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The Effect of MDA and MDMA ("Ecstasy") Isomers in Combination With Pirenpirone on Operant Responding in Mice

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ROSECRANS, J. A. AND R. A. GLENNON. *The effect of MDA and MDMA ("Ecstasy") isomers in combination with pirenpirone on operant responding in mice.* PHARMACOL BIOCHEM BEHAV **28**(1) 39–42, 1987.—The behaviorally disruptive effects of the optical isomers of 1-(3,4-methylenedioxyphenyl-2-aminopropane) (MDA) and its N-methyl derivative (MDMA) were evaluated in 27 mice trained to bar-press for a liquid food reinforcement. In addition, a second study was conducted in which mice were pretreated with either saline or the 5-HT-2 antagonist, pirenpirone, prior to the administration of either MDMA or MDA using the same behavioral procedure. The results indicated that the behaviorally disruptive effects produced only by R(−)-MDA, but not those of S(+)-MDA, R(−)-MDMA, nor of S(+)-MDMA, were significantly attenuated by pirenpirone. These findings support previous research findings which indicate that this isomer may be producing its behaviorally disruptive effects via an action on 5-HT-2 receptors.

MDA MDMA Serotonin Hallucinogens Pirenpirone Schedule-controlled behavior

MDMA [N-methyl 1-(3,4-methylenedioxyphenyl)-2-aminopropane] is a novel phenylisopropylamine derivative that has been claimed to be of benefit as an adjunct to psychotherapy. This issue, however, is controversial in light of recent evidence that MDMA may possess abuse potential [15] and, that at high doses, MDMA is neurotoxic in animals (e.g., [13,16]). MDMA is the N-monomethyl analog of MDA or 1-(3,4-methylenedioxyphenyl)-2-aminopropane. In humans, MDA is a hallucinogenic agent with a strong central-stimulant component of action [14]. Comparable results have been obtained in drug discrimination studies in that rats trained to discriminate MDA from saline recognize (i.e., generalize to) hallucinogenic agents such as 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and lysergic acid diethylamide (LSD) and central stimulants such as cocaine and the unsubstituted phenylisopropylamine amphetamine [4,5]. Conversely, stimulus generalization occurs with MDA in animals trained to discriminate either DOM or amphetamine from saline [3]. Evidence suggests that the R(−)-isomer of MDA is primarily responsible for the hallucinogenic or DOM-like effects, whereas the S(+)-isomer is responsible for the central stimulant or amphetamine-like effects [3,12]. In amphetamine-trained animals, stimulus generalization also occurs with MDMA; however, in animals trained to discriminate DOM from vehicle, stimulus generalization did not occur with MDMA or with either of its optical isomers [3], suggesting that its effects are more amphetamine-like than DOM-like.

To date, the mechanism of action of MDMA is unknown. However, we have recently proposed that the mechanism of action of hallucinogenic phenylisopropylamines involves an agonist interaction at a particular subpopulation of serotonin (5-HT) receptors (i.e., 5-HT-2 receptors) [8]. Furthermore, upon investigating the structure-activity relationships of psychoactive phenylisopropylamines, we have found that N-monomethylation decreases the behavioral potency of hallucinogenic phenylisopropylamines, whereas it has little effect on (or can actually enhance) the potency of amphetamine-like phenylisopropylamines [3,10]. These results are consistent with the above-mentioned drug discrimination studies which suggest that MDMA is primarily an amphetamine-like agent.

Recently, we reported that disruption of schedule-controlled responding of mice might be a useful technique for the investigation of the optical isomers of behaviorally-active agents [6]. In the present study, we have employed this technique to compare the relative potencies of the optical isomers of MDA with those of MDMA. In addition, we wished to determine if the disruptive effects of any of these isomers might involve a 5-HT-2 mechanism by conducting antagonism studies using the 5-HT-2 selective antagonist pirenpirone.

METHOD

Operant Training

A total of twenty-seven mice were used in the present

study; eleven of these animals were those previously trained for the purpose of studying the optical isomers of MDMA [6]. The remaining mice were trained in a similar manner. That is, sixteen naive male ICR mice (30–35 g), housed in standard animal facilities with a 12-hr light/dark cycle, were maintained at constant weight by restricting their diet. The animals were trained to respond under an FR-20 schedule of reinforcement in a single-lever operant procedure; sessions were 15 min in duration. Animals were not limited to the number of reinforcements obtained during test and/or training sessions. The apparatus and training schedule have been previously described in detail [6,11]. Once the animals had learned the behavioral task, they were challenged with saline on five consecutive days in order to establish baseline responding. Subsequently, the mice received intraperitoneal injections of saline except on test days (Wednesdays and Saturdays); on these days, the animals were administered one of the test drug combinations.

Disruption Studies: Experiment I

Doses of racemic, R(−)- and S(+)-MDA were evaluated in groups of 5 to 10 animals. These studies employed the original group of eleven animals that had been previously used to evaluate MDMA and its optical isomers. Results are expressed as percent of vehicle response rate. Each animal served as its own control and the vehicle response rates are the averages of the response rate after administration of saline on the day prior to and the day after a test session. All drugs were administered via intraperitoneal injection 15 min prior to a 15-min test session. ED50 values were calculated from the dose-response data by the method of Finney [2].

Antagonism Studies: Experiment II

These studies involved only the newly trained group of sixteen mice; studies were conducted in a manner similar to that described above except that the mice were pretreated either with saline or pirenpirone prior to a dose of agonist. Preliminary studies were first conducted in order to establish an optimal dose, time course, and route of administration for pirenpirone. In these preliminary studies, the effects of DOM (an agent whose behavioral effects have been previously antagonized by pretreatment with pirenpirone [7]) on schedule-controlled responding was investigaed. Intraperitoneal doses of 1.0 to 4.0 mg/kg of DOM were administered 15 min prior to testing (ED50=2.7 mg/kg). It was subsequently determined (by evaluating various doses, times and routes of administration) that the subcutaneous injection of 0.1 mg/kg of pirenpirone 60 min prior to administration of 3.0 mg/kg of DOM resulted in complete antagonism (i.e., in 91% of baseline responding; n=8) relative to the effect of 3.0 mg/kg of DOM in combination with saline (i.e., 36% baseline responding; n=8). Furthermore, administration of 0.1 mg/kg of pirenpirone (by itself) 75 min prior to testing had no effect on the animals' response rates. Higher doses, e.g., 0.2 mg/kg and above, severely disrupted behavior. Thus, in the antagonism studies involving the isomers of MDA and MDMA, 0.1 mg/kg of pirenpirone was administered to groups of 5 to 10 animals via the subcutaneous route 60 min prior to the intraperitoneal administration of doses of S(+)-MDA, R(−)-MDA, S(+)-MDMA or R(−)-MDMA. In control experiments, 1.0 ml/kg of 0.9% sterile saline was administered 60 min prior to the administration of the same doses of the isomers of MDA and MDMA; mice were placed in the operant chamber 15 min later. The determination for the evaluation of % disruption was similar to that described above.

In these challenge experiments, individual studies were conducted using preliminary ED50 disruption data obtained in Experiment I. Experiments were conducted in which half of the animals to be tested received saline and the other half pirenpirone (5–8 mice in each group) 60 min prior to a specific dose of MDA or MDMA isomer; Student's *t*-tests were conducted to determine level of significance within each experiment. These studies were run such that the calculated ED50 dose of each isomer vs. pirenpirone was evaluated first. After this phase, higher and lower doses of each agonist were given pre-pirenpirone to obtain a more complete evaluation of these antagonism studies. Doses and drug combination assignments were randomized amongst the mice to be tested on a given day.

TABLE 1

EFFECT OF MDA AND ITS ISOMERS ON SCHEDULE-CONTROLLED RESPONDING

Agent	Dose	Percent of Baseline Responding (±SEM)	ED-50 (mg/kg) 95% C.I.
(±)-MDA	1.0	74 (3)	
	2.0	59 (4)	
	4.0	28 (10)	2.2 (1.1–4.7)
S(+)-MDA	1.0	86 (5)	
	2.0	58 (9)	
	4.0	29 (10)	2.5 (1.3–4.8)
R(−)-MDA	2.0	80 (6)	
	4.0	91 (12)	
	6.0	48 (10)	
	8.0	16 (5)	5.9 (4.3–8.3)
(±)MDMA*			4.1
S(+)-MDMA*			3.1
R(−)-MDMA*			11.6

*Data previously reported [6]; included for comparative purposes.

Drugs

Racemic MDA and MDMA and their optical isomers were prepared as the hydrochloride salts in our laboratories as previously described [6]. DOM hydrochloride and pirenpirone were gifts from NIDA and Janssen Pharmaceutica, Belgium, respectively. Solutions of all drugs were prepared fresh daily in (with the exception of pirenpirone) 0.9% sterile saline; pirenpirone was dissolved in an equivalent of 0.1 N hydrochloric acid and then diluted with sterile saline to the desired concentrations.

RESULTS

A significant finding of the present study was that MDA (Table 1), like MDMA [6], disrupts operant responding, and

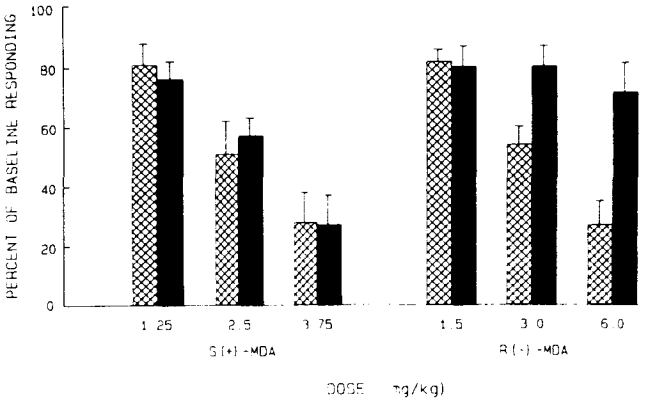

FIG. 1. Effects of S(+)- and R(−)-MDA on schedule-controlled responding. Mice were pretreated with either saline (hatched bars) or pirenpirone (0.2 mg/kg, SC) 60 min prior to the administration of MDA isomers. Results obtained with pirenpirone in combination with 3.0 ($df=13$) and 6.0 mg/kg ($df=14$) of R(−)-MDA are significantly different from those obtained with saline in combination with the same doses of R(−)-MDA; t values >0.05. Comparisons were made at each dose level (N at each dose=13–14 mice).

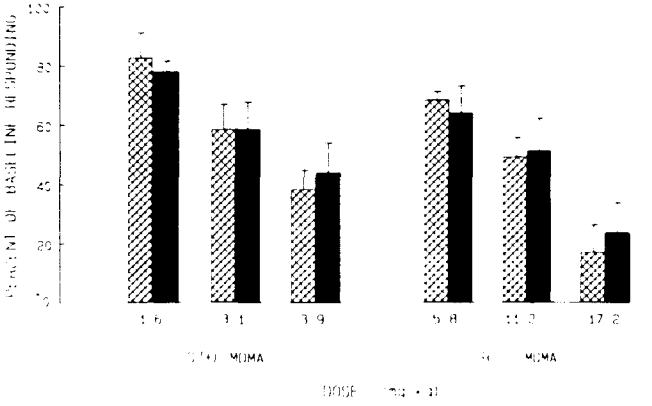

FIG. 2. Effect of S(+)- and R(−)-MDMA on schedule-controlled responding. Mice were pretreated with pirenpirone and saline as described in Fig. 1; statistical procedures were also similar, and the Ns as each dose level averaged 10–14 mice.

that in both cases, the S(+)-isomer is more potent than the R(−)-enantiomer. A comparison of the dose response data reveals that S(+)-MDA is about 2.5 times more potent than R(−)-MDA and that S(+)-MDMA is nearly 4 times more potent than its R(−)-enantiomer (Table 1). Furthermore, pretreatment of the animals with 0.1 mg/kg of pirenpirone effectively antagonized the effects of R(−)-MDA (Fig. 1), whereas it had no significant effect on S(+)-MDA, R(−)-MDMA, or S(+)-MDMA (Fig. 2). In addition pirenpirone antagonized DOM-induced disruption by more than 90% (see the Method section). It should finally be noted that pirenpirone doses above 0.2 mg/kg disrupted behavior, thus making it difficult to analyze higher doses of this antagonist.

DISCUSSION

In humans, R(−)-MDA is relatively similar in potency to, or is slightly more potent than, S(−)-MDA [14]. However, evidence suggests that the isomers of MDA produce a qualitatively dissimilar effect; the R(−)-isomer is primarily responsible for the hallucinogenic or DOM-like effects whereas the S(+)-isomer appears responsible for the amphetamine-like effects [3, 4, 12]. In contrast, S(+)-MDMA is more potent than R(−)-MDMA in humans [1], rats [9], and mice ([6] and Table 1). In amphetamine-trained animals, both MDA and MDMA resulted in stimulus generalization [3]. However, in contrast to MDA, MDMA does not result in stimulus generalization in DOM-trained animals; similar results were obtained with the individual optical isomers of MDMA. Taken together, these findings suggest that both isomers of MDMA are more like S(+)-MDA than like R(−)-MDA (which is DOM-like).

The results of the present study are in accord with these findings. That is, the 5-HT-2 antagonist pirenpirone is able to antagonize the effects of R(−)-MDA (Fig. 1) at a dose comparable to that which blocks the effects of DOM (see the Results section). On the other hand, this dose of pirenpirone (0.1 mg/kg) was unable to antagonize the effects of S(+)-MDA or of either optical isomer of MDMA (Figs. 1 and 2). Apparently, the disruption of behavior produced by the isomers of MDA is via a different mechanism. In addition, the disruptive effects produced by the R(−)-isomer of MDMA appear to be via a mechanism that differs from that implicated for R(−)-MDA (i.e., a 5-HT-2 mechanism).

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