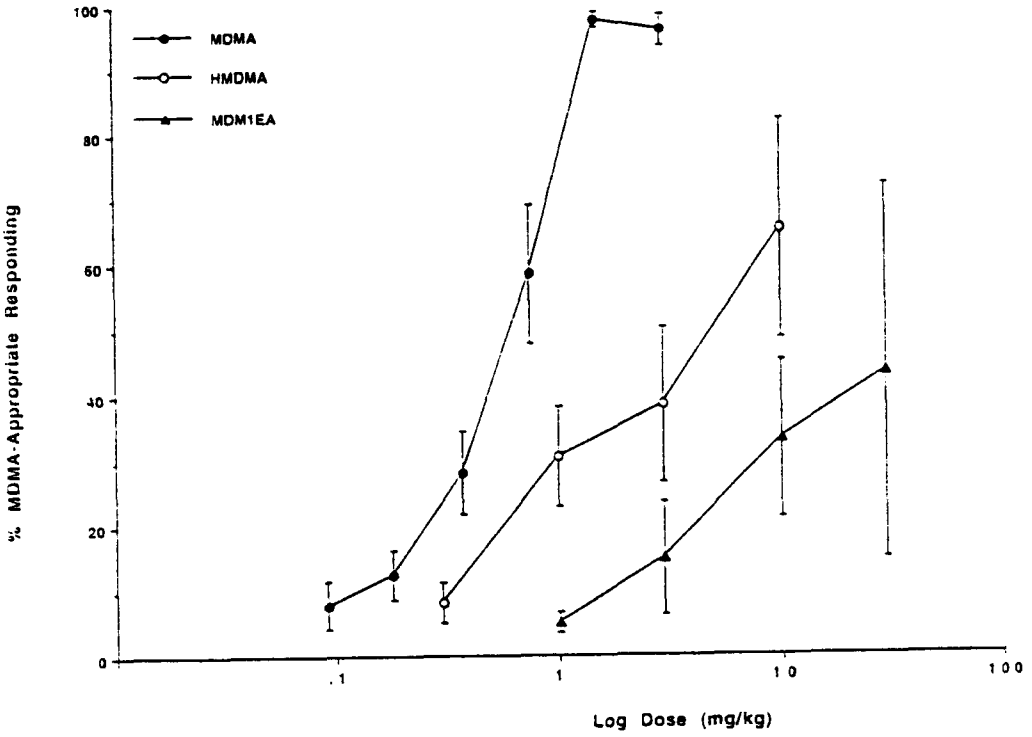


Fig. 2. Effects of MDMA, MDM1EA and HMDMA in rats trained to discriminate 1.5 mg/kg MDMA from saline. Each point represents the mean ± S.E. for 7 rats. Each dose was determined twice.

3.2. Chick embryos

Table 1 shows the results of MDMA, HMDMA and MDM1EA on body, brain and liver weights of newly hatched chickens.

Body weight. One way ANOVA revealed a significant treatment effect on body weight at hatch in the HMDMA and MDM1EA groups, $F(4,112) = 5.161$, $P = 0.0007$ and $F(3,76) = 3.082$, $P = 0.0323$, respectively. Fishers PLSD revealed that the difference was due to significantly increased body weights in chickens that had received HMDMA 4 and 8 mg/kg egg and decreased body weights in the MDM1EA 32 mg/kg egg pretreatment group when compared to contemporaneous water-injected controls, $P < 0.05$.

Table 1
Effects of water, MDMA, HMDMA and MDM1EA administered on day 14 in ova, on body weight at hatch, as well as brain and liver weights, expressed as percent of body weight

Treatment in ova (mg/kg)	n	Body weight (g)	Brain (% body wt.)	Liver (% body wt.)
Water	23	39.35 (0.682)	2.043 (0.061)	2.630 (0.102)
MDMA (8)	20	40.20 (0.613)	1.998 (0.037)	2.531 (0.103)
MDMA (16)	24	38.42 (0.874)	2.042 (0.052)	2.719 (0.136)
MDMA (32)	22	39.41 (0.538)	2.014 (0.038)	2.704 (0.094)
Water	21	36.45 (0.579)	2.179 (0.046)	2.905 (0.063)
HMDMA (4)	19	41.08 (0.734)*	2.038 (0.037)	2.395 (0.067)*
HMDMA (8)	32	38.41 (0.548)*	2.018 (0.040)	2.458 (0.058)*
HMDMA (16)	21	37.31 (0.933)	2.107 (0.059)	2.597 (0.078)*
HMDMA (32)	24	38.25 (0.749)	2.131 (0.057)	2.633 (0.085)*
Water	20	40.18 (0.929)	1.976 (0.070)	2.515 (0.134)
MDM1EA (8)	24	38.63 (0.461)	2.046 (0.030)	2.569 (0.059)
MDM1EA (16)	19	38.97 (0.960)	1.905 (0.070)	2.477 (0.081)
MDM1EA (32)	17	36.59 (0.924)*	2.077 (0.091)	2.814 (0.127)

*Significantly different from water pretreatment, $P < 0.05$.

Brain weight. There was no difference in brain weights among the various pretreatment groups.

Liver weight. One way ANOVA revealed a significant treatment effect on liver weight in the HMDMA group ($F(4,112) = 7.3365$, $P = 0.0001$. Fishers LSD showed that all doses of HMDMA resulted in decreased liver weights, expressed as percent of body weight, when compared to contemporaneous water-injected controls, $P < 0.05$.

4. Discussion

MDMA did not produce any noticeable adverse effects in the chicken embryo or the adult rat at the doses examined, whereas low doses of HMDMA increased body weight of newly hatched chickens, and the highest dose of MDM1EA decreased body weight as compared to controls. HMDMA also decreased liver weight in the chicken embryo. The decrease in liver weight observed in the HMDMA group may have been due to the fact that control animals in this group weighed less than control groups for the other drugs, and liver weight was presented as percent of body weight. This seems unlikely, however, as there was no difference in body weight among controls and the 16 and 32 mg/kg HMDMA-treated embryos, but there was still a significant decrease in liver weight in 16 and 32 mg/kg HMDMA groups. Furthermore, when liver weight in mg was used for statistical analysis (rather than liver weight as percent of body weight), there was still a significant decrease in all HMDMA-treated groups when compared to control, $F(4,112) = 3.761$, $P = 0.0066$. A final argument is that for each drug group there was a contemporaneous control group, i.e. all eggs were set to contain embryos that would receive each of the five treatments. This was done because it is common to find seasonal variability in hatchability, weight at hatch, etc.

These results contribute to and expand studies on embryonic exposure to other drugs of abuse. Chicken embryos exposured to methadone and morphine had decreased liver weight and brain protein, and high doses of methadone resulted in a dose-dependent failure to develop (Jacubovic et al., 1978). These effects resemble those seen in humans in which decreases in birth weight, head circumference, length and gestational age have been reported in offspring of women that tested positive for cocaine, amphetamine or opioids when compared to offspring of women who had negative drug screens (Gillogley et al., 1990). The effects of ethanol on the human embryo (Finnegan, 1981) and chicken embryo (Stockard, 1914) are also similar. Thus, the current findings that HMDMA was hepatotoxic in the chicken embryo, even at low doses, is a cause for concern.

In drug naive rats, HMDMA produced seizures at only 1/2 log unit higher dose than that which produced full substitution in the drug discrimination procedure. This confirms another report that HMDMA is more toxic than MDMA in mice (Davis and Borne, 1984). HMDMA could easily be synthesized accidentally and sold as MDMA on the streets. If HMDMA and MDMA share subjective effects in humans, as they do in rats, then there is a likelihood that HMDMA could be purchased as a substitute for MDMA. Although the stimulus involved in drug discrimination and that involved in self-administration may not be identical, the drug discrimination procedure has been shown to be a good predictor of the abuse libility of drugs (Foltin and Fischman, 1991; Preston and Jasinski, 1991). The very small margin between the dose of HMDMA that substituted for MDMA and that which produced seizures indicates a significant risk associated with use of this drug.

In contrast to HMDMA, MDM1EA substituted for MDMA in the drug discrimination procedure in only 2 rats and then only at a dose that significantly decreased responding. Like HMDMA, MDM1EA could also be sold on the streets as MDMA. In the chicken embryo, a single, high (32 mg/kg) dose of MDM1EA administered on day 14 of embryogenesis resulted in a significant decrease in body weight at hatch. Whether MDM1EA is also toxic to the human fetus remains to be determined. Because of limited substitution with MDM1EA, however, it seems unlikely that this drug would be used repeatedly to obtain an MDMA-like effect.

Other investigators have examined the effects of a variety of MDMA-related compounds in the drug discrimination procedure and have found that a rather large number of them share stimulus properties with MDMA (Glennon and Misenheimer, 1989; Nichols and Oberlender, 1990). To date, virtually all studies have been conducted in male rats. With the increased clandestine synthesis and illicit use of MDMA derivatives (Noggle, et al., 1988), it is important to study the effects of the various compounds in female rats as well. Despite attempts to increase awareness of the dangers of drug use during pregnancy, there is still a substantial number of pregnant women using drugs. Therefore, it is not unlikely that MDMA and its designer analogues/homologues will be used by pregnant women as well. While MDMA had no effect on brain, body or liver weight of newly hatched chickens in the present study, this drug is known to produce serotonergic neurotoxicity in a number of species (Ricuarte, 1989) and those effects would not have been detected in the present experiments. The effects of MDMA-related compounds such as HMDMA and MDM1EA have not been extensively studied, but our findings, combined with those Davis and Borne (1984), suggest that these compounds may be toxic as well.

As noted above, very few drug discrimination procedures employ female subjects. In view of the fact that 2 of the drugs used in the present study were embryotox-

ic, studies of the discriminative stimulus effects of these drugs in females may be justified.

Acknowledgments

This research was supported in part by NIDA grant DA06637.

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