Effects of the Selective 5-HT$_{2A}$ Receptor Antagonist MDL 100,907 on MDMA-Induced Locomotor Stimulation in Rats

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($\pm$)3,4-Methylenedioxymethamphetamine (MDMA) releases dopamine and serotonin in vivo and stimulates locomotor activity. Previous work demonstrated that MDMA-stimulated dopamine release could be reduced by the selective 5-HT$_{2A}$ receptor antagonist [R-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol] (MDL 100,907). In the present study MDL 100,907 significantly reduced MDMA-stimulated locomotion without affecting basal levels of locomotion. Other agents with 5-HT$_{2A}$ antagonist activity (ritanserin, clozapine, MDL 28,133A, or methiothepin), as well as agents that block 5-HT$_{1A}$-(propranolol), D$_2$-(haloperidol), or D$_1$ receptors (SCH 23390) also reduced MDMA-stimulated locomotion. Intraventricularly administered

*5,7-dihydroxytryptamine decreased regional 5-HT levels and attenuated MDMA-stimulated locomotion. These data support the conclusion that serotonin released onto 5-HT$_{2A}$ receptors contributes to MDMA-stimulated locomotion and suggest that MDMA-stimulated locomotion may be useful as an in vivo behavioral measure of 5-HT$_{2A}$ antagonism. The data also support previous reports of contributions of 5-HT$_{1A}$, D$_1$ and D$_2$ receptors to MDMA-stimulated locomotion. A preliminary time-course analysis indicating time-dependent contributions of different receptors to MDMA-stimulated locomotion suggests the potential utility of this model for characterizing potential atypical antipsychotic compounds. [**Neuropsychopharmacology 15:116–124**]*

In vivo models utilizing stimulants have been widely used for identifying and characterizing putative psychotherapeutic agents. For example, the behavioral effects of amphetamine are studied to identify and characterize potential antipsychotics (Megens et al. 1992). Antagonism of a stimulant-induced behavioral effect may provide an in vivo assessment of a specific pharmacological action (i.e., D$_2$ receptor antagonism) as well as predicting a specific therapeutic action (i.e. antipsy-

chotic). Stimulants that affect multiple neurotransmitters may offer unique advantages as behavioral models. The present study focuses on the use of the stimulant ($\pm$)3,4-methylenedioxymethamphetamine (MDMA) as a behavioral model that may be useful for studying 5-HT$_{2A}$ antagonists, and, more generally, antipsychotic compounds.

MDMA, like d-amphetamine, releases dopamine, but it differs from d-amphetamine in being a potent 5-HT releaser as well (Schmidt and Kehne 1990). This additional 5-HT releasing component might account for the differences in MDMA's behavioral effects relative to d-amphetamine. For example, compared to d-amphetamine, MDMA produces a different pattern of locomotor stimulation (Paulus and Geyer 1991) and suppresses (rather than stimulates) rearing behavior (Gold et al. 1988; Callaway et al. 1990). MDMA-stimulated locomotor activity is mediated, at least in part, by 5-HT recep-

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tor activation, and the contributions of 5-HT_1-like receptors have been demonstrated (Callaway et al. 1992; Rempel et al. 1993). Studies using the $5\text{-HT}_{2A/2C}$ antagonist ritanserin suggested minimal contribution of 5-HT_2 receptors to these effects.

The reported lack of a 5-HT_2 contribution by Rempel et al. (1993) was surprising given previous neurochemical evidence that 5-HT_{2A} antagonism attenuated neurochemical effects of MDMA administration (Schmidt et al. 1992a; 1992b). These studies used [R-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol] (MDL 100,907, Figure 1), a potent and selective 5-HT_{2A} antagonist (Palfreyman et al. 1993; Kehne et al. in press) and a putative atypical antipsychotic (Sorensen et al. 1993; Schmidt et al. 1995; Moser et al. 1996; Kehne et al. in press) as a pharmacological tool to block 5-HT_{2A} receptors. MDL 100,907 reduced the serotonergic neurotoxicity produced by MDMA (Schmidt et al. 1992b). Furthermore, in vivo microdialysis experiments found that MDL 100,907 reduced the stimulation of dopamine release produced by MDMA (Schmidt et al. 1992b). Therefore, despite its lack of affinity for D_2 receptors (Palfreyman et al. 1993; Kehne et al. in press), MDL 100,907 attenuates excessive dopaminergic activity by agents such as MDMA (Schmidt et al. 1993). Both D_2 and 5-HT_2 receptors have been suggested to contribute to antipsychotic actions (Meltzer et al. 1989; Schmidt et al. 1993). MDL 100,907, which is currently in development for the treatment of schizophrenia, should be the first agent to definitively test the hypothesis that selective 5-HT_{2A} stimulation is sufficient to produce antipsychotic actions in man (Schmidt et al. 1995).

Given previous evidence for an interaction between MDL 100,907 and MDMA (Schmidt et al. 1992b), the primary goal of the present study was to evaluate MDL 100,907's activity in reducing MDMA locomotor stimulation in rats. Comparisons were made to compounds that have 5-HT_{2A} antagonism as part of their profile (the $5\text{-HT}_{2A/2C}$ antagonist ritanserin, the broad-spectrum receptor antagonist/atypical antipsychotic cloza-

pine, the mixed $5\text{-HT}_2/D_2$ antagonist MDL 28,133A; the $5\text{-HT}_{1/2}/D_2$ antagonist methiothepin), as well as compounds that affect dopamine receptors (the D_2 antagonist haloperidol, the D_1 antagonist SCH 23390) and other 5-HT receptors (the $5\text{-HT}_{1A}/\beta$-adrenergic antagonist propranolol). Intraventricular administration of the 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) was used to lesion 5-HT terminals to evaluate the importance of 5-HT terminals for the expression of MDMA-stimulated locomotion.

METHODS AND MATERIALS

Animals

Adult male CD rats purchased from Charles River Laboratories (Wilmington, MA) at a weight of 125–150 g were used in each experiment. The colony room was on a 14:10 light/dark cycle (lights on at 6:00 a.m.) and temperature was controlled to 74–78°F. The animals were housed four per cage with free access to food and water. All animals were acclimated for at least 1 week from the date of receipt before beginning the experiments.

Test Compounds

[R-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol] (MDL 100,907; Hoechst Marion Roussel, Inc.), haloperidol (Janssen), clozapine (Sandoz), ritanserin (Janssen), SCH 23390 (Schering-Plough), methiothepin, and (1-(4-fluorophenyl)-2-[4-[(4methanesulfonamidophenyl)carbonyl]-1-piperidinyl]-ethanone hydrochloride (MDL 28,133A; Hoechst Marion Roussel, Inc.) were prepared as suspensions in distilled water using 1% Tween 80®. MDMA refers to the racemic compound. Propranolol and MDMA (National Institute of Drug Abuse) were prepared as solutions in distilled water. MDMA was administered subcutaneously (SC). All other test compounds were given intraperitoneally (IP). Injection volumes were 1 ml/kg body weight.

Receptor Binding

The methods used to determine the affinity of MDL 28,133A for 5-HT_2-, D_2-, 5-HT_{2C}-, α_1-adrenergic-, and β-adrenergic receptors have been previously referenced (Palfreyman et al. 1993).

Activity Measurement

Activity testing was carried out using a photocell-based system ("Autotrack System®"; Columbus Instruments, Columbus, Ohio). The parameters for locomotion were defined by the Autotrack software as follows: "Distance
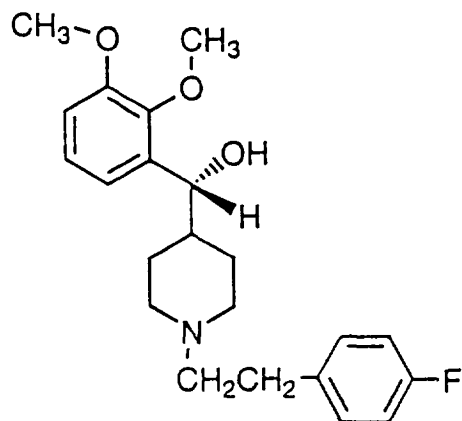

Figure 1.   The structure of MDL 100,907.

Traveled" (DT) was the parameter used to measure forward locomotion. A rat had to break four consecutive photocell beams to register. Thus, more localized, "stereotyped" movements would not be counted as forward locomotion. "Vertical Time" (VT) was the amount of time that the rat engaged in vertical rearing behaviors as measured by a separate bank of elevated photocells.

5,7-Dihydroxytryptamine (5,7-DHT) Lesions of Central 5-HT Terminals

Rats were tested 2 weeks after intracerebroventricular (ICV) administration of 200 µg 5,7-DHT. Brains were removed 1 week later for assay of 5-HT. Procedures used have been previously described in detail (Kehne et al. 1992).

Testing Procedure

In the first experiment, rats were injected SC with vehicle or MDMA and then were placed singly in clear Plexiglas® boxes (16 × 16 × 8 inches) in the activity monitors for 120 minutes of activity testing. In subsequent experiments, rats were dosed IP with vehicle or the antagonist and placed in the Plexiglas® boxes and allowed to acclimatize for 30 minutes. Following accli-

matization, the rats were dosed with vehicle or MDMA SC and tested in the activity monitors for 60 minutes.

Experimental Protocols and Data Analyses

The first experiment characterized the effects of vehicle or a range of doses (1, 2, 4, 10, 20, and 40 mg/kg) of MDMA on locomotion and rearing behavior and was used to help choose a single dose of MDMA for subsequent studies. Statistical analyses on the 2-hour session means were carried out using a one-way analysis of variance (ANOVA) with dose as a between-subjects factor. Individual comparisons were made using Fisher's Protected Least-Significant Difference test ($p < .05$). In the first experiment, 3 rats were used at each dose. Replications of the vehicle-treated group resulted in a total of nine rats. The second experiment evaluated the effects of MDL 100,907 (1 mg/kg) or other treatments on MDMA-stimulated locomotion and rearing. A 1-hour test session was used ($n = 5$ in each group). In addition, the effects of various other antagonists on MDMA locomotor stimulation were measured. The additional treatments included: the D_2 antagonist haloperidol (0.2 mg/kg), the 5-HT_2/D_2 antagonist clozapine (4 mg/kg), the 5-$HT_{2A/2C}$ antagonist ritanserin (2 mg/kg), the 5-HT_{1A}/β-adrenergic antagonist propranolol (20 mg/kg); the D_1
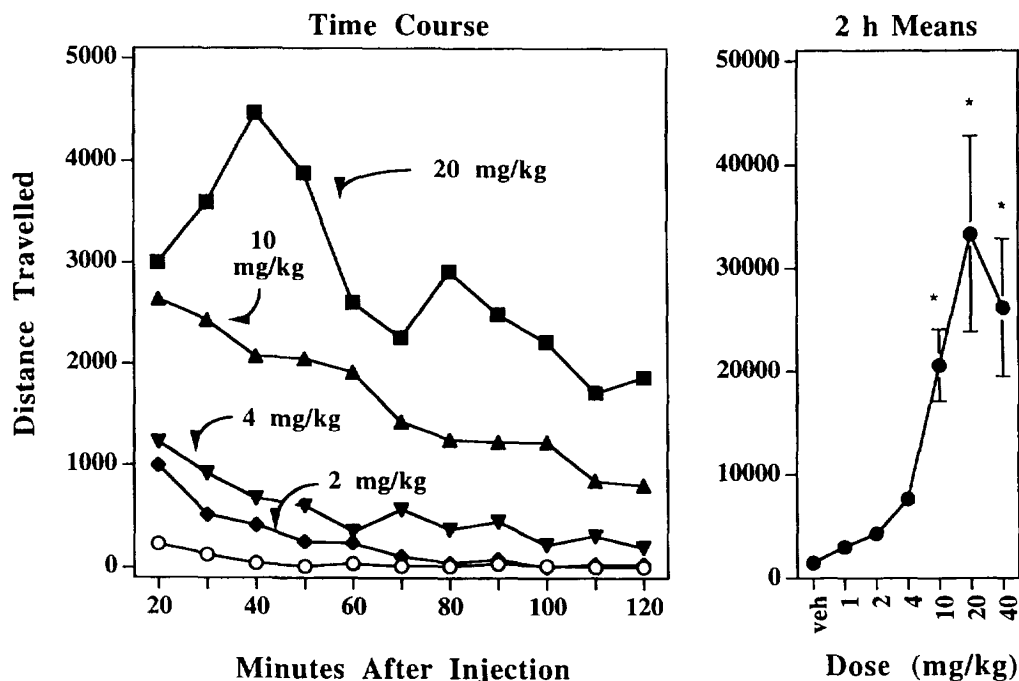

Figure 2. The effects of vehicle (VEH) or MDMA (1, 2, 4, 10, 20, and 40 mg/kg, SC) on locomotion in rats. In the right panel each point represents the group mean blocked over 2 hours of testing. The left panel shows the time course for the effects or locomotion of vehicle (*open circles*), 2 mg/kg (*diamonds*), 4 mg/kg (*inverted triangles*), 10 mg/kg (*triangles*), and 20 mg/kg (*squares*), blocked over 10-minute intervals. *$p < .05$ or less, relative to vehicle-injected controls. For vehicle group, $n = 9$; for all other groups, $n = 3$.

antagonist SCH 23390 (0.1 mg/kg); the mixed 5-HT$_2$/D$_2$ antagonist, MDL 28,133A (1 mg/kg; Schmidt et al. 1992a); the mixed D$_2$/5-HT$_{1/2}$ antagonist methiothepin (1 mg/kg). In addition, the 5-HT neurotoxin, 5,7-DHT (200 μg given into the right lateral ventricle 2 weeks before testing) was administered to evaluate the importance of 5-HT terminals to MDMA-stimulated locomotion. The doses of these treatments were chosen from literature references and from pilot studies. For each compound evaluated, an MDMA control consisting of rats injected with vehicle/vehicle or vehicle/MDMA was included. Two scores (0–30 minute and 30–60-minute session scores) were generated for each rat. Statistical analyses on these scores were carried out using treatment as a between-subjects factor and time as a within-subjects factor. Contrast tests were used for individual comparisons ($p < .05$).

RESULTS

Receptor Binding Profile of MDL 28,133A

Calculation of IC$_{50}$s for various receptors were as follows: 5-HT$_{2A}$, [^{3}H]ketanserin = 59.8 ± 15 nM; D$_2$, [^{3}H]spiroperidol = 240 ± 100 nM; 5-HT$_{2C}$, [^{3}H]mesulergine = 586 ± 40 nM; α$_1$-adrenergic receptor, [^{3}H]prazosin = 700 ± 30 nM; β-adrenergic receptor, [^{3}H]DHA > 10,000 nM. Thus, relative to its affinity at 5-HT$_{2A}$ receptors, MDL 28,133A was 4-fold less potent at the D$_2$ receptor, 10-fold less potent at the 5-HT$_{2C}$ receptor, 12-fold less potent at the α$_1$-adrenergic receptor, and 167-fold less potent at the β-adrenergic receptor.

Effects of MDMA on Locomotor Activity and Rearing

Figure 2 summarizes the effects of vehicle ($n = 9$) or MDMA (1, 2, 4, 10, 20, and 40 mg/kg; $n = 3$ in each group) on locomotor activity in rats. In addition, Figure 2 shows the time course of the effects of vehicle, 2, 4, 10, and 20 mg/kg MDMA on locomotor activity, with the data blocked over 10-minute intervals for a total of 2 hours of testing. MDMA, at doses of up to 20 mg/kg, produced a dose-related increase in locomotion. A one-way ANOVA of the 2-hour means with Dose as a between-subjects factors revealed a significant MDMA effect [$F(6,20) = 13.86, p < .0001$]. Subsequent individual comparisons [Fisher's protected least-significant difference (LSD) test; $p < .05$] revealed significant effects of the 10-, 20-, and 40-mg/kg doses relative to vehicle-injected controls. Significant differences were seen between the 4- and 10-, and the 10- and 20-mg/kg doses, indicating that the stimulatory effect of MDMA was dose-related. The 20- and 40-mg/kg doses were not significantly different from each other, indicating that a maximal effect was achieved with the 20-mg/kg dose.

MDMA also produced a dose-related decrease in rearing behavior. Means (2-hour test period) and SEM were: vehicle (396 ± 101); 1 mg/kg (405 ± 33); 2 mg/kg (509 ± 143); 4 mg/kg (219 ± 81); 10 mg/kg (168 ± 33); 20 mg/kg (45 ± 38); and 40 mg/kg (37 ± 36). A one-way ANOVA using dose as a between-subjects factor revealed a marginally significant effect of MDMA [$F(6,20) = 2.56, p = .053$]. Post hoc comparisons (Fisher's protected LSD test, $p < .05$) revealed significant MDMA reductions of rearing at the 20-mg/kg and 40-mg/kg

Figure 3. The effects of MDL 100,907 (1 mg/kg, IP) or vehicle given 0.5 hours before MDMA (20 mg/kg, SC) or vehicle on locomotion in rats. Panel A shows data blocked over 10-minute intervals. Panel B summarizes data blocked over two 30-minute bins. *$p < .05$ or less, vs. vehicle/MDMA in same time bin; #p < .05 or less, versus vehicle/vehicle in same time bin; @ $p < .05$ or less, versus MDL 100,907/vehicle in same time bin; ^$p < .05$ or less, versus MDL 100,907/MDMA in other time bin.
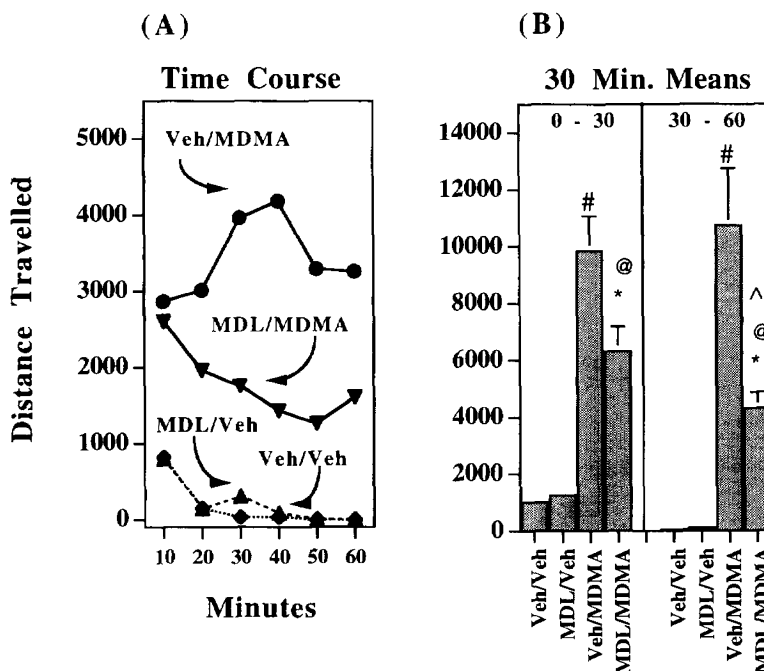
(A)
Time Course

(B)
30 Min. Means

Table 1. Effect of Various Treatments on MDMA (20 mg/kg)-Stimulated Locomotion (distance traveled, DT) in Rats ($n = 5$ in each group)

Antagonist Used (dose, mg/kg, IP)	Overall ANOVA F Ratios (df)			0–30-minute Mean ± SEM				30–60-minute Mean ± SEM			
	Treatment	Time	Treatment-by-Time Interaction	Vehicle	Control (%)	MDMA	Control (%)	Vehicle	Control (%)	MDMA	Control (%)
Vehicle	31.82 (3,16) $p < .0001$	4.08 (1,16) $.05 < p < .1$	2.33 (3,16) NS	1,005 ± 111	100	9,847 ± 1,237[c]	100	41 ± 32	100	10,737 ± 2,004[c]	100
MDL 100,907 (1)				1,248 ± 124	124	6,332 ± 865[b,d]	64	104 ± 68	254	4,311 ± 568[b,d,f]	40
Vehicle	83.24 (3,16) $p < .0001$	18.45 (1,16) $p < .0006$	11.05 (3,16) $p < .0004$	1,063 ± 122	100	10,224 ± 628[c]	100	536 ± 416	100	5,727 ± 925[c,e]	100
Haloperidol (0.2)				838 ± 91	79	6,282 ± 639[b,d]	61	248 ± 245	46	6,432 ± 537[d]	112
Vehicle	19.90 (3,16) $p < .0001$	0.77 (1,16) NS	1.14 (3,16) NS	1,591 ± 264	100	9,770 ± 676[c]	100	50 ± 46	100	11,734 ± 3,710[c]	100
Clozapine (4)				1,046 ± 294	66	9,065 ± 1,611[d]	93	342 ± 255	684	5,729 ± 1,235[b,d]	49
Vehicle	20.60 (3,16) $p < .0001$	0.51 (1,16) NS	4.39 (3,16) $p < .02$	1,180 ± 88	100	6,716 ± 688[c]	100	74 ± 54	100	9,616 ± 2,341[c,e]	100
Ritanserin (2)				1,212 ± 158	103	8,284 ± 1,474[d]	123	15 ± 15	20	6,165 ± 805[b,d]	64
Vehicle	12.80 (3,16) $p < .0002$	0.30 (1,16) NS	1.48 (3,16) NS	1,186 ± 139	100	10,787 ± 1,496[c]	100	9 ± 5	100	10,268 ± 2,856[c]	100
Propranolol (20)				1,327 ± 188	112	6,876 ± 1,703[b,d]	64	6 ± 4	67	8,619 ± 2,292[d]	84
Vehicle	19.42 (3,16) $p < .0001$	3.06 (1,16) $.05 < p < 0.1$	7.42 (3,16) $p < .0025$	1,159 ± 286	100	9,199 ± 1,315[c]	100	6 ± 6	100	10,268 ± 1,935[c]	100
SCH 23390 (0.1)				1,089 ± 312	94	6,460 ± 1,013[b,d]	70	96 ± 85	16	10,849 ± 2,314[d,f]	106
Vehicle	14.12 (3,16) $p < .0001$	8.58 (1,16) $p < .01$	0.25 (3,16) NS	1,802 ± 235	100	9,562 ± 1,548[c]	100	200 ± 119	100	7,451 ± 1,695[c]	100
Methiothepin (1)				888 ± 465	49	4,328 ± 1,652[b,d]	45	35 ± 16	18	2,808 ± 1,232[b,d]	38
Vehicle	16.31 (3,16) $p < .0001$	2.13 (1,16) NS	4.74 (3,16) $p < .015$	1,720 ± 259	100	9,872 ± 805[c]	100	425 ± 170	100	17,677 ± 4,366[c,e]	100
MDL 28,133A (1)				1,785 ± 132	104	6,051 ± 1,223[d]	61	593 ± 166	140	6,488 ± 2,012[b,d]	37
Vehicle	20.36 (3,16) $p < .0001$	1.21 (1,16) NS	4.86 (3,16) $p < .014$	1,398 ± 258	100	7,314 ± 981[c]	100	74 ± 34	100	9,911 ± 2,348[c,e]	100
5,7-DHT (0.2[a])				990 ± 143	71	3,217 ± 463[b,d]	44	86 ± 61	140	4,582 ± 478[b,d]	46

[a] Dose expressed in mg, compound administered i.c.v.
[b] $p < .05$ or less vs. vehicle/MDMA in same time bin.
[c] $p < .05$ or less vs. vehicle/vehicle in the same time bin.
[d] $p < .05$ or less vs. antagonist/vehicle in the same time bin.
[e] $p < .05$ or less vs. vehicle/MDMA in other time bin.
[f] $p < .05$ or less vs. antagonist/MDMA in other time bin.

doses. The 20- and 40-mg/kg doses did not differ significantly from each other.

On the basis of locomotion data described and on the basis of previous studies (Kehne et al. 1992; Schmidt et al. 1992a; 1992b), a dose of 20 mg/kg MDMA and a test session duration of 1 hour were chosen for further antagonism studies.

Effects of MDL 100,907 on MDMA-Stimulated Locomotion (DT) and Rearing (VT)

Figure 3 and Table 1 summarize the effects of 1 mg/kg MDL 100,907 on 20 mg/kg MDMA-stimulated locomotion. This graph shows that MDL 100,907 reduced MDMA-stimulated locomotion without altering baseline activity. This conclusion was supported by statisti-

cal analyses (summarized in Table 1). Contrast tests showed that for each time bin there was a significant reduction of MDMA-stimulated locomotion by MDL 100,907 relative to the vehicle/MDMA-injected controls. MDMA significantly potentiated locomotion in the MDL 100,907/MDMA group relative to MDL 100,907/vehicle controls, indicating that MDL 100,907 did not fully antagonize MDMA stimulation. Finally, Table 1 also shows that MDL 100,907 produced a significantly greater reduction in the 30–60-minute bin relative to the 0–30-minute bin.

In contrast to its reduction of MDMA-stimulated locomotion, MDL 100,907 did not attenuate MDMA reduction in rearing behavior (vertical time, VT). For each group, the means and SEM for the 0–30 and 30–60-minute bins, respectively, were: vehicle/vehicle (320 ± 71; 13 ± 13); vehicle/MDMA (32 ± 19; 51 ± 21); MDL
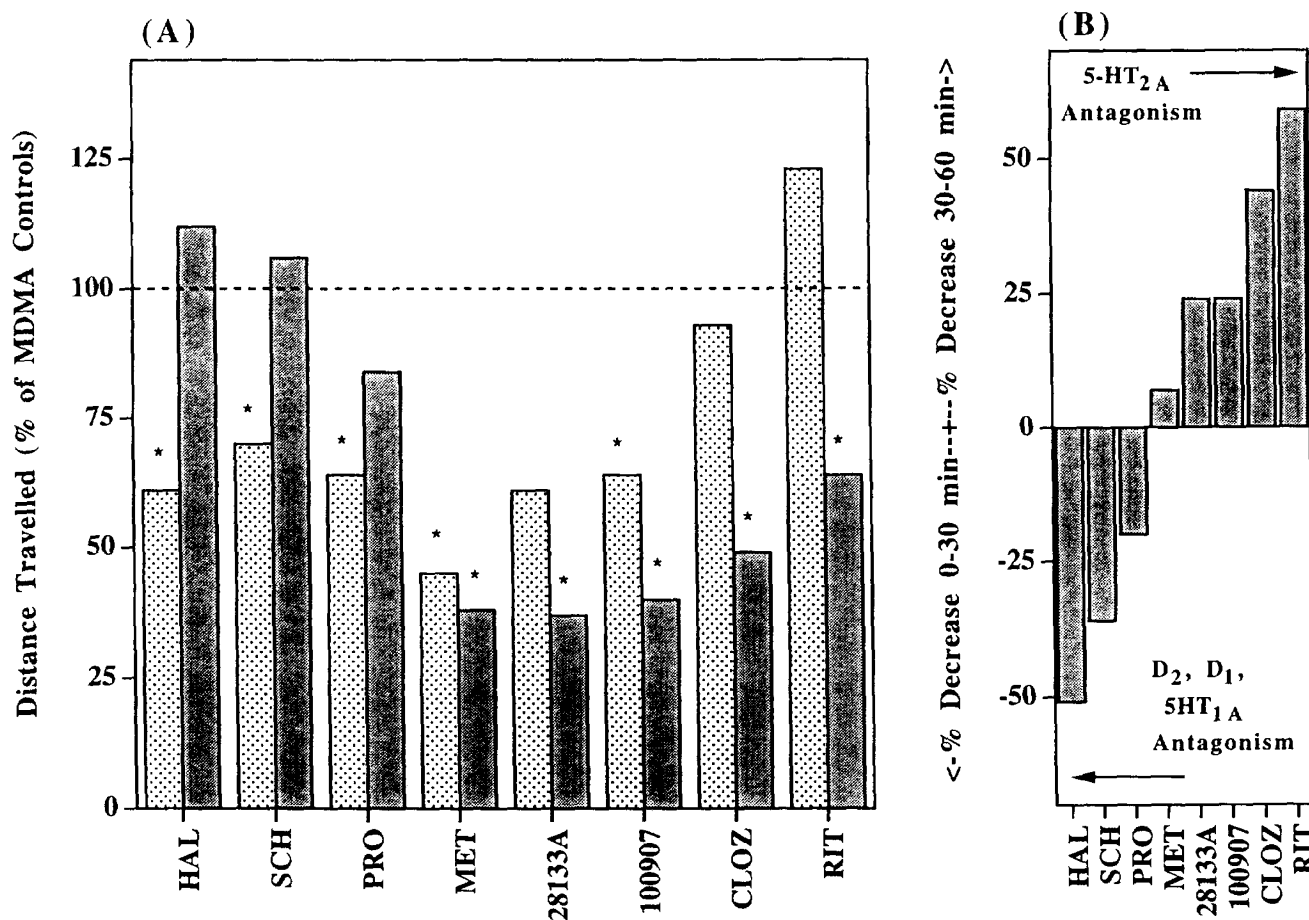

Figure 4. Summary of the effects of a variety of treatments on the early (0–30-minute bin, *dotted bars*) and late (30–60-minute bin, *shaded bars*) component of MDMA (20 mg/kg) locomotor stimulation in rats. **A**, data as percent of MDMA control for the two components. **B**, data for each treatment as a single score by using the following calculation: [−(30–60-minute % score minus the 0–30-minute % score)]. Abbreviations: *HAL* = haloperidol, 0.2 mg/kg; *SCH* = SCH 23390, 0.1 mg/kg; *PRO* = propranolol, 20 mg/kg; *MET*, methiothepin, 1 mg/kg; *28133A* = MDL 28,133A, 1 mg/kg; *100907*, MDL 100,907, 1 mg/kg; *CLOZ*, clozapine, 4 mg/kg; *RIT* = ritanserin, 2 mg/kg. Asterisks represent significant reductions ($p < .05$ or less) by antagonist of MDMA-stimulated locomotion relative to vehicle/MDMA-treated controls (see Table 1 for data and statistical analyses).

100,907/vehicle (281 ± 57; 39 ± 17); and MDL 100,907/ MDMA (48 ± 18; 17 ± 19). A two-way ANOVA with treatment as a between-subjects factor and time as a repeated-measures factor revealed a significant treatment effect [$F(3,16)$ ± 6.32, $p < .005$], a significant time effect {$F(3,16)$ = 46.87, $p < .0001$], and a significant treatment by time interaction effect, [$F(3,16)$ = 14.72, $p < .0001$]. Individual comparisons using contrast tests revealed significant depressant effects ($p < .001$) of MDMA on rearing in the vehicle and MDL 100,907 groups in the 0–30-minute bin only. The lack of significance in the 30–60-minute bin was attributable to a "floor" effect arising from the low level of rearing in the controls. The MDMA suppression of rearing in the 0–30-minute bin was not significantly diminished by MDL 100,907.

Effects of Other Treatments on MDMA-Induced Locomotor Stimulation

Figure 4 and Table 1 summarize the effects of additional treatments on MDMA-stimulated locomotor activity. Contrast tests (summarized in Table 1) revealed that each agent tested reduced at least one of the two 30-minute components of MDMA-induced locomotor stimulation ($p < .05$). Haloperidol, SCH 23390, and propranolol affected only the 0–30-minute bin, whereas MDL 28,133A, ritanserin, and clozapine significantly affected only the 30–60-minute bin. Methiothepin and intraventricular 5,7-DHT significantly reduced both bins.

A further preliminary assessment of the time-course data was carried out by deriving a single score from the percent of MDMA control scores generated for the 0–30- and 30–60-minute bins listed in Table 1. The purpose of this score was to provide a crude representation of the tendency of a compound to reduce one component relative to the other. This score was calculated as follows: [− (30–60-minute % MDMA score minus 0–30-minute % MDMA score)]. Thus, treatments that primarily reduce the late component would have a positive score, treatments that primarily reduce the early component would have a negative score, and those that would affect both components would tend to cancel out each other and therefore yield a score around zero. (Note that treatments that affected neither component would also have a zero score; however, none of the treatments used herein were without effect). Panel B of Figure 4 graphs these scores for each treatment, rank-ordered from the left (most negative, treatments that primarily affect the early component) to the right (most positive, those that primarily affect the late component). One interpretation of these data is that compounds are primarily acting as antagonists for the D_2, 5-$HT_{1A/1B}$, or D_1 receptors cluster to the left, compounds acting primarily as 5-HT_2 antagonists cluster to the right, and agents with multiple actions tend to cluster in the mid-

dle. However, it should be emphasized that this analysis is preliminary. Caution should be taken to avoid overinterpreting these time-course data in that anomalous responses were sometimes seen (i.e., a low vehicle/MDMA response at 30–60 minutes in the haloperidol experiment; a high vehicle/MDMA response at 30–60 minutes in the MDL 28,133A experiment). Increasing sample sizes would help to clarify these anomalies. Nevertheless, the trends seen in the data are worth noting.

Effects of Haloperidol on MDMA-Reduced Rearing

Additional analyses were carried out to determine the effects of haloperidol on MDMA-suppressed rearing (VT). Like MDL 100,907, haloperidol did not attenuate the MDMA reduction in VT. The means and SEM for the 0–30- and 30–60-minute bins, respectively, were: vehicle/vehicle (410 ± 83; 1 ± 1); vehicle/MDMA (32 ± 14; 1 ± 1); haloperidol/vehicle (133 ± 80; 3 ± 2); and haloperidol/MDMA (419 ± 7; 1 ± 1). A two-way ANOVA revealed a significant treatment effect [$F(3,16)$ = 4.67, $p < .016$], a significant time effect [$F(3,16)$ = 19.01, $p < .0005$], and a significant treatment by time interaction [$F(3,16)$ = 4.78, $p < .015$]. Individual comparisons using contrasts revealed significant depressant effects ($p < .001$) of MDMA on rearing in the vehicle and haloperidol groups in the 0–30-minute bin. MDMA suppression of rearing in the 0–30-minute bin was not significantly diminished by haloperidol.

Effects of 5,7-DHT on Regional Brain 5-HT Levels

5,7-DHT treatment depleted 5-HT levels in the brainstem (47% of control), cortex (36%), and hippocampus (45%). The concentrations (ng/g brain tissue) for each area were: brainstem: 906.2 ± 51.4, ICV 5,7-DHT injected, 429.7 ± 191.3; cortex: vehicle 112.1 ± 8.4, 5,7-DHT, 40.1 ± 24.7; hippocampus: vehicle, 207.3 ± 26.3, 5,7-DHT, 94.1 ± 58.1 ($n = 5$ for each injection group).

DISCUSSION

The main finding of the present study was that the selective 5-HT_{2A} antagonist MDL 100,907 significantly reduced the locomotor stimulation produced by MDMA without affecting MDMA-suppressed rearing behavior. This reduction was significantly greater over the later (30–60-minute) component of the 1-hour test session relative to the early (0–30-minute) component. These data generally support the conclusion that 5-HT_{2A} receptors are important for the expression of MDMA-stimulated locomotion and suggest that MDMA-stimulated locomotion may be used as an in vivo behavioral model for evaluation of 5-HT_{2A} antagonist activity.

Consistent with this conclusion, other agents known to have $5-HT_{2A}$ antagonist activity (ritanserin, methiothepin, MDL 28,133A, and clozapine), all significantly reduced MDMA-stimulated locomotion (Table 1, Figure 4). In each case, significant reductions occurred over the late (30–60-minute) component, though conclusions about the precise component affected should be tempered by acknowledging that anomalous MDMA control responses were sometimes seen (i.e., MDL 28,133A and haloperidol experiments). Furthermore, possible contributions of other receptor activities (i.e., D_2 antagonism) might contribute to MDMA reduction. Ex vivo binding studies using clozapine have shown that the ED_{50} for displacing $5-HT_2$ receptors is 1.3 mg/kg SC, whereas the ED_{50} for displacing D_2 receptors is 9 mg/kg, a sevenfold difference (Leysen et al. 1993). By extrapolation, the 4-mg/kg dose of clozapine used in the present study is probably reducing MDMA stimulation by $5-HT_{2A}$ antagonism.

A contribution of D_2 receptor antagonism is also a possibility for MDL 28,133A, which has a $D_2/5-HT_{2A}$ affinity ratio of 4 (present study). Recent work (Tsibulsky et al. 1995; Frank et al. 1995) has shown that, like haloperidol but unlike MDL 100,907, MDL 28,133A attenuates the threshold-lowering effect of d-amphetamine in the brain-stimulation reward paradigm in rats, an effect that was presumed to be D_2-mediated. However, a 5-mg/kg dose (compared to the 1-mg/kg dose used in the present study) was required to achieve its effects in the reward paradigm. Thus, it is likely that MDL 28,133A reduced MDMA stimulation by blocking $5-HT_{2A}$ receptors, although ex vivo binding data are needed to confirm this conclusion.

Ritanserin has a $D_2/5-HT_{2A}$ affinity ratio of 150 (Leysen et al. 1993), suggesting that it reduced MDMA stimulation by selective $5-HT_{2A}$ blockade. Callaway et al. (1992) reported a lack of effect of ritanserin against MDMA-stimulated locomotion. It is not clear why different effects were obtained in the present study, although it should be noted that Callaway et al. used the (+)-isomer of MDMA, whereas the present study used racemic MDMA. Further work is needed to determine if this accounts for the different findings of the two studies.

Intraventricularly administered 5,7-DHT chronically reduced regional 5-HT levels (presumably reflecting a lesion of central 5-HT terminals) and attenuated MDMA-stimulated locomotion. These findings are consistent with the conclusion that intact 5-HT terminals are necessary, at least in part, for the expression of MDMA-stimulated locomotion.

The MDL 100,907 reduction of MDMA-stimulated locomotion supports previous in vivo microdialysis findings that MDL 100,907 reduced MDMA-stimulated dopamine release (Schmidt et al. 1992b). The authors hypothesized that the mechanism involved the blockade of $5-HT_{2A}$ receptors that were "permissive" for

stimulated dopamine release. Thus, $5-HT_{2A}$ receptor activation supports increased synthesis of dopamine under conditions of accelerated demand, as following administration of a releaser such as MDMA. Receptor blockade with MDL 100,907 blocks this permissive role, thereby attenuating MDMA-induced dopamine release. This explanation may account for MDL 100,907's reduction of MDMA-stimulated locomotion, although other mechanisms (i.e., dopamine independent) cannot currently be ruled out.

Consistent with the findings by Callaway et al. (1992), a variety of other treatments that affected D_2 receptors (haloperidol), D_1 receptors (SCH 23390), or $5-HT_{1A}$ receptors (propranolol) diminished MDMA-induced locomotor stimulation. MDMA is a potent releaser of both dopamine and 5-HT, and thus it is not surprising that a number of different receptor subtypes may underlie the reported behavioral stimulation. Furthermore, as illustrated in Panel B of Figure 4, a preliminary analysis suggests that these receptors may mediate an early component involved in MDMA-stimulated locomotion, relative to a later component preferentially affected by $5-HT_{2A}$ antagonists. From an empirical standpoint, this pattern could be useful in characterizing antipsychotic compounds by providing an in vivo assessment of $5-HT_{2A}$ antagonism relative to other actions, a particularly powerful approach if used in conjunction with ex vivo binding evaluations. It should be emphasized that the current limited studies are only suggestive and not sufficient fully to evaluate this hypothesis. Such evaluation will require extensive dose-response and time-course studies with larger sample sizes, as well as additional comparisons with other antagonists (i.e., D_2 antagonists such as eticlopride). It is not clear why MDMA-stimulated locomotion should be differentially affected in such a manner, although time-dependent effects of MDMA on dopamine and 5-HT release (Yamamoto and Spanos 1988; Hiramatsu and Cho 1990) and on discriminative stimulus cues (Schecter 1988) have been described. Thus, an explanation may involve complex, time-dependent interactions between 5-HT and dopamine release.

As reported previously in other labs (Callaway et al. 1990), MDMA decreased the amount of time rats engaged in vertical (rearing) behavior. The present study found that MDL 100,907 and haloperidol both failed to block MDMA-suppressed rearing, suggesting that D_2 or $5-HT_{2A}$ receptors are not involved. Further work is required to elucidate the neurochemical mediation of MDMA-suppressed rearing.

In summary, MDL 100,907 antagonized the locomotor-stimulating effects of MDMA in rats. Further characterization of the MDMA behavioral model may prove its utility as an in vivo index of $5-HT_{2A}$ receptor antagonism and, more generally, as a method for characterizing potential atypical antipsychotic compounds.

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