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Behavioral Effects of N-Ethyl-3,4-Methylenedioxyamphetamine (MDE; "EVE")

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BOJA, J. W. AND M. D. SCHECHTER. *Behavioral effects of N-ethyl-3,4-methylenedioxyamphetamine (MDE; "EVE").* PHARMACOL BIOCHEM BEHAV **28**(2) 153–156, 1987.— Eight male rats were trained to discriminate 2.0 mg/kg N-ethyl-3,4-methylenedioxyamphetamine (MDE) from its vehicle using a two-lever, food-motivated operant discrimination task. Once trained, the rats showed a dose-dependent decrease in discriminative accuracy following administration of decreased doses of MDE (ED_{50}=0.75 mg/kg). Administration of 1.5 mg/kg 3,4-methylenedioxymethamphetamine (MDMA), a recently restricted Schedule I drug, produced 100% MDE-appropriate responding in the MDE-trained rats and decreased discriminative performance was similarly observed following lower doses of MDMA (ED_{50}=0.62 mg/kg). The difference in relative potencies of MDE and MDMA in rats is reminiscent of those seen in human abusers who report effective oral psychotomimetic doses. Time-course data indicated that MDE has a fast onset, 100% drug-correct responding 10 min post-injection, and a peak effect between 10–20 min with declining effect at 60–120 min post-administration. These findings along with those of others show a pharmacological similarity between MDE and MDMA. Implications as to the future scheduling of MDE are discussed.

Drug discrimination Stimulus properties of drugs MDE MDMA Rats Time-course

N-ethyl-3,4-methylenedioxyamphetamine (MDE) is the N-ethyl derivative of the recently restricted drug 3,4-methylenedioxymethamphetamine (MDMA). Both MDMA and MDE are structurally related to the other scheduled drugs methylenedioxyamphetamine (MDA), methamphetamine and mescaline. Much recent attention has been directed toward MDMA due to its increasing non-medical use, currently estimated to be 30,000 doses per month [1], and its July 1, 1985 assignment by the Drug Enforcement Agency (DEA) to Schedule I status [17]. In contrast, there is a paucity of scientific literature on MDE. This lack of information was illustrated at a recent report of the Committee on Problems of Drug Abuse Symposium on Stimulants and Hallucinogens: "The Committee is not aware of any information relevant to the pharmacologic similarity of this compound (MDE) to any currently controlled drug" [5]. One of the possible reasons for this present lack of concern regarding MDE may be the limited number of documented MDE abusers, i.e., between 1973–1983 there were an estimated 40,100 dose units of MDMA produced by clandestine laboratories compared to less than 100 unit doses of MDE [2]. The drug MDE, known as "EVE" on the streets, has not been scheduled by the DEA at this time and its non-medical use is, therefore, still not illegal. Due to the lack of any schedule for MDE and the Schedule I status of MDMA the potential for the growth of MDE abuse is real. This is illustrated by the report that drug suppliers dealing in MDMA were awaiting arrival of MDE immediately following the imposition of the Schedule I status for MDMA [6]. Futhermore, two deaths have been reported to have occurred in the Dallas area alone following MDE intoxication [7].

This laboratory is presently involved in the study of the neurochemical and behavioral properties of MDMA and related drugs [12,13]. In a previous study, acute administration of 15 mg/kg MDE produced a serotonergic-like behavioral syndrome accompanied by an increase in dopamine (DA) and a decrease in both serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in selected brain regions [3]. The purpose of the present investigation was to study the pharmacological properties of MDE in rats utilizing the drug-discrimination paradigm. Rats were trained to discriminate MDE from vehicle in order to determine if MDE produced stimulus properties that are discriminable. MDMA was subsequently administered in order to classify it as similar or dissimilar to MDE in regard to its stimulus properties, potency and time-course.

METHOD

Subjects

Ten male Sprague-Dawley (Zivic-Miller) rats, weighing 200–250 g at the beginning of the experiment, were individually housed in galvanized cages with free access to water except during experimental sessions. They were maintained at 80–85% of their free feeding body weight by restricted feedings of commercial rat chow. The rats were trained 5 days per week at the same time of day (1300–1400). Room temperature was maintained at 20–22°C with lights on from 0600 to 1800.

Apparatus

Ten standard rodent operant test cages (Lafayette Instrument Co., Lafayette, IN) were equipped with two levers mounted 7 cm above the metal grid floor and 7 cm apart. Equidistant between the two levers and 2 cm above the floor was a food pellet receptacle. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9 watt houselight. Solid-state programming equipment (Med Assoc., E. Fairfield, VT) was used to control and record each session and was located in an adjacent room.

Shaping and Discrimination Training

The food-deprived rats were administered vehicle intraperitoneally (IP) 20 min prior to the start of each of the first nine shaping sessions of the experiment and were trained to press either the right (N=5) or left (N=5) lever to receive a food reinforcement (45 mg Noyes pellet) under a fixed-ratio 1 (FR 1) schedule. Shaping continued as the FR schedule was gradually increased to FR 10 over a period of 6 days; this FR 10 schedule was maintained for 3 days. Before each of the subsequent shaping sessions, the rats received (IP) an equal volume (1 ml/kg) of vehicle containing 2.0 mg/ml MDE 20 min prior to the session. The rats were then trained on an FR 1 schedule on the opposite (the drug-correct) lever. The FR schedule was gradually increased over a 4 day period until a stable FR 10 was attained; this schedule was maintained for 3 days. Subsequently, the following biweekly treatment schedule was instituted with either drug (D) or vehicle (V): V-D-D-V-V, D-V-V-D-D. This constitutes the discriminative training (post-shaping) period. The training criterion was achieved when an animal chose the appropriate lever on 8 daily sessions out of 10 consecutive sessions. This 8 out of 10 correct session criterion was required twice before any further testing was conducted.

Dose-Response Testing

After all the rats had met the 8 out of 10 criterion twice, the animals received various doses of MDE (DR) according to the following 2 week schedule; D-DR-V-DR-D, DR-V-DR-D-DR. Thus, each dose was preceded by one session with vehicle and one session with 2.0 mg/kg MDE. Dose-response testing data were treated in the same manner as the substitution data (below). Any animal failing to maintain discriminative performance at criterion levels was eliminated from the study. This occurred with two of the ten animals. All doses were given IP 20 min prior to testing and each animal was allowed to lever press until 10 responses had been recorded on either lever. The rat was then immediately removed from the box without receiving reinforcement and each placed into its respective home cage. This procedure precluded any continued training at a dose other than the training dose. The dose-response relationship to MDMA in rats trained with MDE was then determined using an identical procedure.

Time-Course of MDE Action

To determine the time-course of the MDE discriminative cue, rats were injected wih 2.0 mg/kg MDE, returned to their home cage and allowed to remain there for 5–240 min before testing began. The order of testing the various time delays was randomized between subjects such that each rat received any one post-injection time twice with each preceded by one maintenance session with 2.0 mg/kg MDE and one maintenance session with vehicle, each at 20 min post-injection. The behavioral half-life ($T_{1/2}$) of MDE, i.e., the point in time in which the strength of the MDE cue (as indicated by the precent drug choices) reaches 50%, was determined by linear regression from the terminal linear part of the time-course curve which followed the section of the curve representing peak discriminative performance.

TABLE 1

LEARNING RECORD OF 10 RATS SHAPED TO TWO LEVERS AND SUBSEQUENTLY TRAINED TO DISCRIMINATE 2.0 mg/kg MDE FROM VEHICLE

	Frequency (as percentage) of Responding on the MDE-Appropriate Lever In:			
	Vehicle (DW) Sessions		MDE Sessions	
Weeks	Quantal	Quantitative (SD)	Quantal	Quantitative (SD)
1 and 2	66.0	65.6 (23.21)	62.0	60.7 (22.3)
3 and 4	46.0	49.8 (14.8)	92.0	78.8 (5.2)
5 and 6	24.0	32.9 (9.1)	88.0	81.9 (12.1)
7 and 8	16.0	26.0 (9.2)	94.0	81.0 (3.1)

Measurements and Statistics

The lever pressed 10 times first was designated the "selected" lever. The percentage of rats selecting the lever appropriate for MDE was the quantal measurement of discrimination and quantal data are presented as percent correct first choice on the MDE lever. In addition, the number of lever presses on the MDE-correct lever divided by the total number of responses on both levers prior to 10 responses, times 100, constitutes the quantitative measurement. Mean (and standard deviation) of quantitative measurements were calculated across all rats in any given day. Both measurements are reported as suggested previously [16]. Quantal data were compared by the method of Litchfield and Wilcoxon [10], which employs probit vs. log-dose effects, allows for testing for parallelism and derivation of ED_{50}s. Quantitative data were compared by a two-tailed paired t-test of means ($p<0.05$).

Drugs

The following drugs were used in this study: ±N-ethyl-3,4-methylenedioxyamphetamine HCl and ±3,4-methylenedioxymethamphetamine HCl. All drugs were made fresh daily, because there is no information on the stability of these drugs in solution, by being dissolved in de-ionized water (DW) and were injected (IP) in a constant volume of 1 ml/kg. All doses were calculated as the salt.

RESULTS

Since this is the first reported use of MDE to provide stimulus control in the drug discrimination paradigm, the learning record of the ten rats is provided in Table 1. The training schedule of V-D-D-V-V, D-V-V-D-D- allows for 5 vehicle (V) sessions and 5 drug (D) sessions in each two-week training period. The percent responses on the drug-appropriate lever following vehicle administration is observed to decrease with time; conversely the percent of re-

TABLE 2

TEST SESSION RESULTS INDICATING DISCRIMINATION OF VARIOUS DOSES OF MDE AND GENERALIZATION TO MDMA IN RATS (n=8) TRAINED TO DISCRIMINATE 2.0 mg/kg MDE FROM VEHICLE

Dose (mg/kg)	No. Trials	Quantal	Quantitative (SD)
2.0 MDE	4	91.1	82.5 (10.3)
1.0 MDE	2	72.2	78.8 (2.5)
0.5 MDE	2	25.0	34.5 (5.7)
0.0 (V)	4	6.3	17.5 (9.7)
ED_{50}		0.75	0.62
1.5 MDMA	2	100.0	83.5 (4.9)
1.0 MDMA	2	93.8	79.2 (2.1)
0.75 MDMA	2	62.5	62.4 (28.5)
0.5 MDMA	2	22.2	38.0 (17.0)
ED_{50}		0.65	0.60

Parallelism: Quantal—critical $t=3.18$ > calculated $t=1.04$; Quantitative—critical $t=3.18$ > calculated $t=0.58$.

TABLE 3

TIME COURSE OF THE MDE CUE AS SHOWN BY THE RESULTS OF TEST SESSIONS WITH VARIOUS INJECTION-TO-TESTING DELAYS

Post-Injection Time (min)	No. Trials	Quantal	Quantitative (SD)
5	2	43.8	43.4 (1.7)
10	2	100.0	88.5 (3.5)
20	4	95.8	91.4 (6.9)
60	2	75.0	70.9 (2.6)
120	2	12.5	22.5 (1.9)
240	2	12.5	21.3 (10.3)

sponses on the MDE-appropriate lever following drug administration generally increased. The number of sessions-to-criteria (STC), that is, when 8 sessions correct out of 10 consecutive sessions were reached twice [11], for the ten rats was attained in a mean of 21.3 sessions; thus, all the rats were judged able to correctly discriminate 2.0 mg/kg MDE from vehicle by the 38th training session (19 sessions with MDE and 19 sessions with vehicle).

Two rats were dropped from the study for repeatedly failing to maintain an 80% criterion during the testing phase (first ten presses) of subsequent maintenance sessions. In the remaining 8 rats, 2.0 mg/kg of MDE produced 91.1% quantal responding, whereas vehicle administration produced 6.3% responding on this lever (or 93.7% responses on the vehicle-correct lever) as presented in Table 2. Decreasing doses of MDE resulted in a progressively decreasing frequency of drug lever choices and this yielded a typical dose-response relationship for both the quantal and the quantitative measurement. Probit analysis of the quantal dose-response relationship [10] yielded an ED_{50} of 0.75 mg/kg, while similar analysis of the quantitative measurement resulted in an ED_{50} of 0.62 mg/kg.

Administration of 1.5 mg/kg of MDMA to MDE-trained rats resulted in 100% of the first-choice (quantal) responses on the MDE-appropriate lever, as also presented in Table 2. Decreasing doses of MDMA produced decreasing first choice selections upon the MDE-correct lever and a quantal dose-response relationship. Analysis of the MDE and MDMA dose-response lines indicated that they are parallel (critical $t=3.18$ > calculated $t=1.04$). Analysis of the dose-response data for MDMA yielded a quantal $ED_{50}=0.65$ mg/kg and a quantitative $ED_{50}=0.60$ mg/kg.

The onset of the MDE interoceptive stimulus cue is fast, i.e., 100% quantal responses 10 min following administration of 2 mg/kg MDE, as shown in Table 3. The MDE cue is not long lasting, i.e., 12.5% quantal responding 120 minutes after 2.0 mg/kg MDE administration. The quantitative value at 120 min post-administration is not significantly different from quantitative value for saline responding ($t=0.40$, $p<0.64$). Analysis of the time-course data from 20–120 min yielded a calculated half-life of approximately 60 min.

DISCUSSION

The results indicate that MDE, like MDMA [13], produces stimuli that can serve to control differential discriminative responding in the rat. The dose of MDE utilized, viz., 2.0 mg/kg, apparently produced few if any anorectic effects as shown by the animals' continued willingness to lever-press for food reward; this was a concern as MDMA was first synthesized by Merck and Co. Inc, as an anorectic [8]. The maintenance sessions resulted in a high level of discriminative control for both 2.0 mg/kg MDE (91.1%) and vehicle (93.7%). The discrimination of MDE was shown to be dose-responsive, and the ED_{50} for MDE (0.75 mg/kg) was approximately one-third the training dose.

MDMA, administered at 1.5 mg/kg, produced 100% MDE-like responding and decreasing doses of MDMA produced a dose-response curve parallel to the dose-response curve generated by MDE. Thus, MDMA produces many of the cues that provide the basis for discriminating MDE, and vice versa (unpublished results), suggesting that both MDE and MDMA share the same mechanism/site for producing the discriminative "cue" [9]. A common effect of these two drugs has previously been reported in that acute administration of 10 or 20 mg/kg of MDE produced 5-HT depletion in an amount similar to that produced by acute administration of 10 or 20 mg/kg MDMA [14]. In contrast, the time-course for the MDE cue appears to differ somewhat from that of MDMA. Whereas MDE responding in MDE-trained animals is not significantly different from saline after 120 minutes, MDMA responding in MDMA-trained animals is significantly different from saline until 240 minutes [13].

The calculated half-life of MDE was 60 min as compared to the calculated half-life for MDMA of approximately 100 min [13]. In comparisons of the ED_{50}s of MDMA vs. MDE (0.65 and 0.75, respectively) a ratio of 1.15 was obtained. The slight difference in potency for MDE as compared to MDMA has also been reported in humans [4], i.e., the range of effective oral doses for human psychotomimetic activity for MDMA as compared to MDE was 100–160 mg and 140–200 mg, respectively. Comparison of these human potencies results in a ratio of 1.4 for the lower dose range and 1.25 for the upper dose range. As a side-note, one former MDMA dealer described MDE as "a little milder than MDMA" [6]. Additionally the time course of MDE in humans has been

reported to be "slightly faster acting and shorter lived" than MDMA [15].

Further work will be required to determine what, if any, differences exist between MDE and MDMA regarding pharmacokinetics or neurochemical mediation of their specific stimulus properties. However, we can report that MDE shares similar pharmacological properties with MDMA and, perhaps, the same possible abuse potential. The implications of this finding may play a role in the future scheduling of MDE.

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