*Pharmacology Biochemistry and Behavior*, Vol. 43, pp. 1099–1106, 1992


0091-3057/92 $5.00 + .00


# Further Studies on *N*-Methyl-1(3, 4-Methylenedioxyphenyl)-2-Aminopropane as a Discriminative Stimulus: Antagonism by 5-Hydroxytryptamine$_3$ Antagonists

RICHARD A. GLENNON,[1] RODNEY HIGGS, RICHARD YOUNG AND HASSAN ISSA

*Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0540*

Received 21 January 1992

GLENNON, R. A., R. HIGGS, R. YOUNG AND H. ISSA. *Further studies on N-methyl-1(3, 4-methylenedioxyphenyl)-2-aminopropane as a discriminative stimulus: Antagonism by 5-hydroxytryptamine$_3$ antagonists.* PHARMACOL BIOCHEM BEHAV **43**(4) 1099–1106, 1992.—Using a standard two-lever operant paradigm, male Sprague-Dawley rats were trained to discriminate 1.5 mg/kg *N*-methyl-1(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA) from saline using a variable-interval 15-s schedule of reinforcement for food reward. Tests of stimulus antagonism were conducted to further define the mechanism of action of MDMA as a discriminative stimulus. Low doses of the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) antagonist NAN-190, the 5-HT$_2$ antagonist pirenperone, and the dopamine antagonist haloperidol were able to somewhat attenuate the MDMA stimulus; however, none of these agents decreased MDMA-appropriate responding to less than 46%. The 5-HT$_3$ antagonists zacopride and LY 278584 ($ID_{50}$ = 0.02 μg/kg) antagonized the MDMA discriminative stimulus. Zacopride also attenuated the stimulus effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) in DOM-trained animals but not those of (+)amphetamine in (+)amphetamine-trained animals. Several possible mechanistic interpretations are provided but it is concluded that MDMA produces its stimulus effects via a complex mechanism involving both dopaminergic and serotonergic components.

MDMA Drug discrimination 5-HT$_2$ 5-HT$_3$ Dopamine Pirenperone Zacopride LY 278584 DOM Amphetamine

3,4-METHYLENEDIOXYAMPHETAMINE (MDA) and its *N*-monomethyl derivative 3,4-methylenedioxymethamphetamine (MDMA) represent schedule I drugs that possess a common amphetamine backbone. In drug discrimination studies, MDA produces both amphetamine-like and hallucinogen-like effects (13,18), that is, MDA results in stimulus generalization in rats trained to discriminate the stimulant (+)amphetamine and the hallucinogen 1-(2,5-dimethoxy-4-methyphenyl)-2-aminopropane (DOM) from saline. Conversely, MDA-trained rats recognize both (+)amphetamine and DOM (13). These effects are stereoselective (although perhaps not stereospecific) with amphetamine-like properties residing primarily with the *S*(+)isomer and with the DOM-like effects being attributable primarily to the *R*(−)isomer of MDA. Both racemic MDMA and *S*(+)MDMA, but not *R*(−)MDMA, produce amphetamine-like stimulus effects; *S*(+)MDMA and (±)MDMA are approximately one sixth and one twentieth as potent as (+)amphetamine, respectively. Unlike MDA, MDMA typically produces only partial generalization in DOM-trained animals, followed by disruption of behavior at slightly higher doses (21). [See (13) for a recent review of the stimulus properties of amphetamine-related designer drugs).] Because a) DOM is thought to produce its stimulus via a 5-hydroxytryptamine$_2$ (5-HT$_2$) serotonin mechanism (13), b) MDMA and its isomers bind (albeit with modest affinity) at 5-HT$_2$ receptors (27), and c) at high doses MDMA disrupts DOM-trained animals, it cannot be ruled out that MDMA may possess some DOM-like (i.e., 5-HT$_2$) character. MDMA has also been demonstrated to release stores of 5-HT (28,33) and dopamine (24,25,28). It would appear then that MDMA produces certain of its effects via an amphetamine-like (or dopaminergic) mechanism, and it is possible that certain other of its effects may involve a serotonergic mechanism. Indeed, it has recently been shown that MDMA induces a tail-flick response in rats that involves a serotonergic, but not a dopaminergic, mechanism (29), and that the hyperthermic

[1] To whom requests for reprints should be addressed.

effects (22), elevation of certain neuroendocrine levels (31), and hyperlocomotor actions (3) of MDMA in rodents also involve a serotonergic mechanism. It has also been argued that MDMA produces a unique nonamphetamine nonhallucinogenic effect (32); thus, MDMA may (in addition to any dopaminergic and serotonergic effects) in addition produce a third type of effect. Support for this concept is that certain agents lacking either amphetamine or hallucinogenic activity result in stimulus generalization in MDMA-trained animals (13,32). This third effect may involve an as yet unknown mechanism of action or may simply reflect a combination of serotonergic and dopaminergic mechanisms.

The effects of MDMA are complex and may involve multiple mechanisms. Because 5-HT and/or dopamine receptors may be involved in the actions of MDMA, we attempted to attenuate its discriminative stimulus effects in rats with several standard 5-HT antagonists and the dopamine antagonist haloperidol to further define its mechanism(s) of action.

## METHOD

### *Drug Discrimination Studies*

We previously trained groups of rats to discriminate each of the three training drugs used in the present study (15,17,21); identical training procedures were used in the present investigation. The subjects were 16 male Sprague-Dawley rats weighing 250–300 g at the start of the study. Animals were first trained to lever press for sweetened milk reward using standard two-lever operant chambers [Coulbourn Instruments (Lehigh Valley, PA) Model E10-10] housed within sound- and light-attenuating outer chambers. Once lever-pressing behavior was acquired, animals were divided into three groups and trained to discriminate IP injections of either MDMA (1.5 mg/kg), (+)amphetamine (1.0 mg/kg), or DOM (1.0 mg/kg) from 0.9% sterile saline (1.0 ml/kg). Training of animals to discriminate MDMA from saline has been reported (16); it was these same six animals that were used in the present study. A similar procedure was followed to train animals to discriminate (+)amphetamine ($n$ = 5) and DOM ($n$ = 5) from saline. Briefly, rats were trained to respond on a variable-interval 15-s (VI 15-s) schedule of reinforcement. Once rates of responding stabilized, animals received an injection of drug or saline 15 min prior to each session. Drug or saline was administered on a double alternation schedule (i.e., 2 days drug, 2 days saline) and training sessions were of 15 min duration. On every fifth day, learning was assessed during an initial 2.5-min nonreinforced (extinction) period followed by a 12.5-min training session. Data collected during the extinction period included percent drug-appropriate lever responding (i.e., the number of responses on the drug-designated lever/total number of responses, expressed as a percent) and total responses made during the 2.5-min session (expressed as responses/min).

Once rats consistently (i.e., for 3 consecutive weeks) made >80% of their responses on the drug-appropriate lever after administration of drug and <20% of their responses on the same lever after injection of saline, stimulus generalization and antagonism studies were begun. During these investigations, test sessions were interposed among the training sessions; however, after the 2.5-min extinction period animals were returned to their home cages. During generalization tests rats were injected with doses of a substitute compound and, 15 min later, tested under extinction conditions. Stimulus generalization was said to have occurred when animals made ≥80% of their responses on the drug-appropriate lever. During antagonism tests, rats were injected with purported antagonists 45 min (15 min in the case of the 5-$HT_3$ antagonists) prior to administration of a training drug. Stimulus antagonism was said to have occurred when animals made approximately 20% of their responses on the drug-appropriate lever. $ID_{50}$ (inhibition dose 50%) values were determined by the method of Finney (10).

### *Drugs*

MDMA HCl and DOM HCl were obtained from NIDA. Ketanserin (free base) and pirenperone tartarate were gifts from Janssen Pharmaceutica (Beerse, Belgium) and zacopride HCl was a gift from A. H. Robins (Richmond, VA). Haloperidol for injection (Haldol) was purchased from the MCV Hospital Pharmacy (Richmond, VA). NAN-190, as its HBr salt, was purchased from Research Biochemicals, Inc. (Natick, MA) and (+)amphetamine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO). LY 278584 or 1-methyl-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide was prepared according to the method of Fludzinski et al. (12) and converted to the maleate salt as described by Robertson et al. (37). The melting point of the maleate salt (mp 193–195°C) was identical to the literature (37) value after recrystallization from a mixture of ethyl acetate and ethanol. All solutions were prepared fresh daily in sterile saline and administered via the intraperitoneal route in a 1-ml/kg injection volume. Ketanserin was first dissolved in 1 equivalent of 0.01 N HCl before dilution to the desired concentrations with saline.

## RESULTS

### *Effects of the 5-$HT_2$ Antagonists Ketanserin and Pirenperone on the MDMA Stimulus*

Doses of ketanserin up to 1 mg/kg only partially attenuated the stimulus effects of the training dose of MDMA (Table 1); administration of higher doses of ketanserin (1.5 and 2 mg/kg) resulted in disruption of behavior. In contrast, at a dose of 0.01 mg/kg pirenperone reduced the effect of MDMA by nearly 50% (i.e., administration of 0.01 mg/kg pirenperone in combination with the training dose of MDMA reduced drug-appropriate responding to 46%). This reduction in MDMA-appropriate responding was accompanied by a severe depression of animals' response rates relative to controls and higher doses of pirenperone (0.03 and 0.1 mg/kg) produced disruption of behavior. Pirenperone (0.03 mg/kg) was also examined using a shorter (15 vs. 45 min) presession injection interval without any difference in results, that is, three of four animals failed to respond (data not shown).

### *Effect of the Dopamine Antagonist Haloperidol on the MDMA Stimulus*

Table 1 shows that administration of 0.001 mg/kg haloperidol in combination with the training dose of MDMA had essentially no effect on MDMA-appropriate responding. Haloperidol at a dose of 0.01 mg/kg reduced drug-appropriate responding to 46%; higher doses of haloperidol (0.03 and 0.05 mg/kg) in combination with MDMA resulted in disruption of behavior.

TABLE 1

RESULTS OF STIMULUS ANTAGONISM STUDIES WITH MDMA-TRAINED RATS

| Agent | Dose (mg/kg) | n* | % Drug-Appropriate Responding† | Responses/min† |
|---|---|---|---|---|
| MDMA | 1.5 | 6/6 | 94 (2) | 11.7 (3.1) |
| Saline (1.0 ml/kg) | | 6/6 | 7 (3) | 10.8 (2.2) |
| Ketanserin | 0.1 | 4/4 | 83 (8) | 16.4 (1.0) |
| | 0.3 | 4/4 | 78 (10) | 15.3 (5.6) |
| | 0.6 | 3/4 | 82 (9) | 19.6 (8.5) |
| | 0.8 | 4/4 | 69 (14) | 5.2 (2.8) |
| | 1.0 | 3/3 | 69 (19) | 7.3 (1.9) |
| | 1.5 | 1/4 | –‡ | |
| | 2.0 | 0/4 | –‡ | |
| Pirenperone | 0.005 | 4/4 | 82 (10) | 10.1 (3.4) |
| | 0.01 | 3/4 | 46 (14) | 2.9 (0.4) |
| | 0.03 | 1/4 | –‡ | |
| | 0.1 | 0/3 | –‡ | |
| Haloperidol | 0.001 | 4/4 | 82 (6) | 14.8 (4.1) |
| | 0.01 | 4/6 | 46 (10) | 16.1 (6.4) |
| | 0.03 | 1/3 | –‡ | |
| | 0.05 | 0/3 | –‡ | |
| Haloperidol + ketanserin | 0.01<br>1.0 | 3/4 | 76 (17) | 7.7 (2.2) |
| Haloperidol + pirenperone | 0.01<br>0.01 | 4/6 | 53 (13) | 7.0 (2.8) |
| NAN-190 | 0.05 | 5/6 | 80 (12) | 10.0 (2.1) |
| | 0.2 | 5/6 | 68 (11) | 3.8 (0.6) |
| | 0.6 | 3/6 | 57 (30) | 2.3 (0.1) |
| | 1.0 | 0/6 | –‡ | |
| LY 278584 | 0.000001 | 3/4 | 87 (9) | 4.0 (1.2) |
| | 0.00001 | 4/4 | 52 (13) | 7.3 (1.4) |
| | 0.0001 | 4/4 | 41 (16) | 10.6 (3.4) |
| | 0.0005 | 3/6 | 19 (12) | 3.6 (1.0) |
| | 0.001 | 0/3 | –‡ | |
| | 0.01 | 0/4 | –‡ | |
| Zacopride | 0.00001 | 3/4 | 82 (16) | 8.6 (1.6) |
| | 0.0001 | 4/5 | 72 (12) | 7.2 (2.6) |
| | 0.0004 | 5/6 | 68 (28) | 11.9 (3.4) |
| | 0.0005 | 4/4 | 58 (20) | 14.7 (6.4) |
| | 0.001 | 3/4 | 23 (14) | 13.6 (3.9) |

*Number of animals responding/number receiving drug.

†Data collected during 2.5-min extinction session followed by SEM in parentheses.

‡Disruption of behavior; majority of animals tested failed to make > five total responses during the entire 2.5-min extinction session.

*Effect of Haloperidol in Combination With Ketanserin or Pirenperone on the MDMA Stimulus*

It was theorized that if pirenperone and haloperidol both reduce MDMA-appropriate responding by 50% (implicating involvement of both a 5-HT$_2$ and dopaminergic mechanism) a combination of the two may completely antagonize the MDMA stimulus. The highest nondisruptive dose of haloperidol (0.01 mg/kg) administered in combination with the highest nondisruptive dose of pirenperone (0.01 mg/kg) reduced the effect of the training dose of MDMA to 53% MDMA-appropriate responding. Because these results (Table 1) are similar to those where either haloperidol or pirenperone was administered singly with MDMA, their effects do not appear to be additive. A similar experiment, using haloperidol in combination with the highest nondisruptive dose of ketanserin (1 mg/kg), resulted in 76% MDMA-appropriate responding.

*Effect of the Putative 5-HT$_{1A}$ Antagonist NAN-190 on the MDMA Stimulus*

Table 1 shows that 0.2 and 0.6 mg/kg NAN-190, in combination with the training dose of MDMA, somewhat attenuate the stimulus effects of MDMA (i.e., they result in 68 and 57%, respectively, drug-appropriate responding). At 0.6 mg/kg, responding varied widely. Response rates were severely depressed at these dose combinations (Table 1), and administration of a higher dose (1 mg/kg) of NAN-190 resulted in disruption of behavior.

*Effect of 5-HT$_3$ Antagonists on the MDMA Stimulus*

Initial administration of 0.01 mg/kg of the 5-HT$_3$ antagonist LY 278584 in combination with the training dose of MDMA resulted in disruption of behavior (Table 1). Due to

the potent disruptive effect of such a low dose of drug, we examined several lower doses of this 5-$HT_3$ antagonist. Table 1 shows that doses of 0.00001–0.0005 mg/kg LY 278584 [$ID_{50}$ dose = 0.02 (0.003–0.26) μg/kg] attenuate the stimulus effects of MDMA. To obtain supporting data for this effect, we additionally examined the effect of zacopride, a representative member of another structural class of 5-$HT_3$ antagonists. Zacopride at doses of 0.0001–0.001 mg/kg was also capable of attenuating the stimulus effects of MDMA (Table 1).

### *Effect of the 5-$HT_3$ Agonist 2-Methyl 5-HT on the MDMA Stimulus*

Because LY 278584 and zacopride antagonized the MDMA stimulus, we examined the effect of the 5-$HT_3$ agonist 2-methyl 5-HT in tests of stimulus generalization. Doses of 2.5, 5.0, and 7.5 mg/kg 2-methyl 5-HT in MDMA-trained animals resulted in <26% drug-appropriate responding (Table 2). Because response rates were severely depressed at 7.5 mg/kg, additional doses were not examined. The ability of a single dose of 2-methyl 5-HT to antagonize the MDMA stimulus was also examined. Upon administration of 2-methyl 5-HT (7.5 mg/kg) in combination with the training dose of MDMA, two animals failed to respond and the other two made 100% of their responses on the drug-appropriate lever, with individual response rates being 2.8 and 3.2 responses per minute (data not shown).

### *Effect of the 5-$HT_3$ Antagonist Zacopride on the DOM and Amphetamine Stimulus*

Interestingly, at doses of 0.00001–0.001 mg/kg zacopride administered in combination with the training dose of DOM to DOM-trained rats attenuated, but did not completely antagonize, the effect of this phenylisopropylamine hallucinogen (Table 3). At 0.001 mg/kg zacopride in combination with the training dose of DOM, animals made 30% of their responses on the DOM-appropriate lever. At doses of 0.004 and 0.006 mg/kg zacopride in combination with DOM, DOM-appropriate responding was 41 and 67%, respectively. Comparable doses of zacopride (i.e., 0.0001–0.01 mg/kg) in combination with (+)amphetamine in rats trained to discriminate 1 mg/kg (+)amphetamine had no effect on drug-lever selection. Higher doses of zacopride (0.05 and 0.1 mg/kg) in combination with (+)amphetamine resulted in disruption of behavior (Table 3). The highest nondisruptive dose of zacopride (i.e., 0.01 mg/kg) was administered to five (+)amphetamine-trained rats in the absence of a subsequent injection of (+)amphetamine. Although zacopride produced saline-appropriate responding (16 ± 13% drug-appropriate responding), animals' response rates were increased and erratic (48.7 ± 22.3 responses/min) relative to control rates (data not shown).

## DISCUSSION

The phenylisopropylamine hallucinogen DOM, unlike the phenylisopropylamine stimulant amphetamine, produces stimulus effects that are potently antagonized by the 5-$HT_2$ antagonists ketanserin and pirenperone (20). Other 5-$HT_2$ antagonists also attenuate the stimulus effects of DOM (15). Table 1 shows that doses of ketanserin capable of antagonizing the DOM stimulus have relatively little effect on the MDMA stimulus. The 5-$HT_2$ antagonist pirenperone partially antagonizes the MDMA stimulus. Using the same training dose of MDMA but a different schedule of reinforcement and different presession injection intervals, Schechter (40) recently demonstrated that pirenperone can attenuate the MDMA stimulus. Pirenperone (0.32 mg/kg) administered in combination with the training dose of MDMA resulted in 28.6% (quantal) and 40.4% (quantitative) drug-appropriate responding(40). Although the quantal score suggests a greater degree of antagonism than that observed in the present study, the quantitative score (which is obtained in a manner that is more analogous to that presented here) is similar to the maximal antagonism reported in Table 1. The Schechter study also employed higher doses of pirenperone than those used in the present investigation and pirenperone was administered 15 min prior to administration of MDMA, whereas in the present study pirenperone was administered 45 min prior to MDMA. We evaluated the effect of 0.03 mg/kg pirenperone using the shorter 15-min presession injection interval and, as with the 45-min interval, observed disruption of behavior. Although we cannot explain the differences between the Schechter study (40) and the present results, both demonstrate that 5-$HT_2$ antagonists can at least partially attenuate the stimulus effects of MDMA.

Haloperidol potently antagonizes the stimulus effects of amphetamine but not those of DOM (13). Haloperidol can partially antagonize the MDMA stimulus (maximum 46% MDMA-appropriate responding; Table 1). While our work was in progress, it was demonstrated that haloperidol has little effect in rats trained to discriminate 1.5 mg/kg MDMA but that it can partially antagonize (46.2%) the effect of MDMA in rats trained to discriminate 2.5 mg/kg of the training drug using a 105-min presession injection interval (40). Although once again there were procedural differences in the two studies, both demonstrate that haloperidol can partially antagonize the MDMA stimulus. Haloperidol and ketanserin in combination with MDMA have relatively little effect (Table 1); haloperidol and pirenperone in combination with MDMA do

TABLE 2

RESULTS OF STIMULUS GENERALIZATION STUDIES WITH MDMA-TRAINED RATS

| Agent | Dose (mg/kg) | n* | %Drug-Appropriate Responding† | Responses/min† |
|---|---|---|---|---|
| 2-Me 5-HT | 2.5 | 4/4 | 9 (3) | 10.8 (3.1) |
| | 5.0 | 2/4 | 19 (19) | 7.8 (3.8) |
| | 7.5 | 2/3 | 25 (0) | 2.0 (0.0) |

*Number of animals responding/number of animals receiving drug.

†Data collected during 2.5-min extinction session followed by SEM in parentheses.

TABLE 3

EFFECT OF ZACOPRIDE IN RATS TRAINED TO DISCRIMINATE EITHER 1 mg/kg DOM OR 1 mg/kg (+)AMPHETAMINE FROM SALINE

| Training Drug | Zacopride Dose | $n$* | % Drug-Appropriate Responding† | Responses/min† |
|---|---|---|---|---|
| (+)Amphetamine | 0.0 | 5/5 | 94 (3) | 9.2 (1.4) |
| | 0.0001 | 3/3 | 91 (9) | 8.1 (3.9) |
| | 0.001 | 3/3 | 82 (14) | 20.5 (16.8) |
| | 0.01 | 3/3 | 84 (16) | 6.4 (3.6) |
| | 0.05 | 1/3 | −‡ | |
| | 0.1 | 2/5 | −‡ | |
| DOM | 0.0 | 5/5 | 87 (5) | 8.8 (2.1) |
| | 0.00001 | 4/5 | 62 (20) | 5.7 (2.0) |
| | 0.0001 | 3/4 | 43 (21) | 9.1 (3.6) |
| | 0.001 | 6/7§ | 30 (15) | 8.8 (3.6) |
| | 0.004 | 4/5 | 41 (8) | 7.3 (2.1) |
| | 0.006 | 3/3 | 67 (13) | 14.8 (5.0) |
| | 0.010 | 0/3 | −‡ | |

*Number of animals responding/number of animals receiving drug.

†Data collected during the 2.5-min extinction session followed by SEM in parentheses.

‡Disruption of behavior; majority of animals tested failed to make > five total responses during the entire 2.5-min extinction session.

§Three animals were used on two separate occasions.

not result in antagonism greater than that observed with either haloperidol or pirenperone given singly with MDMA.

Millan and Colpaert (29) have shown that several 5-HT$_{1A}$ antagonists, including NAN-190, potently antagonize spontaneous tail-flicks induced in restrained rats by MDMA. Thus, it seemed logical to attempt antagonism of the MDMA stimulus using NAN-190. Although there was some evidence for stimulus attenuation at the doses evaluated (Table 1), the disruptive effects of NAN-190 precluded evaluation of higher doses of this agent.

5-HT$_3$ receptors have been implicated as modulators of dopamine release (1,4,23). Because MDMA is known to release stores of dopamine, and because 5-HT$_3$ antagonists can apparently antagonize such release (23), an attempt was made to antagonize the stimulus effects of MDMA using the 5-HT$_3$ antagonist LY 278584. As shown in Table 1, LY 278584 at doses as low as 0.00001 mg/kg can attenuate the effect of MDMA and at 0.0005 mg/kg results in saline-appropriate responding when given in combination with MDMA; the calculated dose for 50% antagonism ($ID_{50}$ dose) is 0.02 μg/kg. The 5-HT$_3$ antagonist zacopride produces a similar effect. Because 5-HT$_3$ agonists have been reported to induce release of dopamine (1), we attempted stimulus generalization studies with the 5-HT$_3$ agonist 2-methyl 5-HT; however, administration of 2.5–7.5 mg/kg 2-methyl 5-HT failed to result in stimulus generalization (Table 2) and had a severe disruptive effect on animals. Although it has been suggested that this agent does not readily penetrate the blood–brain barrier, we recently demonstrated that 2-methyl 5-HT, at a dose of 5 mg/kg, serves as a training drug in drug discrimination studies and that its effects are most likely centrally mediated (19). We have also shown that the 2-methyl 5-HT stimulus is potently antagonized by a 5-HT$_3$ antagonist (19).

If the 5-HT$_3$ antagonists block the stimulus effects of MDMA by modulating the release of dopamine, they a) may also antagonize the stimulus effects of (+)amphetamine in amphetamine-trained animals and b) should have no effect on the stimulus effects of DOM in DOM-trained animals. This is not what was observed. Zacopride, at 10 times the dose that completely antagonized the MDMA stimulus, had no effect on (+)amphetamine-appropriate responding (Table 3), and yet zacopride, at certain doses, appears to attenuate the stimulus effects of DOM in DOM-trained animals. This latter effect was biphasic. Zacopride (at 0.001 μg/kg) in combination with the training dose of DOM resulted in 30% DOM-appropriate responding; higher doses of zacopride resulted in 41 and 67% DOM-appropriate responding. It is difficult to explain the unanticipated attenuation of DOM-appropriate responding by zacopride. DOM does not bind at 5-HT$_3$ receptors ($K_i$ > 10,000 nM; Teitler, personal communication) and zacopride displays low affinity for 5-HT$_2$ receptors ($IC_{50}$ = 3,200 nM) (30). Further, zacopride does not bind at other populations of 5-HT receptors nor does it bind at either $D_1$ or $D_2$ dopamine receptors ($IC_{50}$ > 10,000 nM) (30). In addition, the dopamine antagonist haloperidol antagonizes the stimulus effects of (+)amphetamine but not those of DOM (13). The U-shaped dose–response curve for zacopride in combination with DOM is also difficult to explain, but such actions are not without precedent. For example, the 5-HT$_3$ receptor antagonist ICS-205,930 has been shown to produce a U-shaped dose–effect function when tested for anxiolytic-like activity in a two-compartment light/dark apparatus [(26); and R. Young, unpublished data]. Taken together with the inability of zacopride to antagonize the (+)amphetamine stimulus (Table 3), it seems unlikely that zacopride's ability to antagonize the MDMA stimulus is related to a simple blockade of 5-HT$_2$ receptors, blockade of $D_1$ or $D_2$ dopamine receptors, or modulation of dopamine release.

Several mechanistic interpretations are possible for the results obtained in the present investigation, but it appears that the MDMA stimulus is complex and involves both dopaminergic and serotonergic components. In this regard, our conclusions are in general similar to those of Schechter (40) but differ in that Schechter finds evidence for dopaminergic

involvement only after a longer (>30 min) presession injection interval.

Specifically, how can the present results be explained on a mechanistic basis? At this time, it is unknown whether the stimulus effects of MDMA are due to MDMA itself or to a metabolite of MDMA. Therefore, several mechanistic explanations are possible. MDMA is metabolized to MDA both in vitro (2,5) and in vivo (5,11,42) and it is possible that certain effects of MDMA may arise from its metabolic conversion to MDA. The behavioral effects of the more 5-$HT_2$-like or DOM-like isomer of MDA [i.e., *R*(−)MDA], but not those of *S*(+)MDA, *R*(−)MDMA, or *S*(+)MDMA, are attenuated by pretreatment of animals with the 5-$HT_2$ antagonist pirenperone (38). Thus, conversion of MDMA to MDA could explain the partial antagonism of the MDMA stimulus by pirenperone observed in the present study. However, the half-life for conversion of MDMA to MDA in rats is reported to be >60 min (5). Given the 15-min presession injection interval employed in the present study, it is unlikely (although not altogether impossible) that significant concentrations of MDA would be formed. It might be noted parenthetically that antagonism of the MDMA stimulus with pirenperone 105 min after administration of MDMA, as reported by Schechter (40), would be consistent with this concept. Additional evidence arguing against a significant stimulus role for MDA is that the MDA stimulus, but not the MDMA stimulus, generalizes to DOM (13). MDMA is also metabolized to other metabolites [see (16) and (42) and references therein], and the possibility exists that one or more of these metabolites may also account for, or contribute to, the stimulus properties of MDMA.

Assuming that the stimulus effects of MDMA are not due to MDA, the partial antagonism of the MDMA stimulus by haloperidol suggests a role for dopamine. A dopaminergic mechanism is also implicated by the results with the 5-$HT_3$ antagonists. However, although 5-$HT_3$ receptors may modulate dopamine release the ability of zacopride and LY 278584 to antagonize the MDMA stimulus via this mechanism is an unsatisfactory explanation when zacopride lacks a similar effect in amphetamine-trained animals. While this article was in preparation, Pan and Wang (35,36) reported that MDMA acts indirectly to decrease the firing rate of spontaneously active neurons in the medial prefrontal cortex of rats by releasing endogenous 5-HT. They further report that this effect is reversed by the nonselective 5-$HT_1$/5-$HT_2$ antagonist metergoline and, although in a less consistent manner, by the 5-$HT_3$ antagonists zacopride and granisetron (36). 5-HT-induced phosphoinositide hydrolysis is thought to be mediated (at least in the frontal cortex) primarily via a 5-$HT_2$ mechanism; this same group of investigators provided evidence that in some brain regions phosphoinositide hydrolysis may be mediated both by 5-$HT_2$ and 5-$HT_3$ receptors (9). They have shown that this 5-HT-induced effect is only partially blocked by 5-$HT_2$ antagonists but is completely blocked by various 5-$HT_3$ antagonists (9). This, either by itself or coupled with any modulatory effects on dopaminergic mechanisms, offers a possible explanation for the antagonism noted in the present study.

We recently reported that the interaction of serotonergic agents at a particular population of 5-HT receptors can modulate the effects of interaction of another serotonergic agent on a different population of 5-HT receptors [reviewed in (15)]. For example, activation of 5-$HT_{1A}$ or 5-$HT_{1B}$ receptors can attenuate the behavioral effects of 5-$HT_2$ receptor activation (7,8,14). This offers a third explanation. An alternative but closely related explanation for the present results, then, is that by virtue of its ability to release stores of 5-HT (which would presumably interact at multiple populations of 5-HT receptors) MDMA's actions at 5-$HT_2$ receptors would not appear to be as robust as those of agents that lack this ability to release 5-HT. Although both MDMA and MDA release stores of 5-HT (28,33), the higher 5-$HT_2$ affinity of MDA relative to MDMA (27,39,41) may be sufficient to overcome these effects (i.e., higher doses of MDMA would be required to produce DOM-like stimulus effects and such effects may not be manifested at doses below those that disrupt animals' behavior). This explanation is also consistent with antagonism of the MDMA stimulus by 5-$HT_3$ antagonists, that is, if the MDMA stimulus involves a multiple 5-HT mechanism resulting from release of 5-HT, inhibition of this release (coupled, perhaps, with any modulatory effects on a dopaminergic mechanism) would be sufficient to antagonize the MDMA stimulus.

On the basis of the above discussion, it is concluded that both a serotonergic and a dopaminergic mechanism are at least partially responsible for the stimulus effects produced by MDMA. The serotonergic component may not be mediated solely by one specific population of 5-HT receptors but likely involves multiple populations of 5-HT receptors. Evidence in support of this view is that the MDMA stimulus does not generalize to the 5-$HT_{1C}$/5-$HT_2$ agonist DOM (13), the 5-$HT_3$ agonist 2-methyl 5-HT (Table 2), or the 5-$HT_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (40), but does generalize to the nonselective 5-HT agonists TFMPP (40) and quipazine (40) and to the 5-HT releasing agent norfenfluramine (40). Agents capable of blocking only a specific portion of the serotonergic component (e.g., pirenperone) reduce, but do not completely antagonize, MDMA-appropriate responding. Evidence for the involvement of a dopaminergic mechanism is that the MDMA stimulus generalizes or partially generalizes to dopaminergic agents such as amphetamine or apomorphine (13,34,40) and is at least partially antagonized by the dopamine release inhibitor CGS 10746B (40) and the dopamine antagonist haloperidol (Table 1). The results with the 5-$HT_3$ antagonists may reflect their unique ability to modulate both the serotonergic and dopaminergic aspects of MDMA. However, it must be realized that the effects of coadministration of various agents with MDMA on the rate of distribution and metabolism of MDMA are unknown at this time. Nevertheless, antagonism of the MDMA stimulus with 5-$HT_3$ antagonists supports previous arguments (6) for the possible clinical utility of such agents in the treatment of drug abuse.

ACKNOWLEDGEMENT

This work was supported in part by DA-01642.

## REFERENCES

1. Blandina, P.; Goldfarb, J.; Craddock-Royal, B.; Green, J. P. Release of endogenous dopamine by stimulation of 5-hydroxytryptamine$_3$ receptors in rat striatum. J. Pharmacol. Exp. Ther. 251:803–809; 1989.
2. Brady, J. F.; Di Stefano, E. W.; Cho, A. K. Spectral and inhibitory interactions of (±)3,4-methylenedioxymethamphetamine (MDMA) with rat hepatic microsomes. Life Sci. 39:1457–1464; 1986.
3. Callaway, C. W.; Wing, L. L.; Geyer, M. A. Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J. Pharmacol. Exp. Ther. 254:456–464; 1990.

4. Carboni, E.; Acquas, E.; Leone, P.; Perezzani, L.; DiChiara, G. 5-HT3 receptor antagonists block morphine- and nicotine-induced place-preference conditioning. Eur. J. Pharmacol. 151: 159–160; 1988.
5. Cho, A. K.; Hiramatsu, M.; Di Stefano, E. W.; Chang, A. S.; Jenden, E. J. Stereochemical differences in the metabolism of 3,4-methylenedioxymethamphetamine in vivo and in vitro: A pharmacokinetic analysis. Drug Metab. Disp. 18:686–691; 1990.
6. Costall, B.; Naylor, R. J.; Tyers, M. B. The psychopharmacology of 5-HT3 receptors. Pharmacol. Ther. 47:181–202; 1990.
7. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. Do functional relationships exist between 5-HT1A and 5-HT2 receptors? Pharmacol. Biochem. Behav. 36:901–906; 1990.
8. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. Pharmacological characterization of ear-scratch response in mice as a behavioral model for selective 5-HT2 receptor agonists and evidence for 5-HT1B- and 5-HT2-receptor interactions. Pharmacol. Biochem. Behav. 37:95–99; 1990.
9. Edwards, E.; Harkins, K.; Ashby, C. R.; Wang, R. Y. Effect of 5-hydroxytryptamine-3 receptor agonists on phosphoinositides hydrolysis in the rat fronto-cingulate and entorhinal cortices. J. Pharmacol. Exp. Ther. 256:1025–1032; 1991.
10. Finney, D. J. Probit analysis. London, UK: Cambridge University Press; 1952.
11. Fitzgerald, R. L.; Blanke, R. V.; Narasimhachari, N.; Glennon, R. A.; Rosecrans, J. A. Identification of 3,4-methylenedioxyamphetamine (MDA) as a major urinary metabolite of 3,4-methylenedioxymethamphetamine (MDMA). Presented at the CPDD Meeting, Philadelphia, PA, 1987.
12. Fludzinski, P.; Evard, D. A.; Bloomquist, W. E.; Lacefield, W. B.; Pfeifer, W.; Jones, N. D.; Deeter, J. B.; Cohen, M. L. Azoles as indole bioisoteres: Synthesis and evaluation of the tropanyl ester and amide of indole-3-carboxylate as antagonists of the 5-HT3 receptor. J. Med. Chem. 30:1535–1537; 1987.
13. Glennon, R. A. Stimulus properties of hallucinogenic phenalkylamines and related designer drugs: Formulation of structure activity relationships. In: Ashgar, K.; De Souza, E., eds., Pharmacology and toxicology of amphetamine and related designer drugs. NIDA Research Monograph 94. Washington, DC: U.S. Government Printing Office; 1989:43–67.
14. Glennon, R. A.; Darmani, N. A.; Martin, B. R. Multiple populations of serotonin receptors may modulate the behavioral effects of serotonin agents. Life Sci. 48:2493–2498; 1991.
15. Glennon, R. A.; Hauck, A. E. Mechanistic studies on DOM as a discriminative stimulus. Pharmacol. Biochem. Behav. 23:937–941; 1985.
16. Glennon, R. A.; Higgs, R. An investigation of MDMA metabolites and related agents in rats trained to discriminate MDMA from saline. Pharmacol. Biochem. Behav. (in press).
17. Glennon, R. A.; Misenheimer, B. R. Stimulus effects of *N*-monoethyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDE) and *N*-hydroxy-1-(3,4-methylenedioxyphenyl)-2-aminopropane (*N*-OH MDA) in rats trained to discriminate MDMA from saline. Pharmacol. Biochem. Behav. 33:909–912; 1989.
18. Glennon, R. A.; Young, R. Further investigation of the stimulus properties of MDA. Pharmacol. Biochem. Behav. 20:501–505; 1984.
19. Glennon, R. A.; Young, R.; Dukat, M. The 5-HT3 agonist 2-methyl 5-HT serves as a training drug in drug discrimination studies. Pharmacol. Biochem. Behav. 41:361–364; 1992.
20. Glennon, R. A.; Young, R.; Rosecrans, J. A. Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT2 antagonists. Eur. J. Pharmacol. 91:189–196; 1983.
21. Glennon, R. A.; Young, R.; Rosecrans, J. A.; Anderson, G. M. Discriminative stimulus properties of MDA analogs. Biol. Psychiatry 17:807–814; 1982.
22. Gordon, C. J.; Watkinson, W. P.; O'Callaghan, J. P.; Miller, D. B. Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. Pharmacol. Biochem. Behav. 38:339–344; 1991.
23. Imperato, A.; Angelucci, L. 5-HT3 receptors control dopamine release in the nucleus accumbens of freely moving rats. Neurosci. Lett. 101:214–216; 1989.
24. Johnson, M. P.; Hoffman, A. H.; Nichols, D. E. Effect of the enantiomers of MDA, MDMA and related analogues on [$^3$H]serotonin and [$^3$H]dopamine release from superfused rat brain slices. Eur. J. Pharmacol. 132:269–276; 1986.
25. Kalix, P.; Yousif, M. Y.; Glennon, R. A. Differential effect of the enantiomers of methylenedioxymethamphetamine (MDMA) on the release of radioactivity from [$^3$H]dopamine-prelabeled rat striatum. Res. Comm. Subst. Abuse 9:45–52; 1988.
26. Kilfoil, T.; Michael, A.; Montgomery, D.; Whiting, R. L. Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. Neuropharmacology 28:901–905; 1989.
27. Lyon, R. A.; Glennon, R. A.; Titeler, M. 3,4-Methylenedioxymethamphetamine (MDMA): Stereoselective interactions at brain 5-HT1 and 5-HT2 receptors. Psychopharmacology (Berl.) 88:525–526; 1986.
28. McKenna, D. J.; Guan, X.-M.; Shulgin, A. T. 3,4-Methylenedioxyamphetamine (MDA) analogues exhibit differential effects on synaptosomal release of $^3$H-dopamine and $^3$H-5-hydroxytryptamine. Pharmacol. Biochem. Behav. 38:505–512; 1991.
29. Millan, M. J.; Colpaert, F. C. Methylenedioxymethamphetamine induces spontaneous tail-flicks in the rat via 5-HT1A receptors. Eur. J. Pharmacol. 193:145–152; 1991.
30. Munson, H. R., Jr.; Smith, W. L.; Alphin, R. S.; Boswell, R. F.; Jagman, J. E., Jr.; Lo, Y. S.; Kilpatrick, B. F.; Johnson, D. N.; Dannenberg, W. N. The synthesis and gastrointestinal regulatory activities of *N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamides. A new series of selective prokinetic and antiemetic agents. Abstract 16, 194th National Meeting of the American Chemical Society, New Orleans, LA, 1987.
31. Nash, J. F.; Meltzer, H. Y.; Gudelsky, G. A. Elevation of serum prolactin and corticosterone concentrations in the rat after the administration of 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 245:873–879; 1988.
32. Nichols, D. E. Differences between the mechanism of action of MDMA, MBDB, and the classical hallucinogens. Identification of a new therapeutic class: Entactogens. J. Psychoact. Drugs 18: 305–313; 1986.
33. Nichols, D. E.; Lloyd, D. H.; Hoffman, A. J.; Yim, G. K. W. Effects of certain hallucinogenic amphetamine analogues on the release of [$^3$H]serotonin from rat brain synaptosomes. J. Med. Chem. 25:530–534; 1982.
34. Oberlender, R.; Nichols, D. E. Drug discrimination studies with MDMA and amphetamine. Psychopharmacology (Berl.) 95:71–76; 1988.
35. Pan, H. S.; Wang, R. Y. MDMA: Further evidence that its action in the medial prefrontal cortex is mediated by the serotonergic system. Brain Res. 539:332–336; 1991.
36. Pan, H. S.; Wang, R. Y. The action of (±)MDMA on medial prefrontal cortical neurons is mediated through the serotonergic system. Brain Res. 543:56–60; 1991.
37. Robertson, D. W.; Bloomquist, W.; Cohen, M. L.; Reid, L. K.; Schenck, K.; Wong, D. T. Synthesis and biochemical evaluation of tritium-labeled 1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*indazole, a useful radioligand for 5-HT3 receptors. J. Med. Chem. 33:3176–3181; 1990.
38. Rosecrans, J. A.; Glennon, R. A. The effect of MDA and MDMA ("ecstacy") isomers in combination with pirenperone on operant responding in mice. Pharmacol. Biochem. Behav. 28:39–42; 1987.
39. Sadzot, B.; Baraban, J. M.; Glennon, R. A.; Lyon, R. A.; Leonhardt, S.; Jan, C.-R.; Titeler, M. Hallucinogenic drug interactions at human brain 5-HT2 receptors: Implications for treating LSD-induced hallucinogenesis. Psychopharmacology (Berl.) 98: 495–499; 1989.
40. Schechter, M. D. Serotonergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). Pharmacol. Biochem. Behav. 31:817–824; 1989.
41. Titeler, M.; Lyon, R. A.; Glennon, R. A. Radioligand binding evidence implicates the brain 5-HT2 receptor as a site of action

for LSD and phenylisopropylamine hallucinogens. Psychopharmacology (Berl.) 94:213–216; 1988.
42. Yousif, M. Y.; Fitzgerald, R. L.; Narasimhachari, N.; Rosecrans, J. A.; Blanke, R. V.; Glennon, R. A. Identification of metabolites of 3,4-methylenedioxymethamphetamine in rats. Drug Alcohol Depend. 26:127–135; 1990.