Pharmacology Biochemistry & Behavior, Vol. 39, pp. 509–512. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

RAPID COMMUNICATION

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

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Received 8 March 1991

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 MDMA MDL72222 5-HT3 receptors Conditioned place preference Affect Reward

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 = MDL72222. Numbers following MDL refer to the dose in μg/kg.

Drugs

±-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 (MDL72222) (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). MDL72222 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 seperate 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 seperate assessments of MDL72222'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 MDL72222 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 MDL72222 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 relevent data assessing MDL72222's effects on a MDMA

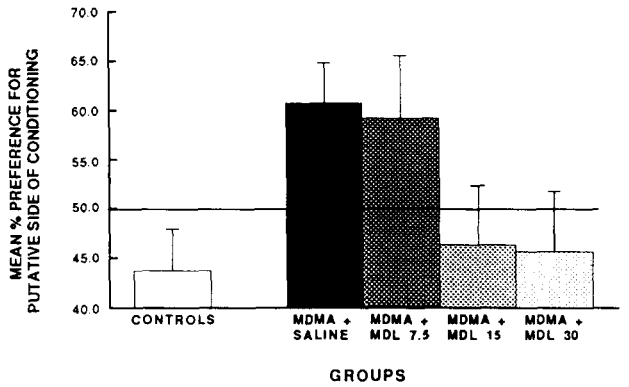

FIG. 1. Test 2 scores are depicted as mean % time spent on side of putative conditioning for each group. A score greater than 50.0% reflects more time spent on side of putative conditioning. For example, rats conditioned with MDMA (plus saline) spent on average 1094 out of a possible 1800 s on their 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.

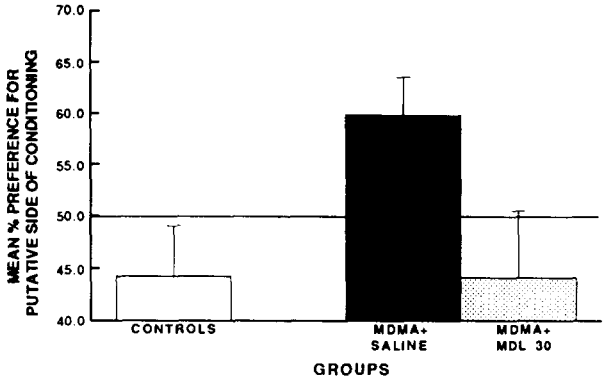

FIG. 2. The results of the second assessment are depicted as groups' mean % time spent on side of putative conditioning, for Test 2. Bars represent standard errors of the mean.

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 prefered 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 between 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.

ACKNOWLEDGEMENTS

We thank Chris Hubbell, Mike Nichols and John Delconte for their help and Dr. Michael Kalsher for the use of his computing facilities. This work was supported, in part, by Grant DA 04440 from the National Institute on Drug Abuse (NIDA). MDMA was provided by the NIDA Drug Supply Program: Research Technology Branch.

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