

RAPID COMMUNICATION

MDL72222, a Serotonin 5-HT₃ Receptor Antagonist, Blocks MDMA's Ability to Establish a Conditioned Place Preference

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BILSKY, E. J. AND L. D. REID. MDL72222, a serotonin 5-HT₃ receptor antagonist, blocks MDMA's ability to establish a conditioned place preference. PHARMACOL BIOCHEM BEHAV 39(2) 509-512, 1991.—Methylenedioxymethamphetamine (MDMA) has previously been shown to produce a positive conditioned place preference (CPP) among rats. Here the effects of doses of a specific 5-HT₃ antagonist, MDL72222, on MDMA's ability to produce a CPP were assessed. A dose of MDL72222 (0.03 mg/kg) blocked the establishment of a MDMA CPP. These results support the suggestions that compounds affecting the 5-HT₃ receptor may be of particular interest in studying the pharmacology of self-administered drugs.

Methylenedioxymethamphetamine
Affect Reward

MDMA

MDL72222

5-HT₃ receptors

Conditioned place preference

THE amphetamine analogue methylenedioxymethamphetamine (MDMA) has recently been the target of pharmacological, toxicological and behavioral investigations. It is apparent that MDMA produces a state that might be characterized as rewarding (16,18) and which leads to its self-administration (17,18). Furthermore, MDMA is able to produce conditioned place preferences (CPPs) among rats, an index of a drug's rewarding properties (3,20). There still remains, however, questions concerning the mechanisms of MDMA's reinforcing properties.

Since raised dopaminergic activity in the mesolimbic pathway is thought to be critical to the reinforcing properties of many drugs of abuse (6,24), and MDMA increases levels of dopaminergic activity (13,21), such enhanced activity may be sufficient to account for MDMA's rewarding effects. The mechanisms that lead to this increased activity are, however, open to question.

Unlike amphetamine and cocaine, MDMA has low affinity for D-1, D-2 and dopamine uptake sites (1,2), making it unlikely that MDMA's reinforcing properties occur through direct effects on dopamine receptors. MDMA also causes the release of serotonin (7,22) and serotonin's release may augment dopamine's release (5, 14, 15). Although the exact mechanisms of serotonin's neuromodulatory action are unclear, experimental results indicate that serotonin's facilitation of dopamine release is through activation of 5-HT₃ receptors (15). Furthermore, 5-HT₃ antagonists can inhibit the release of dopamine associated with the administration of many drugs of abuse and attenuate dopamine-induced hyperactivity in the mesolimbic pathway (10,11).

Given that 5-HT₃ antagonists might attenuate the raised dopaminergic activity produced by self-administered drugs, 5-HT₃ antagonists may attenuate the positivity of these drugs. In concordance with this possibility, selective 5-HT₃ antagonists can block the establishment of CPPs with morphine, nicotine and in some instances amphetamine (8,9). Since MDMA is a drug that is self-administered and which facilitates both the release of dopamine and serotonin, a 5-HT₃ antagonist might block MDMA's reinforcing properties. The CPP test is one method that can be used to assess the neurochemical coding of a drug's reinforcing properties (4). In the present study, we tested MDL72222, a selective 5-HT₃ antagonist (12) previously shown to block morphine and nicotine CPPs (8), as a putative agent capable of altering a MDMA CPP.

METHOD

Subjects

Ninety experimentally naive, male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were used in these assessments. Rats weighed between 175 and 200 g upon their arrival at the laboratory. They were housed individually in standard hanging metal cages in a windowless colony room. The colony was maintained at 22°C with 12 h of artificial light a day (lights on at 0700 h). Food (standard laboratory chow) and water were always available in the rats' home cages.

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TABLE 1

GROUP ASSIGNMENTS AND SCHEDULES OF DRUG ADMINISTRATION
FOR THE 1ST ASSESSMENT ARE DEPICTED

Group	No. of Subjects	Putative Side Inj.	Alternate Side Inj.
Saline control	10	SAL/SAL	SAL/SAL
MDL control	10	MDL30/SAL	MDL30/SAL
MDMA control	10	SAL/MDMA	SAL/SAL
MDMA/MDL7.5	10	MDL7.5/MDMA	MDL7.5/SAL
MDMA/MDL15	10	MDL15/MDMA	MDL15/SAL
MDMA/MDL30	10	MDL30/MDMA	MDL30/SAL

Putative side inj. refers to the injections each group received prior to being placed on their putative side while alternate side inj. refers to the injections administered prior to being placed on the other side. The label to the left of the slash is the type of injection administered first. The labels correspond to the following injections: SAL=saline; MDMA=MDMA at a dose of 6.3 mg/kg; MDL=MDL7222. Numbers following MDL refer to the dose in $\mu\text{g/kg}$.

Drugs

$\pm$ -3,4-Methylenedioxymethamphetamine (MDMA) was dissolved in physiological saline and administered in a dose of 6.3 mg/kg bodyweight, a dose known to produce a reliable CPP in our apparatus (3). 3-Tropanyl-3,5-dichlorobenzoate (MDL7222) (Research Biochemicals, Natick, MA) was dissolved by adding a few drops of glacial acetic acid to the powder, taking the mixture up to one-half volume in physiological saline, adjusting the pH to 7 with NaOH and then bringing the solution to final volume with physiological saline. Doses used were 0.0075, 0.015 and 0.03 mg/kg.

All injections were administered subcutaneously in a volume of 1 ml/kg. Injection times were based upon previous research indicating that the drugs' effects would be extant during conditioning (3,8). MDL7222 was injected 20 min prior to conditioning while MDMA and its placebo were injected 10 min prior to conditioning.

Apparatus

The apparatus, described in detail elsewhere (19), was 12 nearly identical alleys, each housed in a sound-attenuating outer shell. Each alley was divided into two equal halves having distinct visual (solid grey or black and white striped sides) and textural cues (flooring made of steel rods running either parallel or perpendicular to the length of the alley). A wooden barrier, with sides that matched the respective halves of the alley, was used to separate the distinct environments. An alley tilted slightly when a rat moved to either side of a center support, completing a circuit that was monitored by a personal computer.

Each side of the alley had an adjustable lightbulb overhead. The amount of reflected light on each side of the alley was adjusted so that the side of putative conditioning was slightly brighter than the alternate side.

Procedure

There were two separate assessments of MDL7222's effects on a MDMA CPP. The procedures in each assessment were nearly identical to each other in terms of handling, conditioning

and testing. Upon arrival at the laboratory, all rats were individually housed in their home cages. On the following day, rats began a 3-week long schedule of habituation, conditioning and testing. All procedures took place between 0900 and 1300 h.

Days 1–5 comprised the handling phase of the experiment in which rats were habituated to the general procedures. Rats were weighed daily, as they were on every day of the formal experiment, and placed into a mobile cart (12 cages/cart, 1 rat/cage). The cart was then wheeled into an adjacent room which contained the CPP apparatus and each rat was handled briefly before being returned to its home cage.

On Days 6–7, each rat was placed into its respective alley and allowed access to either side for 30 min. The time spent on the side of putative conditioning was recorded on Day 7 and served as a baseline measure. Rats were subsequently assigned to groups so that each group was roughly equal in terms of baseline preference scores and number of rats assigned the grey or striped side as side of putative conditioning. A treatment was then randomly assigned to each of the groups. On Days 8–9, rats were given no special treatment.

Formal conditioning began on Day 10 with rats being given their assigned injections (see Table 1) before being placed into their side of putative conditioning for 30 min. These procedures were repeated on Days 11–12. On Day 13, rats received two injections (see Table 1) and were placed into the alternate side of the alley. The 14th day served as a test with rats being placed into the alley with access to both sides for 30 min. Two days of no special intervention followed the test. The procedure of 3 days of putative conditioning, 1 day of alternate conditioning and a test was repeated once more.

Briefly, the results with the 60 rats of the first assessment, though promising, only approached standards of statistical significance. In order to further clarify the main findings of the first assessment, an additional 30 rats were conditioned and tested. They were divided into four groups having the same regimen of injections as some of the groups of Table 1, i.e., there was: (a) a Saline control group ($n=5$), (b) a MDL7222 control group ($n=5$), (c) a MDMA group ($n=10$) and (d) a MDMA/MDL30 group ($n=10$).

Data Reduction and Statistics

The design of the first assessment conforms to a 6 by 2 by 3 ANOVA with factors of Group (see Table 1), Side of putative conditioning (Grey or Striped) and Tests (Baseline, Test 1 and Test 2), respectively. Since the factor of Side failed to be a reliable source of variance by itself or to interact with the other factors ($ps>0.21$), it was subsequently dropped from further analyses. Furthermore, since rats were assigned to groups based on their Baseline scores (and, therefore, did not differ) and these scores were as expected (approximately a 42% preference for putative side), consideration of Baseline scores was dropped from final analyses. The scores associated with Test 1 were also dropped from final analyses, since none of the groups showed any indication of a conditioned drug effect with the limited conditioning prior to Test 1.

Further analyses revealed no differences between the saline control and the MDL7222 control groups at either Baseline or Test 2 ($ps>0.74$). Since the best indicator of what the other rats would do without a conditioned drug effect are the scores associated with the two control groups, the data of these two groups were collapsed into one group. Furthermore, as expected, the control groups did not exhibit any gross change in preferences between Baseline and Test 2. With these conditions met, the relevant data assessing MDL7222's effects on a MDMA

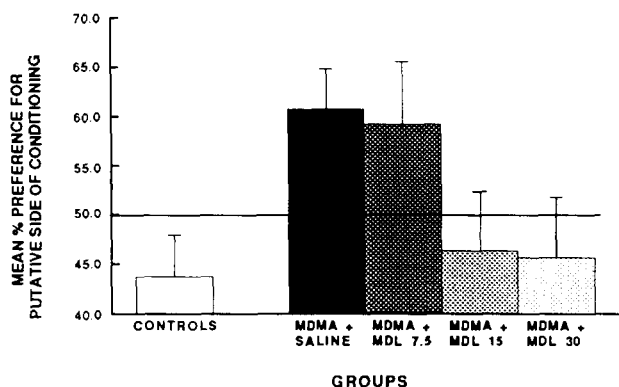


FIG. 1. Test 2 scores are depicted as mean % time spent on side of putative conditioning for each group. A score greater than 50.0% reflects more time spent on side of putative conditioning. For example, rats conditioned with MDMA (plus saline) spent on average 1094 out of a possible 1800 s on their side of putative conditioning which translates to a 60.77% preference. Groups are labelled according to the two injections they received on day of putative conditioning, e.g., MDMA/MDL 7.5 refers to the group which received conditioning with 6.3 mg/kg MDMA in combination with a 0.0075 mg/kg dose of MDL72222 (see Table 1). Bars represent standard errors of the mean.

CPP conformed to a one-way ANOVA across the scores of Test 2. The analysis of the second assessment followed that of the first.

RESULTS

The results of the first assessment are depicted in Fig. 1. An ANOVA of the data yields an $F(4,55) = 2.49$, $p = 0.054$. Despite the p -value not meeting the conventional standard for concluding there is a reliable effect of treatment ($p < 0.05$), selected t -tests were done. A comparison of the scores of the Control group and the MDMA group indicates that the group conditioned with MDMA preferred the side of MDMA experience, $t(28) = 2.60$, $p = 0.015$, replicating previous research (3). The low dose of MDL72222 had no apparent effect on MDMA's ability to establish a positive CPP; that group's mean score is very similar to the group getting MDMA (plus placebo) on side of putative conditioning, $t(28) = 0.2$, $p = 0.84$, and reliably different from the control group, $t(28) = 2.10$, $p = 0.045$.

There are indications that the two higher doses of MDL72222 (0.015 and 0.03 mg/kg) did block the establishment of a MDMA CPP. The mean scores of the two groups getting MDMA in combination with one of the higher doses of MDL72222 are very similar to those of the control group ($ps > 0.7$). Furthermore, t -tests comparing the MDMA group with the MDMA/MDL15 and MDMA/MDL30 groups yield $ts(28) = 2.0$ and 2.36 , $ps = 0.06$ and 0.03 , respectively.

As mentioned, the results of the first assessment only approached standards of statistical significance ($p < 0.05$) for the critical F -value from the ANOVA even though planned comparisons indicated reliable differences between some of the groups. The second assessment was designed to further assess the finding that MDMA produced a CPP and that the 0.03 mg/kg dose of MDL72222 blocked that CPP.

The results of the second assessment are depicted in Fig. 2. As in the first assessment, an ANOVA of Test 2 data produced a p -value that only approached standards for reliability, $F(2,27) = 2.74$, $p = 0.083$. Nevertheless, Student's t -tests be-

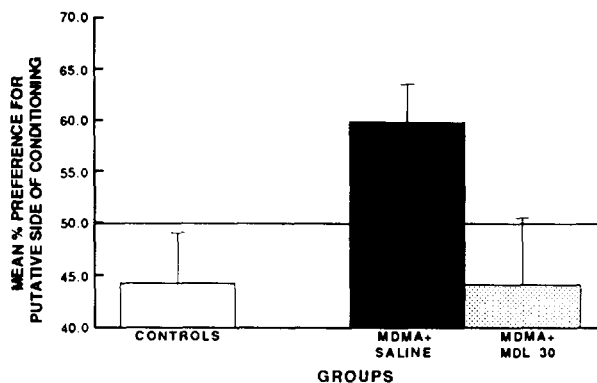


FIG. 2. The results of the second assessment are depicted as groups' mean % time spent on side of putative conditioning, for Test 2. Bars represent standard errors of the mean.

tween the Control group and the MDMA group revealed that the MDMA group spent reliably more time on side of putative conditioning, $t(18) = 2.26$, $p = 0.04$. Furthermore, the 0.03 mg/kg dose of MDL72222 blocked the effects of MDMA as indexed by the comparison between the MDMA/MDL30 and the MDMA/SAL groups, $t(18) = 2.11$, $p = 0.049$, and the comparison between the MDMA/MDL30 group and Control group, $t(18) = 0.015$, $p = 0.99$.

Using the procedure suggested by Winer (23) for assessing the statistical significance of two tests of the same hypothesis, it was confirmed that MDMA produced a positive CPP that was blocked by a dose of MDL72222. The comparison of the MDMA groups to the controls yields a $\chi^2(4) = 15.04$, $p < 0.005$. Further analysis revealed a reliable difference between the MDMA groups and the MDMA/MDL30 groups, $\chi^2(4) = 13.06$, $p < 0.025$, while the Control groups and the MDMA/MDL30 groups showed no such difference, $\chi^2(4) = 0.52$, $p > 0.50$.

DISCUSSION

MDMA is capable of establishing a positive CPP [Assessments 1 and 2 and (3,20)]. These data support the conclusion that MDMA's ability to establish a positive CPP is blocked by doses of MDL72222, a 5-HT₃ antagonist. It can also be concluded with some confidence that MDL72222's effects were specific. First, MDL72222's effects were paired with both sides of the alley and, therefore, any nonspecific effects are apt to be conditioned to each side of the alley. This is reflected in the mean score of the MDL72222 control group which was no different than the saline control group (consequently, these two groups were combined to form a larger Control group), a finding similar to that found by others (8). Furthermore, in a similar procedure (unpublished results), a dose of 0.03 mg/kg of MDL72222 was paired with only the putative side of conditioning and at testing there was no indication that MDL72222 produced reliable shifts in preference compared to a placebo group.

There is an extant theoretical framework for explaining MDMA's positivity and MDL72222's ability to block that positivity. Supposedly, MDMA causes the release of dopamine (13,21), the primary neurotransmitter modulating the reinforcing effects of self-administered drugs (6,24). Because of MDL72222's reputed specificity to the 5-HT₃ receptor (12), and because MDL72222 blocked MDMA's capability to establish a CPP, it can be concluded that MDMA achieves at least some of its rewarding effects by acting, either directly or indirectly (e.g., by

way of its modulation of serotonin), at the 5-HT₃ receptor. Given that activity following activation of 5-HT₃ receptors affects the release of dopamine, this cascade of events, in turn, may be critical to MDMA's positivity.

Because MDMA affects dopaminergic systems and because 5-HT₃ receptors affect the release of dopamine, these data are concordant with the dopaminergic hypothesis of reinforcement. Furthermore, these results support the suggestions that compounds affecting 5-HT₃ receptors may be of particular interest

in studying the pharmacology of self-administered drugs.

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REFERENCES

- Battaglia, G.; Brooks, B. P.; Kulsakdinum, C.; De Souza, E. B. Pharmacological profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. *Eur. J. Pharmacol.* 149:159-163; 1988.
- Battaglia, G.; Zaczek, R.; De Souza, E. B. MDMA effects in brain: Pharmacological profile and evidence of neurotoxicity from neurochemical and autoradiographic studies. In: Peroutka, S. J., ed. *Ecstasy: The clinical, pharmacological and neurotoxicological effects of the drug MDMA*. Boston: Kluwer Academic Publishers; 1990:171-199.
- Bilsky, E. J.; Hui, Y.; Hubbell, C. L.; Reid, L. D. Methylenedioxymethamphetamine's capacity to establish place preferences and modify intake of an alcoholic beverage. *Pharmacol. Biochem. Behav.* 37:633-638; 1990.
- Bilsky, E. J.; Marglin, S. H.; Reid, L. D. Using antagonists to assess neurochemical coding of a drug's ability to establish a conditioned place preference. *Pharmacol. Biochem. Behav.* 37:425-431; 1990.
- Blandina, P.; Goldfarb, J.; Green, J. P. Activation of a 5-HT₃ receptor releases dopamine from striatal slice. *Eur. J. Pharmacol.* 155:349; 1988.
- Bozarth, M. A. Neural basis of psychomotor stimulant and opiate reward: Evidence suggesting the involvement of a common dopaminergic system. *Behav. Brain Res.* 22:107-116; 1986.
- 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(2): 456-464; 1990.
- Carboni, E.; Acquas, E.; Leone, P.; DiChiara, G. 5-HT₃ receptor antagonists block morphine and nicotine- but not amphetamine-induced reward. *Psychopharmacology (Berlin)* 97:175; 1989.
- Cooper, S. J.; Van Der Hoek, G.; Jones, B. J.; Tyers, M. B. Antagonism of d-amphetamine induced place preference conditioning by the 5-HT₃ receptor antagonist GR38032F. *Psychopharmacology (Berlin)*; in press.
- Costall, B.; Domeney, A. M.; Naylor, R. J.; Tyers, M. B. Effects of the 5-HT₃ receptor antagonist GR38032F on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. *Br. J. Pharmacol.* 92:881-890; 1987.
- Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increased synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. USA* 85:5274; 1988.
- Fozard, J. R. MDL72222: A potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn Schmiedeberg Arch. Pharmacol.* 326:36-44; 1984.
- Gibb, J. W.; Stone, D.; Johnson, M.; Hanson, G. R. Neurochemical effects of MDMA. In: Peroutka, S. J., ed. *Ecstasy: The clinical, pharmacological and neurotoxicological effects of the drug MDMA*. Boston: Kluwer Academic Publishers; 1990:133-150.
- Guan, X.-M.; McBride, W. J. Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. *Brain Res. Bull.* 23:541-547; 1989.
- Hagan, R. M.; Butler, A.; Hill, J. M.; Jordan, C. C.; Ireland, S. J.; Tyers, M. B. Effect of the 5-HT₃ receptor antagonist GR38032F, on the response to injection of neurokinin agonist into the ventral tegmental area of the rat brain. *Eur. J. Pharmacol.* 138:303; 1987.
- Hubner, C. B.; Bird, M.; Rassnick, S.; Kornetsky, C. The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. *Psychopharmacology (Berlin)* 95:49-51; 1988.
- Lamb, R.; Griffiths, R. Self-injection of d,l-3,4-methylenedioxymethamphetamine (MDMA) in the baboon. *Psychopharmacology (Berlin)* 91:268-272; 1987.
- Peroutka, S. J. Recreational use of MDMA. In: Peroutka, S. J., ed. *Ecstasy: The clinical, pharmacological and neurotoxicological effects of the drug MDMA*. Boston: Kluwer Academic Publishers; 1990:53-62.
- Reid, L. D.; Marglin, S. H.; Mattie, M. E.; Hubbell, C. L. Measuring morphine's capacity to establish a place preference. *Pharmacol. Biochem. Behav.* 33:765-775; 1989.
- Schechter, M. D. Effect of MDMA neurotoxicity upon its conditioned place preference and discrimination. *Pharmacol. Biochem. Behav.* 38:539-544; 1991.
- Schmidt, C. J.; Taylor, V. L. Neurochemical effects of methylenedioxymethamphetamine in the rat: Acute versus long-term changes. In: Peroutka, S. J., ed. *Ecstasy: The clinical, pharmacological and neurotoxicological effects of the drug MDMA*. Boston: Kluwer Academic Publishers; 1990:151-169.
- Steele, T. D.; Nichols, D. E.; Yim, G. K. W. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [3H] monoamines into synaptosomes from different regions of rat brain. *Biochem. Pharmacol.* 36:2297-2303; 1987.
- Winer, B. J. *Statistical principles in experimental design*. New York: McGraw-Hill; 1971:49-50.
- Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. *Psychol. Rev.* 94:469-492; 1987.