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Further Investigation of the Discriminative Stimulus Properties of MDA

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GLENNON, R. A. AND R. YOUNG. *Further investigation of the discriminative stimulus properties of MDA.* PHARMACOL BIOCHEM BEHAV **20**(4) 501–505, 1984.—Rats trained to discriminate either (+)-amphetamine or (±)-MDA from saline in a two-lever drug discrimination task, were used to study the stimulus effects of MDA and its two optical isomers. Amphetamine-stimulus generalization occurred to S(+)-MDA, but not to its enantiomer R(−)-MDA. This, coupled with our earlier finding of DOM-stimulus generalization to R(−)-MDA but not to S(+)-MDA, suggests that the stimulus effects of S(+)-MDA are predominantly amphetamine-like while those of R(−)-MDA are more DOM-like. Thus, animals trained to discriminate racemic MDA from saline can apparently recognize members of both classes of agents.

MDA 3,4-Methylenedioxyamphetamine Amphetamine Hallucinogens DOM CNS stimulants Drug discrimination

WE have recently found that racemic 1-(3,4-methylenedioxyphenyl)-2-aminopropane (3,4-methylenedioxyamphetamine; MDA) is capable of producing a dual stimulus effect in rats [3]. That is, animals trained to discriminate MDA from saline in a two-lever drug discrimination procedure elicit MDA-appropriate responses when administered certain doses of either the CNS stimulant (±)-amphetamine or the hallucinogenic agent 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). In tests of discriminative control of behavior, stimulus cues are ordinarily considered to be rather specific [6]; MDA appears to be an exception to this concept. In animals trained to discriminate racemic DOM from saline, stimulus generalization occurs upon administration of racemic MDA [5], but not upon administration of amphetamine [3,17]. Using animals trained to discriminate (+)-amphetamine sulfate from saline, administration of racemic MDA [3] results in stimulus generalization, while administration of DOM does not [14,17]. Although the stimulus properties of DOM and amphetamine apparently differ, racemic MDA appears to mimic both. This is consistent with the results of other investigations, using human [1,15] or non-human [10,11] subjects, in that MDA can produce effects that are both amphetamine-like and hallucinogenic-like (i.e., LSD- or DOM-like).

MDA exists as a mixture of optical isomers; hence, the possibility exists that one isomer may be responsible for the amphetamine-like effects while the hallucinogenic or psychotomimetic effects may be the result of the other isomer. The present investigation was conducted to explore this possibility, and to further explore the stimulus properties of the optical isomers of MDA.

METHOD

The animals used in this study were twenty-one 150-day old male Sprague-Dawley rats that weighed between 250–300 g (mean=281 g) at the beginning of training. The discrimination training procedure used for these animals has been reported [3]. The animals were reduced to approximately 80% of their free-feeding weight by partial food deprivation, and were then trained to press one of two levers in a standard operant chamber (Coulbourn Instruments) for food (0.01 ml of sweetened condensed milk) reward under a fixed-ratio 1 schedule of reinforcement. The schedule of reinforcement was gradually increased to a variable interval 15-sec (VI-15) schedule of reinforcement; training was continued until rates of responding stabilized. At this time, the rats were administered, via intraperitoneal injection, either (+)-amphetamine sulfate (1.0 mg/kg, N=14) or (±)-MDA (1.5 mg/kg, N=7), paired with saline (1 ml/kg), 15 min before each session. For approximately half of the animals in each of the two groups, responses on the right lever were reinforced after drug administration, while for the other half, responding on the left lever was reinforced after drug administration. Discrimination training sessions were of 15 min duration; drug or saline was administered on a double alternation schedule. Each block of four training sessions is represented (for MDA) in Fig. 1. as a "Session." On every fifth day, discrimination learning was assessed during an initial 2.5 min extinction session; this was followed by a 12.5 min training session. The animals were said to have achieved criteria when greater than 85% of their total responses were made on the drug-designated lever following administration of drug, and fewer than 15% of total responses were made on the same lever following administration of saline.

During the substitution investigation, test sessions, where the animals were allowed 2.5 min of non-reinforced lever responding and were then returned to their individual home cages, were interposed between training sessions. Throughout the substitution studies, maintenance of the discrimination was insured by the random administration of training drug or saline (except on testing days), with the constraint

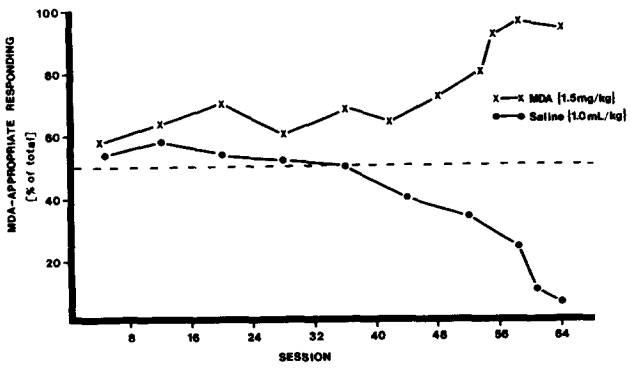

FIG. 1. Time-course for the acquisition of the MDA (1.5 mg/kg) vs. saline discrimination.

that no more than two consecutive administrations of training drug or saline were allowed. Substitution testing investigated the ability of the rats trained to (+)-amphetamine or (±)-MDA to show transfer to members of a series of phenylisopropylamine derivatives.

Drugs

Racemic, S(+)-, and R(−)-MDA hydrochloride were gifts from NIDA. Racemic, S(+)- and R(−)-amphetamine were used as their sulfate salts while S(+)-methamphetamine was employed as the hydrochloride salt. Racemic N-methyl MDA hydrochloride [i.e., N-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane; MDMA] was prepared according to literature methods [1]. All solutions were prepared fresh daily in sterile saline.

RESULTS AND DISCUSSION

The MDA discrimination was a difficult task for the animals to learn, as judged by the length of time necessary to acquire the discrimination (Fig. 1). Nevertheless, at the end of 64 sessions, the animals consistently made greater than 85% of their total responses on the drug-designated lever after administration of racemic MDA, and fewer than 15% on the same lever after administration of saline. Administration of S(+)- and R(−)-MDA, as well as the N-methyl derivative of MDA, i.e., racemic MDMA, to the MDA-trained animals resulted in stimulus generalization (Table 1). In the (+)-amphetamine-trained animals, stimulus generalization occurred to both optical isomers of amphetamine, S(+)-methamphetamine, S(+)-MDA and (±)-MDMA, but not to R(−)-MDA or S(+)-DOM (Table 2). Saline administration to either group of animals consistently produced less than 10% drug-appropriate responding. The animals response rates after administration of saline ranged from 12–15 (MDA-trained group), and 13–16 (amphetamine-trained group) responses/min.

Although (±)-, (+)- and (−)-amphetamine have been used as training drugs in previous drug discrimination studies (see

TABLE 1

RESULTS OF GENERALIZATION STUDIES USING (±)-MDA AS TRAINING DRUG

Agent	Dose (mg/kg)	N*	MDA-Appropriate Responding† (±SEM)	Mean Resp/ Min† (±SEM)	ED_{50}‡ (mg/kg)
(±)-MDA§					0.65
S(+)-MDA	0.25	4/4	34% (14.1)	10.8 (1.1)	
	0.5	4/4	42% (18.5)	10.6 (1.4)	
	0.75	4/4	46% (23.1)	11.0 (1.0)	
	1.0	4/4	85% (7.7)	9.7 (1.5)	0.51 (0.22–1.15)
R(−)-MDA	1.0	4/4	29% (8.5)	10.4 (2.3)	
	1.25	4/4	66% (18.6)	11.4 (1.9)	
	1.5	4/4	71% (14.4)	10.3 (1.8)	
	2.0	4/4	83% (10.3)	11.0 (2.4)	1.18 (0.79–1.75)
(±)-Amphetamine§					1.93
(±)-MDMA	0.75	3/3	31% (15.1)	10.7 (2.2)	
	1.0	3/3	50% (21.4)	7.9 (1.0)	
	1.1	3/3	62% (16.0)	9.3 (1.3)	
	1.5	6/7	83% (10.8)	7.7 (1.0)	
	1.65	1/4	—¶		
	1.75	1/4	—		
	2.0	1/4	—		
	2.5	1/4	—		0.96 (0.68–1.36)

*Number of animals responding/number of animals receiving drug.
†Data obtained during 2.5-min extinction sessions.
‡Followed by 95% confidence limits.
§Data previously reported [3]; included for comparative purposes.
¶Disruption of behavior (i.e., no responding).

TABLE 2

RESULTS OF GENERALIZATION STUDIES USING (+)-AMPHETAMINE AS TRAINING DRUG

Agent	Dose (mg/kg)	N*	Amphetamine Appropriate Responding (±SEM)	Mean Resp/ Min† (±SEM)	ED_{50}‡ (mg/kg)
(±)-Amphetamine§					0.62
S(+)-Amphetamine	0.25	4/4	15% (5.7)	13.1 (1.3)	
	0.35	5/5	48% (9.0)	17.8 (3.3)	
	0.50	5/5	61% (13.2)	13.5 (1.7)	
	0.75	5/5	79% (10.6)	14.6 (1.8)	
	1.0	14/14	94% (3.1)	14.9 (1.4)	0.42 (0.29–0.62)
R(−)-Amphetamine	1.0	4/4	38% (10.8)	12.8 (3.5)	
	2.0	4/4	72% (18.4)	14.3 (3.2)	
	2.0	4/4	95% (4.7)	12.0 (1.7)	1.23 (0.73–2.09)
S(+)-Methamphetamine	0.25	4/4	19% (14.3)	11.2 (1.3)	
	0.50	4/4	48% (17.3)	10.5 (2.1)	
	0.75	4/4	100%	10.3 (1.9)	0.40 (0.23–0.68)
(±)-MDA§					2.29
S(+)-MDA	0.75	4/4	37% (9.0)	12.7 (1.9)	
	1.0	7/8	61% (12.3)	8.5 (2.0)	
	1.25	7/8	61% (14.9)	8.2 (2.7)	
	1.35	3/4	89% (10.0)	5.3 (2.4)	
	1.4	3/4	93% (5.4)	4.8 (2.1)	
	1.5	0/4	—¶		0.90 (0.70–1.17)
R(−)-MDA	1.5	4/4	23% (9.0)	8.8 (1.3)	
	2.0	3/4	25% (5.6)	5.5 (2.1)	
	2.25	1/4	—¶		
	2.5	0/4	—		—
(±)-MDMA	1.0	4/4	10% (5.8)	10.3 (1.2)	
	1.75	4/4	45% (18.4)	5.0 (1.0)	
	2.0	3/4	77% (12.5)	4.3 (1.5)	
	2.25	3/4	82% (11.0)	3.3 (0.9)	
	3.0	1/4	—¶		1.64 (1.19–2.27)
(±)-DOM§			—¶		—
S(+)-DOM	1.5	4/4	13% (6.4)	10.0 (1.7)	
	3.0	3/4	15% (3.5)	6.0 (2.7)	
	3.5	3/4	10% (4.6)	5.1 (1.8)	
	4.0	3/4	9% (5.7)	6.0 (2.8)	
	5.0	3/4	26% (14.5)	7.0 (2.5)	
	6.0	2/4	46% (18.9)	4.3 (1.4)	
	6.15	1/4	—¶		
	6.3	1/4	—		
	6.5	1/4	—		—

*Number of animals responding/number of animals receiving drug.
†Data obtained during 2.5-min extinction session.
‡Followed by 95% confidence limits.
§Data previously reported [3]; included for comparative purposes.
¶Disruption of behavior (i.e., no responding).

[8,16] for a review), this is the first time that the activity of the racemate has been compared, in the same study, with the activities of the individual amphetamine isomers. S(+)-Amphetamine was found to be approximately three times more active than its R(−)-enantiomer, and half again more active than the racemic mixture (Table 2); previous drug discrimination studies have yielded enantiomeric potency ratios of two to five [9, 13, 16]. This is consistent with the results of various other pharmacological studies that have found, depending on the particular parameter being measured, that the central potency of S(+)-amphetamine is two to ten times that of its enantiomer [12]. In similar studies,

N-methylation of amphetamine did not have a detrimental effect on, and even somewhat enhanced, the central activity of amphetamine [12]. As shown in Table 2, the potencies of S(+)-amphetamine and S(+)-methamphetamine are essentially identical. It has been demonstrated, using animals trained to discriminate amphetamine from saline, that stimulus generalization does not occur upon administration of (±)-DOM [3,14], or either isomer of DOM [17]; the results shown in Table 2 for S(+)-DOM are consistent with these prior findings. As with amphetamine, S(+)-MDA is more potent than its racemate (Table 2); however, administration of R(−)-MDA failed to produce amphetamine-appropriate responding. Finally, N-methylation of racemic MDA (i.e., MDMA) resulted in a slight enhancement in potency. Taken together, these results support the suggestion by Marquardt *et al.* [10] that the S(+)-isomer of MDA is responsible for the amphetamine-like effects of racemic MDA. This is particularly convincing in light of our earlier report that, using animals trained to discriminate either the phenalkylamine hallucinogen DOM, or the indolealkylamine hallucinogen 5-methoxy-N,N-dimethyltryptamine from saline, stimulus generalization occurs with (±)- and R(−)-MDA, but not with S(+)-MDA, [4,5]. Furthermore, we have reported that N-methylation of hallucinogenic phenalkylamines decreases their potency in tests of discriminative control of behavior [7], and we have speculated that N-methylation of MDA might decrease its "hallucinogenic" potency while, at the same time, have little effect on the stimulant component of its activity. In other words, N-methylation might unveil the amphetamine-like character of MDA [6]. While DOM-stimulus generalization occurred with (±)-MDA, it did not occur with (±)-MDMA [5]; on the other hand, racemic MDMA is somewhat more active than racemic MDA in amphetamine-trained animals (Table 2).

Although a racemic DOM-stimulus generalized to both R(−)-DOM and S(+)-DOM [6,17] and while an amphetamine-stimulus was demonstrated to generalize to neither isomer of DOM [17], it was thought worthwhile to examine more closely the stimulus properties of S(+)-DOM in order to determine whether or not amphetamine-stimulus generalization might occur as it did with S(+)-MDA. However, as shown in Table 2, administration of 6.0 mg/kg of S(+)-DOM resulted in only partial generalization (46% amphetamine-appropriate responding), while higher doses resulted in disruption of behavior.

Animals trained to discriminate racemic MDA from saline apparently recognize both aspects of the stimulus cue (i.e., the effects produced by S(+)-MDA and the effects produced by R(−)-MDA). This suggestion is supported by the finding that MDA-stimulus generalization occurs to both (±)-amphetamine and (±)-DOM [3]. However, because S(+)-MDA is twice as potent as R(−)-MDA, it appears that the stimulant portion of the stimulus cue is more readily recognized than the "hallucinogen" component. And, while a DOM-stimulus did not generalize to (±)-MDMA [5], the MDA-stimulus does (Table 1).

Racemic MDA has been demonstrated to produce a stimulus cue in rats that is dualistic in nature. In other words, the stimulus appears to be both amphetamine-like and DOM-like. At first appearance, this seems to argue against the concept of cue specificity. However, based on the results of the current study, it seems likely that each of the individual isomers is capable of producing a distinct stimulus cue; the effect of S(+)-MDA is predominantly amphetamine-like while the effect of R(−)-MDA is predominantly DOM-like.

In essence, training animals to discriminate (±)-MDA from saline is probably akin to training animals to discriminate a drug combination of a CNS stimulant and a hallucinogenic agent from saline. It might be anticipated that it would be difficult for the animals to learn such a stimulus cue; indeed, training the animals to discriminate MDA from saline required more than twice the time (Fig. 1) necessary to train rats to discriminate DOM from saline [18]. Nevertheless, the results of this study seem to parallel the results of human studies; R(−)-MDA is more active than either its racemate or S(+)-isomer in producing subjective effects in man, while there is a considerable stimulant component associated with S(+)-MDA [15]. The next logical step in studying the discriminative stimulus properties of MDA would be to train animals to either discriminate isomers of MDA from saline, or to discriminate between the isomers themselves.

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REFERENCES

1. Anderson, G. M., G. Braun, U. Braun, D. E. Nichols and A. T. Shulgin. Absolute configuration and psychotomimetic activity. In: *Quantitative Structure Activity Relationships of Analgesics, Narcotic Antagonists and Hallucinogens,* edited by G. Barnett, M. Trsic and R. E. Willette. Washington, DC: U. S. Government Printing Office, 1978, pp. 8–15.
2. Finney, D. J. *Probit Analysis.* London: Cambridge University Press, 1952.
3. Glennon, R. A. and R. Young. MDA: A psychoactive agent with dual stimulus effects. *Life Sci* **34:** 379–383, 1984.
4. Glennon, R. A., J. A. Rosecrans and R. Young. Behavioral properites of psychoactive phenylisopropylamines in rats. *Eur J Pharmacol* **76:** 353–360, 1981.
5. Glennon, R. A., R. Young, J. A. Rosecrans and G. M. Anderson. Discriminative stimulus properties of MDA and related agents. *Biol Psychiatry* **17:** 807–814, 1982.
6. Glennon, R. A., J. A. Rosecrans and R. Young. Drug-induced discrimination: A description of the paradigm and a review of its specific application to the study of hallucinogenic agents. *Med Res Rev* **3:** 289–340, 1983.
7. Glennon, R. A., R. A. Young and J. M. Jacyno. Indolealkylamine and phenalkylamine hallucinogens: Effect of α-methyl and N-methyl substituents on behavioral activity. *Biochem Pharmacol* **32:** 1267–1273, 1983.
8. Ho, B. T. and P. B. Silverman. Stimulants as discriminative stimuli. In: *Stimulus Properties of Drugs: Ten Years of Progress,* edited by F. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier North Holland Biomedical Press, 1978, pp. 53–68.
9. Jones, C. N., H. F. Hill and R. T. Harris. Discriminative response control by d-amphetamine and related compounds in the rat. *Psychopharmacologia* **36:** 347–356, 1974.
10. Marquardt, G. M., V. Distefano and L. L. Ling. Pharmacological effects of (±)-, (S)- and (R)-MDA. In: *Psychopharmacology of Hallucinogens,* edited by R. C. Stillman and R. E. Willette. New York: Pergamon Press, 1978, pp. 84–104.
11. Martin, W. R., D. B. Vaupel, J. W. Sloan, J. A. Bell, M. Nozaki and L. D. Bright. The mode of action of LSD-like hallucinogens and their identification. In: *Psychopharmacology of Hallucinogens,* edited by R. C. Stillman and R. E. Willette. New York: Pergamon Press, 1978, pp. 84–104.

12. Moore, K. E. Amphetamines: Biochemical and behavioral actions in animals. In: *Handbook of Psychopharmacology*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1978, pp. 41–98.
13. Schechter, M. D. Stimulus properties of d-amphetamine as compared to *l*-amphetamine. *Eur J Pharmacol* **47**: 461–464, 1978.
14. Shannon, H. E. MDA and DOM: Substituted amphetamines that do not produce amphetamine-like discriminative stimuli in the rat. *Psychopharmacology (Berlin)* **67**: 311–312, 1980.
15. Shulgin, A. T. Psychotomimetic Drugs: Structure-Activity relationships. In: *Handbook of Psychopharmacology*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1978, pp. 243–331.
16. Silverman, P. B. and B. T. Ho. Characterization of discriminative response control by psychomotor stimulants. In: *Discriminative Stimulus Properties of Drugs*, edited by H. Lal. New York: Plenum Press, 1977, pp. 107–119.
17. Silverman, P. B. and B. T. Ho. The discriminative stimulus properties of 2,5-dimethoxy-4-methylamphetamine (DOM). Differentiation from amphetamine. *Psychopharmacology (Berlin)* **68**: 209–215, 1980.
18. Young, R., R. A. Glennon and J. A. Rosecrans. Discriminative stimulus properties of the hallucinogenic agent DOM. *Commun Psychopharmacol* **4**: 501–506, 1981.