Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1533–1537, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Discriminative Profile of MDMA

MARTIN D. SCHECHTER

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

Received 25 November 1985

SCHECHTER, M. D. *Discriminative profile of MDMA*. PHARMACOL BIOCHEM BEHAV **24**(6) 1533–1537, 1986.— Groups of rats were trained to discriminate the stimulus properties of dopaminergically and/or serotonergically active drugs, viz., apomorphine, fenfluramine, tetrahydro-β-carboline (THBC) and *l*-cathinone. Once trained, these animals were given several doses of drugs used in training and dose-response relationships and ED50 values were generated. Subsequently, each group of trained rats was administered various doses of 3,4-methylenedioxymethamphetamine (MDMA) to test generalization of the interoceptive cue of the drug used for training to MDMA. Rats trained to fenfluramine, THBC, and *l*-cathinone were observed to discriminate MDMA in a manner similar to the drug state to which they had been trained. Analysis of dose-response curves suggested that MDMA may be acting both as an indirect dopaminergic agonist and as a serotonergic receptor agonist. This duality of effect of MDMA has been evidenced by other studies and may account for its present abuse potential.

MDMA Fenfluramine Drug discrimination Apomorphine Cathinone
Tetrahydro-β-carboline Dopamine Serotonin

MDMA (3,4-methylenedioxymethamphetamine) is a hallucinogenic drug that was first synthesized in 1960 [4]. It has recently received much attention in the lay press (e.g., [1, 2, 24]) as a result of its July 1, 1985 assignment, by the Drug Enforcement Administration (DEA), as a highly restricted Schedule 1 drug. The drug, known on the streets as "ecstasy," is chemically related to both amphetamine and mescaline and users maintain that it intensifies emotional feelings without sensory distortion, and increases perceptions of self-insight, empathy, and esthetic awareness. Indeed, MDMA's apparent ability to relax inhibitions and enhance communication has been recognized by psychotherapists in their practices and it is estimated that 35 to 200 physicians were using the drug on their patients prior to the DEA ban [2]. Non-medical MDMA use has, in addition, been estimated to have reached 30,000 doses per month in 1985 [2]. Although some scientific investigations have been conducted on the behavioral effects of the MDMA analogue, 3,4-methylenedioxyamphetamine or MDA [6, 9, 16], there are few published scientific reports concerning either the psychopharmacological action of MDMA in man or its behavioral effects in laboratory animals [3, 18, 25].

The behavioral paradigm that employs the discriminative stimulus effect of a psychoactive drug has been shown to be stable, sensitive, and specific in determining the mechanism of drug action. Within this technique, which is essentially a drug detection procedure, animals are trained to discriminate between a drug state and a non-drug state using operant behavioral techniques. The usefulness of this procedure in determining the mechanism of drug action resides in the animals' learning and retaining the acquired drug-induced discrimination or interoceptive cue. Once animals learn to make a differential response to the discriminative stimulus produced by one drug, they can be tested with a second drug in order to test their ability to detect the latter agent. The specificity of this detection has allowed classification of drugs and this evidence suggests that the discriminative stimulus effect of a particular drug is a result of a specific drug-receptor interaction. Thus, for two drugs to produce a similar discriminable effect, i.e., transfer, generalize or substitute for each other, they need to mutually act at similar receptors sites in the brain [7].

This laboratory has been extensively involved in the use of this behavioral paradigm to study drugs that are proported to act upon dopaminergic and/or serotonergic neurons and, thus, numerous groups of rats have been trained to discriminate between the effects of a specific drug and its (non-drug) vehicle (saline). The purpose of this investigation was to employ these well-trained groups of animals, by administering various doses of MDMA to them, in order to classify this agent as similar or dissimilar to the trained drug and to generate suggestive evidence as to the mechanism of MDMA action.

METHOD

Subjects

The rats used to investigate the effects of MDMA had been previously trained to discriminate the stimulus properties of other drugs. Thus, groups of rats were trained to discriminate each of these agents from the (non-drug) vehicle: apomorphine [11], fenfluramine [21], tetrahydro-β-carboline [22] and *l*-cathinone [23]. While each trained group of rats varied in number, sex, weight and age (see Table 1), the level of discriminative training was maintained at a specified criterion level (see below) and this training was employed as the "starting point" to test MDMA in its ability to generalize from the trained drug cue.

All rats were housed in individual living cages and their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80–85% of their free-feeding weights. Water was continuously available in the home

TABLE 1

DISCRIMINATIVE EFFECT OF MDMA IN RATS TRAINED TO DISCRIMINATE OTHER DRUGS

Drug	Dose mg/kg	Quantal	Quantitative (SD)	No. rats; Sex (No. Trials Each)	Delays:min Mean (Range)
Apomorphine	0.16	90.0	82.6 (7.2)	10; ♂ (4)	
Saline	—	12.5	17.9 (9.7)	(4)	
MDMA	1.0	25.0	35.9 (0.2)	(2)	
	1.5	65.0	56.1 (3.0)	(2)	
	2.0	45.0	51.8 (6.6)	(2)	36.4 (8–183)
Apomorphine	0.16	90.5	76.1 (13.2)	7; ♀ (4)	
Saline	—	0.0	2.5 (0.7)	(4)	
MDMA	1.0	0.0	12.0 (0.8)	(2)	
	1.5	42.9	48.6 (11.0)	(2)	
	2.0	50.0	50.7 (6.6)	(2)	9.9 (5–30)
Fenfluramine	2.0	88.0	85.6 (14.4)	5; ♂ (5)	
Saline	—	0.0	5.6 (5.1)	(5)	
MDMA	0.5	20.0	31.2 (18.7)	(2)	
	1.0	70.0	68.0 (8.1)	(2)	
	1.5	80.0	77.9 (23.3)	(2)	
	2.0	90.0	78.5 (6.8)	(2)	0.5 (0–5)
THBC	20.0	98.0	94.6 (5.0)	10; ♂ (4)	
Vehicle	—	4.0	11.2 (4.6)	(4)	
MDMA	1.0	35.0	41.5 (14.3)	(2)	
	1.5	75.0	66.5 (0.5)	(2)	
	2.0	95.0	85.5 (0.9)	(2)	5.5 (0–35)
l-Cathinone	0.6	97.4	92.1 (3.6)	9; ♂ (5)	
Saline	—	3.7	15.3 (5.9)	(5)	
MDMA	1.0	16.7	27.7 (7.6)	(2)	
	1.5	44.4	53.0 (12.3)	(2)	
	2.0	55.6	53.8 (8.8)	(2)	
	2.5	88.9	65.2 (2.5)	(2)	18.6 (0–45)

cages, which were kept at a constant temperature (20–22°C) and maintained on a 12-hour light/12 hour dark daily cycle.

Apparatus

The apparatus consisted of eight identical standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN) each equipped with two operant levers located 7 cm apart and 7 cm above the gridded floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the two levers. The operant chamber was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9 W house light. Solid-state programming equipment (Med Associates, E. Fairfield, VT) was used to control experimental contingencies and record responses, and was located in an adjacent room.

Discriminative Training

Drug discrimination training in each group of rats was based upon procedures described in detail elsewhere [11, 21–23]. In all cases, there were two training phases. In the first phase, the food-deprived rats learned to press the lever indicating saline administration and received a food reward (45 mg Noyes pellet) for each correct response, on a fixed-ratio 1 (FR1) schedule. This schedule was made progressively lengthened, in daily 15 min sessions over 8–10 days, until a FR10 schedule was achieved, i.e., the rat had to press the lever 10 times to receive reinforcement. Throughout lever press training, all rats received daily intraperitoneal (IP) injections of saline (0.9% sodium chloride, 1 ml/kg) 15–30 min (according to the drug used in training) prior to being placed into the two-lever operant chamber. Immediately following saline administration training, the opposite lever was activated and rats received a food reward for each correct response (FR1 schedule) after the IP administration of an equal volume of saline containing one of the training drugs. The number and sex of the rats and the time of testing/training after injection for each training drug were: apomorphine—0.16 mg/kg, 10 males, 7 females, 20 min; fenfluramine—2.0 mg/kg, 10 males, 30 min; tetrahydro-β-carboline (THBC)—20 mg/kg, 10 males, 30 min; *l*-cathinone—0.6 mg/kg, 9 males, 15 min. Daily sessions of 15 min duration, with drug administration, were conducted until a FR10 schedule was attained. In order to minimize the effects due to any position preference, the rats in each group were divided into two equal (or unequal in the cases of odd number) subgroups. For one subgroup, responding on the left lever was reinforced by delivery of food pellets in every session following drug injection, whereas the other subgroup was reinforced with food after responding on the right lever following drug injection. Responses on the opposite lever were reinforced with food pellets after saline administration.

The second phase of drug adminstration then began. The rats were trained 5 days per week with reinforcement in a pseudorandom sequence. Thus, in each two-week period, there were five days with drug lever (D) and five days with saline lever (S) correct. The pattern was D,S,S,D,D,;

TABLE 2
ED50 FOR TRAINING DRUGS AND MDMA SUBSTITUTION

Drug Trained	Reference	ED50 (95% conf. limits)	MDMA ED50	Parallelism [15]
Apomorphine ♂	[11]	0.051 (0.01–0.12)	1.972 (0.85–3.30)	
Apomorphine ♀	[11]	0.049 (0.02–0.14)	1.860 (1.38–2.51)	Yes
Fenfluramine	[21]	0.416 (0.19–0.92)	0.820 (0.51–1.32)	Yes
THBC	[22]	3.162 (1.70–5.88)	1.163 (0.94–1.43)	No
l-Cathinone	[23]	0.191 (0.12–0.30)	1.602 (1.26–2.04)	Yes

S,D,D,S,S. The rats had to respond on the appropriate lever to receive food reinforcement. Which lever was correct was dependent upon whether the training drug or saline had been administered prior to the start of the session. Responses upon the inappropriate lever were recorded, but they had no programmed consequences. The training criterion was reached when the animal selected the appropriate lever, according to the drug state imposed at the onset of each training session, on at least eight of ten consecutive sessions.

Dose-Response Relationships to Training Drugs

After the rats attained the discriminative training criterion with the particular drug used to train that group of rats, testing and training sessions of 15 min duration with alternating administrations of either the drug used for training or its vehicle were continued on Mondays, Wednesdays and Fridays. The procedure had the intent of maintaining and ensuring discrimination to the trained drug conditions. On Tuesdays and Thursdays, the rats of each group were injected IP with one of several different doses of the trained drug and, at the same time after injection as used in training, they were placed into the operant chamber. These doses were chosen from the available literature. The rats were allowed to lever press, without receiving reinforcement, until 10 presses were made on either lever. To preclude training at a drug dose different than that employed to train the animals, the rats were immediately removed from the operant chamber once the total responses on one lever reached 10 presses. Each of the test doses of drugs was tested in each animal on two occasions with each test preceded by both a drug and a saline maintenance session.

Transfer of Discrimination to MDMA

After the dose-response experiments, each group of rats was administered one of various doses of MDMA to test generalization to MDMA from the trained drug condition. Upon pressing one lever ten times after administration of the MDMA dose, the rat was removed from the operant chamber without receiving reinforcement. MDMA was administered in a constant volume of 1 mg/ml and tested at the same time after administration as was the training drug.

Measurements and Statistics

The first lever that was pressed 10 times was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for its training drug was the quantal measurement of discrimination. In addition, the total number of lever presses on both levers made before completion of the ten press criterion on either lever was counted constitutes the quantitative measurement of discrimination, i.e., the number of responses on the drug-correct lever divided by total responses made (including the 10 on the drug-correct lever) times 100. The advantage in using both measurements has been discussed by Stolerman and D'Mello [26]. The quantal data for all of the dose-response experiments, i.e., training drugs and MDMA, were analyzed by the method of Litchfield and Wilcoxon [15] which employs probits vs. log-dose effects, generates ED50's and tests for parallelism. Verification of analysis was made on a TRS-80 computer using published computer programs [27].

Drugs

Apomorphine hydrochloride, purchased from Sigma Chemicals, was dissolved in saline, freshly prepared daily and protected from light. The *l*-isomer of cathinone as the hydrochloride salt, as well as (±) MDMA, was provided by Dr. Richard Hawks of the National Institute of Drug Abuse. The hydrochloride salt of tetrahydro-β-carboline was prepared by dissolving norleagnine, purchased from Sigma Chemicals, in absolute ethanol modified with concentrated HCl to a final pH of 3.8 and recrystallized at 4°C. All drugs were dissolved in saline and administered IP in a volume of 1 ml/kg.

RESULTS

The results of testing the generalization of MDMA in rats previously trained to discriminate other drugs is presented in Table 1. For the two groups (of female and male rats) trained to discriminate 0.16 mg/kg apomorphine from saline, no dose of MDMA produced greater than 65% selected lever responding (quantal measurement) upon the apomorphine-correct lever. Doses higher than the highest dose (2.0 mg/kg) of MDMA tested were precluded by the appearance of behavioral disruption (represented as "delays" in Table 1) at that dose.

In contrast, 2.0 mg/kg MDMA produced 90.0 and 95.0% drug-appropriate responding when tested in rats trained to discriminate 2.0 mg/kg fenfluramine and 20 mg/kg THBC, respectively. Once again, this dose of MDMA produced slight behavioral disruption. Decreasing doses of MDMA, administered in random sessions, produced dose-responsive decreases in discriminative performance, both in terms of quantal and quantitative measurements, in these two groups of trained rats.

Lastly, rats trained to discriminate 0.6 mg/kg *l*-cathinone were observed to choose the *l*-cathinone-appropriate lever on 88.9% of sessions after the administration of 2.5 mg/kg

MDMA. Interestingly, the 2.0 mg/kg dose of MDMA produced no behavioral disruption (delays in min) in this group; this was only seen at the next highest dose of 2.5 mg/kg.

Comparison of the ED50 (with 95% confidence limits) derived from best-fitted dose-response curves [15] for the quantal measurements of the training drug (from [11, 21–23]) and for the substituted MDMA (from Table 1) appear in Table 2. In addition, tests for parallelism of the curves [15] are presented. Thus, apomorphine tested at doses of 0.04–0.32 mg/kg [11] produced a standard dose-response relationship in both male and female rats with ED50's of 0.051 and 0.049 mg/kg, respectively. Generalization tests with MDMA (Table 1) produced a dose-response relationship that allowed calculation of an ED50 of 1.97 mg/kg in the male rats and 1.86 mg/kg in the female rats trained to apomorphine. In both sexes, the dose-response lines for apomorphine and MDMA were parallel within 95% statistical limits, i.e., the critical $t <$ calculated t [15].

Likewise, testing of 0.25–2.0 mg/kg fenfluramine in fenfluramine-trained rats [21] yielded an ED50 of 0.416 mg/kg and MDMA testing produced an ED50 of 0.82 mg/kg with a dose-response curve parallel to that generated for fenfluramine. Administration of 1.25–20.0 mg/kg THBC in THBC-trained rats [22] produced an ED50 of 3.162 mg/kg and analysis of MDMA substitutions generated a dose-response curve with an ED50 of 1.163 mg/kg. Comparison of these two curves indicated that they were not parallel, i.e., calculated $t = 0.345 <$ critical $t = 3.182$ [15].

Testing of 0.15–0.6 mg/kg of *l*-cathinone-trained rats was, once again, dose-responsive with lower doses producing fewer drug-appropriate lever selections [23] and yielding an ED50 of 0.191 mg/kg. MDMA administration produced a parallel dose-response curve (calculated $t = 2.48 <$ critical $t = 3.182$; [15]) and an ED50 of 1.602 mg/kg.

DISCUSSION

The drug discrimination paradigm was employed as a detection method in the present study to evaluate the similarity or dissimilarity of the discriminative cue produced by MDMA as compared to the interoceptive cue produced by other drugs used to train rats. Thus, in rats of both sexes trained to discriminate 0.16 mg/kg apomorphine, MDMA produced only partial drug-appropriate responding. This inability of MDMA to generalize completely for apomorphine has been observed to occur with similar tests with amphetamine [12,20], lisiride [29] and bromocriptine [13]. Although "it is inappropriate to express the relative activities to two drugs in terms of potency, unless they. . . exert the same maximum effect" [14], comparison of the dose-response curves of apomorphine and MDMA are parallel (Table 2). These observations would suggest that MDMA is acting by a dopaminergic mechanism as does apomorphine [20]. Furthermore, the ability of MDMA to generalize completely for the indirect-acting dopamine agonist *l*-cathinone would indicate an indirect effect, i.e., MDMA may produce its disciminative stimulus properties (cue) by releasing pre-synaptic dopamine.

Fenfluramine has been observed to produce its discriminable effects in rats by mediation of brain serotonergic systems [10, 17, 21, 28], probably by pre-synaptic release of this neurotransmitter. In the present study, MDMA was shown to generalize to the fenfluramine cue suggesting a serotonergic component to its discrimination properties. This possibility was further evidenced by the generalization of THBC discrimination to MDMA in that THBC is active upon serotonergic neuronal systems [22]. The non-parallelism of the THBC and MDMA dose-response curves in the present study would, furthermore, suggest that MDMA may not be active upon the same subtype of serotonin receptors as is THBC [14]. As THBC may be more active on tryptamine and/or 5HT1 receptors [22], the possibility exists that MDMA is acting specifically at 5HT2 receptors, an action that has been observed in receptor affinity studies using other hallucinogenic drugs [5]. However, the discriminative stimulus effect of a particular drug is not always a result of a specific drug-receptor interaction. In order to ascertain this interaction, attenuation of discrimination should be demonstrated by pretreatment with specific antagonists and this data compared to other data based upon neurochemical assays and electrophysiological tests.

In conclusion, the present study has employed the drug discrimination technique as a screening method to evaluate the possible mechanism of action of MDMA. Results would suggest that MDMA is acting both as an indirect dopaminergic agonist and upon a serotonergic subtype of receptors, viz., 5HT2. This amphetamine-like (dopaminergic) and hallucinogenic-like (serotonergic) duality for the effect of a drug has previously been suggested to occur with the MDMA analogue MDA [8,19] and with MDMA [18]. It is these stimulant and hallucinogen properties that may account for the present abuse potential of MDMA.

ACKNOWLEDGEMENTS

The author would like to thank Denise Lovano-McBurney, Nancy L. Greer and Ron Maloney for their assistance in various aspects of this study. In addition, gratitude is also expressed to Dr. Richard Glennon for his thoughtful suggestion regarding the conduct of these experiments, and to Dr. Richard Hawks of the NIDA for generously supplying MDMA and *l*-cathinone.

REFERENCES

1. Adler, J., P. Abramson, S. Katz and M. Hager. Getting high on "Ecstasy." *Newsweek*, April 15, 1985, p. 96.
2. American Medical News, June 14, 1985, p. 18.
3. Anderson, G. M., III, J. Braun, U. Braun, D. E. Nichols and A. T. Shulgin. *Absolute Configuration and Psychotomimetic Activity. NIDA Research Monograph No. 22*, edited by G. Barnett, M. Trsic and R. Willette, 1978, pp. 8–15.
4. Biniecki, S. and E. Krajewski. Preparation of DL 1-(3,4-methylenedioxyphenyl)-2-(methylamino)-propane and DL 1-(3,4-dimethoxyphenyl)-2-(methylamino)-propane. *Acta Pol Pharm* **17:** 421–426, 1960.
5. Buckholtz, N. S., D. X. Freedman and L. D. Middaugh. Daily LSD administration selectively decreases serotonin$_2$ receptor binding in rat brain. *Eur J Pharmacol* **109:** 421–425, 1985.

6. Davis, W. M. and R. F. Borne. Pharmacologic investigations of compounds related to 3-4 methylenedioxyamphetamine (MDA). *Subst Alcohol Actions Misuse* **5:** 105–110, 1984.
7. Glennon, R. A. and J. A. Rosecrans. Speculations on the mechanism of action of hallucinogenic indolealkylamines. *Neurosci Biobehav Rev* **5:** 197–207, 1981.
8. Glennon, R. A. and R. Young. MDA: An agent that produces stimulus effects similar to those of 3,4 DMA, LSD and cocaine. *Eur J Pharmacol* **99:** 249–250, 1984.
9. Glennon, R. A., R. Young, J. A. Rosecrans and G. M. Anderson. Discriminative stimulus properties of MDA analogues. *Biol Psychiatry* **17:** 807–814, 1982.
10. Gourdie, A. J. Discriminative stimulus properties of fenfluramine in an operant task: An analysis of its cue function. *Psychopharmacology (Berlin)* **53:** 97–102, 1977.
11. Greer, N. L. and M. D. Schechter. D_1 and D_2 dopamine receptor mediation of a behavioral effect of apomorphine. *Life Sci*, submitted.
12. Hernandez, L. L., A. M. Holohean and J. B. Appel. Effects of opiates on the discriminative stimulus properties of dopamine agonists. *Pharmacol Biochem Behav* **9:** 459–463, 1978.
13. Holohean, A. M., F. J. White and J. B. Appel. Dopaminergic and serotonergic mediation of the discriminable effects of ergot alkaloids. *Eur J Pharmacol* **81:** 595–602, 1982.
14. Levine, R. R. *Pharmacology: Drug Actions and Reactions*, second edition. Boston: Little, Brown, and Co., 1978, p. 179.
15. Litchfield, J. T. and F. W. Wilcoxon. A simplified method of evaluating dose-effect relationships. *J Pharmacol Exp Ther* **96:** 99–113, 1949.
16. Marquardt, G. M., V. DiStefano and L. L. Ling. Pharmacological effects of (±)-, (S)-, and (R)-MDA. In: *The Psychopharmacology of Hallucinogens*, edited by R. C. Stillman and R. E. Willette. New York: Pergamon Press, 1984, pp. 84–104.
17. McElroy, J. F. and R. S. Feldman. Discriminative stimulus properties of fenfluramine: Evidence for serotonergic involvement. *Psychopharmacology (Berlin)* **83:** 172–178, 1984.
18. Nichols, D. E., D. H. Lloyd, A. J. Hoffman, M. B. Nichols and G. K. W. Yim. Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes. *J Med Chem* **25:** 530–535, 1982.
19. Nozaki, M., D. B. Vaupel and W. R. Martin. A pharmacologic comparison of 3,4-methylenedioxyamphetamine and LSD in the chronic spinal dog. *Eur J Pharmacol* **46:** 339–349, 1977.
20. Schechter, M. D. Different dopaminergic mechanisms for amfonelic acid, amphetamine and apomorphine. *Pharmacol Biochem Behav* **13:** 497–500, 1980.
21. Schechter, M. D. Differential effects of fenfluramine in obese and lean Zucker rats. *Eur J Pharmacol*, in press.
22. Schechter, M. D. Serotonergic mediation of tetrahydro-β-carboline. *Pharmacol Biochem Behav* **24:** 1209–1213, 1986.
23. Schechter, M. D. Discriminative properties of *l*-cathinone compared to *dl*- and *d*-cathinone. *Pharmacol Biochem Behav* **24:** 1161–1165, 1986.
24. Shafer, J. MDMA: Psychedelic drug faces regulation. *Psychology Today*, May, 1985, pp. 68–69.
25. Shulgin, A. T. Characterization of three new psychotomimetics. In: *The Psychopharmacology of Hallucinogens*, edited by R. C. Stillman and R. E. Willette. New York: Pergamon Press, 1984, pp. 74–83.
26. Stolerman, I. P. and G. D. D'Mello. Role of training conditions in discrimination of central nervous system stimulants by rats. *Psychopharmacology (Berlin)* **73:** 295–303, 1981.
27. Tallarida, R. J. and R. B. Murray. *Manual of Pharmacologic Calculations With Computer Programs*. New York: Springer-Verlag, 1981, pp. 59–63 and 119–121.
28. White, F. J. and J. B. Appel. A neuropharmacological analysis of the discriminative stimulus properties of fenfluramine. *Psychopharmacology (Berlin)* **73:** 110–115, 1981.
29. White, F. J. and J. B. Appel. The role of dopamine and serotonin in the discriminative stimulus properties of lisuride. *J Pharmacol Exp Ther* **221:** 421–427, 1982.