Serotonin$_{1B}$ Receptor Activation Mimics Behavioral Effects of Presynaptic Serotonin Release

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The locomotor hyperactivity induced by 3,4-methylenedioxymethamphetamine (MDMA) and related drugs in rats appears to be due to the drug-induced release of presynaptic serotonin (5-HT). Thus, these drugs increase locomotor activity by acting as indirect 5-HT agonists. The subtype of 5-HT receptor upon which this released 5-HT acts postsynaptically to produce the activating effect of MDMA-like drugs is not known. When tested under conditions in which MDMA increases locomotion, direct agonists at both 5-HT$_{1A}$ and 5-HT$_{1C/2}$ receptors consistently decrease locomotion. Hence, the present experiments tested the hypothesis that the hyperactivity produced by the release of endogenous 5-HT is due to the activation of 5-HT$_{1B}$ receptors. Using the Behavioral Pattern Monitor (BPM), the profile of behavioral effects of a 5-HT$_{1B}$ agonist, 5-methoxy-3(1,2,3,6)tetrahydropyridin-4yl)-1H-indole (RU 24969), was compared to that previously described for MDMA and related indirect 5-HT agonists. The BPM provided detailed information regarding the amount and qualitative patterning of locomotor activity and investigatory responses in rats. Various doses of RU 24969 (1.25 to 5 mg/kg) were administered to naive male rats 10 minutes prior to placement in the test chambers. As previously reported for MDMA, locomotor activity increased with dose, and investigatory rearings and holepokes decreased. The hyperactivity was characterized by repetitive spatial patterns of locomotion that were qualitatively similar to those produced by indirect 5-HT agonists such as MDMA and dissimilar to those produced by indirect dopamine (DA) agonists such as amphetamine. Pretreatment with racemic propranolol but not (+)propranolol antagonized the hyperactivity induced by RU 24959. Fluoxetine, a 5-HT reuptake inhibitor, failed to block the locomotor activating effects of RU 24969. These findings confirm the similarity between the behavioral effects of RU 24969 and indirect 5-HT agonists and suggest that the locomotor hyperactivity produced by both RU 24969 and MDMA is mediated by the activation of 5-HT$_{1B}$ receptors. Although the effects of MDMA on 5-HT$_{1B}$ receptors are secondary to its ability to release presynaptic 5-HT, the activation produced by RU 24969 appears to be a consequence of its direct agonist effects. ***[Neuropsychopharmacology 8:201–211, 1993]***

KEY WORDS: *Locomotor activity; Serotonin; Investigatory behavior; Holeboard; RU 24969; MDMA; Serotonin$_{1B}$ receptors*

Although central serotonergic systems have long been thought to exert inhibitory influences on motoric behavior (Soubrie 1986), recent studies have demonstrated that the predominant effect of indirect serotonin (5-HT) agonists in rats is a profound locomotor activation. The locomotor hyperactivity produced by 3,4-methylenedioxymethylamphetamine (MDMA) and related drugs is not due primarily to dopamine (DA) release, but is attributable instead to the release of presynaptic 5-HT. First, studies using the Behavioral Pattern Monitor

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Received October 11, 1991; revised April 1, 1992 and July 7, 1992; accepted July 9, 1992.

Published by Elsevier Science Publishing Co., Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/93/$6.00

(BPM) to characterize the behavioral profile produced by indirect 5-HT agonists revealed that the hyperactivity produced by these drugs is qualitatively different from that produced by amphetamine-like stimulants acting via DA release (Gold et al. 1988). Second, the MDMA-induced hyperactivity can be prevented by pretreatment with selective 5-HT uptake blockers, including fluoxetine, sertraline, and zimelidine, or with the 5-HT synthesis inhibitor parachlorophenylalanine (Callaway et al. 1990). By contrast, these pretreatments either potentiate or have no effect on the hyperactivity induced by the DA-releasing agent amphetamine. Third, the catecholamine synthesis inhibitor α-methyl-p-tyrosine blocks the locomotor activating effects of amphetamine but does not affect the response to MDMA (Callaway et al. 1990). Fourth, congeners of MDMA that lack the ability to release DA although retaining the ability to release 5-HT also produce marked increases in locomotor activity in rats (Callaway et al. 1991a, 1991b; Paulus and Geyer 1992). For example, N-methyl-1-(1,3-benzodioxol-5yl)-2-butanamine (MBDB), a derivative of MDMA that releases 5-HT without releasing DA or blocking DA reuptake, produced a dose-dependent and MDMA-like locomotor activation that was completely blocked by pretreatment with fluoxetine (Callaway et al. 1991a). Parallel biochemical data confirm that fluoxetine prevents the depletions of brain 5-HT induced acutely by MDMA or MBDB (Callaway et al. 1991a). Therefore, the acute motor-activating properties of these compounds appear to result from facilitating the release of presynaptic 5HT.

Given that these methylenedioxy-substituted phenylalkylamines increase locomotor activity by acting as indirect 5-HT agonists, the question arises as to the 5-HT receptor subtype upon which this released 5-HT acts postsynaptically to produce this effect. Serotonin receptors are classified as 5-HT_1 and 5-HT_2 (Peroutka and Snyder 1979) and at least three subtypes, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} have been characterized in rats (Pedigo et al. 1981; Hoyer et al. 1985; Peroutka 1986). When tested under conditions in which MDMA increases locomotion, direct agonists at both 5-HT_{1A} and 5-$HT_{1C/2}$ receptors consistently decrease locomotor activity. Specifically, both full and partial 5-HT_{1A} agonists, including 8-OH-DPAT, buspirone, gepirone, and ipsapirone, produce dose related and robust reductions in both locomotor and investigatory responses in the BPM (Mittman and Geyer 1989). In addition, both 1-(m)-chlorophenyl-piperazine and 1-(m)-trifluoromethylphenyl-piperazine, putative 5-HT_{1C} agonists (Hoyer 1988), also decrease horizontal locomotion (MA Geyer, unpublished results; Lucki 1989). Similarly, direct agonists at 5-$HT_{1C/2}$ receptors, including quipazine and both indoleamine and phenethylamine hallucinogens, also decrease activity in the BPM, at least when the animals are not familiar with the test environment (Adams and Geyer 1985a, b; Wing et al. 1990). These effects of the direct agonists are selectively antagonized by the appropriate 5-HT_1 or 5-$HT_{1C/2}$ antagonists and can be differentiated behaviorally using the profile of measures provided by the BPM system (Mittman and Geyer 1989; Wing et al. 1990). Hence, it seems unlikely that the hyperactivity produced by the indirect agonists reflect an activation of postsynaptic 5-HT_{1A}, 5-HT_{1C}, or 5-HT_2 receptors.

By contrast, systemic administration of the 5-HT_{1B} agonist 5-methoxy-3(1,2,3,6)tetrahydropyridin-4-yl)-1H-indole (RU 24969) has been shown to increase locomotor activity in rodents (Oberlander and Boissier 1981; Green et al. 1984) apparently by acting at postsynaptic 5-HT_{1B} receptors (Tricklebank et al. 1986; Oberlander et al. 1987). This effect involves coordinated forward locomotion and is readily distinguishable from the myoclonic syndrome produced by some other 5-HT agonists (Green et al. 1984). The agonist RU 24969 has been shown to have a high affinity for both the 5-HT_{1A} and 5-HT_{1B} sites with some selectivity for the 5-HT_{1B} site (Sills et al. 1984; Doods et al. 1985). Because the 5-HT_{1B} site is the only characterized 5-HT receptor associated with increased locomotor activity, it is a likely candidate as the site of action of the endogenous 5-HT released by indirect 5-HT agonists such as MDMA. Therefore, the present study was designed to characterize the hyperactivity produced by RU 24969 by examining patterns of locomotor and investigatory behavior in the BPM in order to compare its effects with those of the indirect 5-HT agonists in the same experimental paradigm. Additional pharmacologic studies were conducted to corroborate evidence that the behavioral effects of RU 24969 are due to its 5-HT_{1B} agonist actions at postsynaptic sites. Specifically, it was hypothesized that the behavioral profile produced by RU 24969 would mimic that characteristic of indirect 5-HT agonists, including increased locomotor activity dominated by straight paths of movement in both directions around the BPM chamber, decreased investigatory holepoking, and decreased rearing behavior. Such a behavioral profile would distinguish the activating effects of RU 24969 from those produced by scopolamine, caffeine, nicotine, and direct or indirect DA agonists (Geyer et al. 1986).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan Labs, Indianapolis, IN) weighing between 250 and 400 g were housed in pairs and maintained in a temperature- and light-controlled environment on a reversed-light cycle (lights on from 1900 to 0700). Except during behavioral testing, animals were given free access to food and water.

In total, 78 animals were used, each animal being tested only once.

Behavioral Measures

Behavior was measured in a BPM system designed to monitor behavioral patterns as described previously (Geyer et al. 1986). Briefly, the BPM chamber is a 30.5 × 61 cm black Plexiglas box with three floor holes and seven wall holes, each equipped with an infrared photobeam for the detection of holepokes. A 4 × 8 grid of photobeams is used to define the position of the animal in an x-y coordinate system with a resolution of 3.8 cm. Rearing against the chamber walls is detected by a touch-plate 15.2 cm above the floor. Chambers are kept dark with the exception of 7.5 watt red light bulbs.

A computer monitors the status of all photobeams and records the duration and nature of all changes with a temporal resolution of 55 msec. Horizontal locomotion is recorded as crossings, a measure of movement between photobeam-defined sectors. Holepokes are recorded as repeated holepokes (pokes occurring at the same hole in a single bout of investigation) or varied holepokes (the initial poke in a bout of investigation). Total holepokes are presented when measures of repeated and varied holepokes show the same effect. Rearings are similarly divided into varied and repeated rearings and presented in the same manner as holepokes. The number of entries into the center region is corrected for activity level by dividing by the total number of crossings and is presented as a center entries to crossings ratio. The spatial character of locomotor activity can also be visualized by means of graphical reconstructions of the animal's path during the session. Additional measures utilized to describe locomotor path are the *CV9* and ***d*** statistics. The *CV9* evaluates the sequences of position change by calculating the number of occurrences of each of the 40 possible transitions between 9 chamber regions (center, 4 corners, and 4 midwall regions [see Geyer 1990]). An increase in *CV9* reflects the tendency to repeat certain paths or region transitions. The sequence of the x-y positions is used to calculate the ***d*** statistic; increases or decreases in ***d*** reflect a rougher or smoother path, respectively. Briefly, ***d***, the spatial scaling exponent, is based on the fact that the measured length of a line depends upon the length of the ruler used for the measurement. Hence, ***d*** reflects the slope of the line relating the measured length to the ruler size. Increases or decreases in ***d*** reflect a rougher or smoother path, respectively. After indirect 5-HT agonists are administered, *CV9* is increased (Gold et al. 1988) and ***d*** is decreased (Callaway et al. 1991b; Paulus and Geyer 1991; Paulus and Geyer 1992) reflecting the relatively predictable and straight locomotor paths exhibited by the drug-treated animals.

Testing Procedure

For each experimental session, naive animals were brought to the laboratory under a black cloth at least 1 hour prior to testing. Following the appropriate pharmacologic manipulation, each animal was gently placed into a chamber. Data were collected for 120 minutes during the dark phase of the animals' light/dark cycle. The chambers were cleaned thoroughly between testing sessions.

Drug Treatments

The RU 24969 succinate (Roussel-Uclaf, Romainville, France), (+/−)propranolol HCl, (+)propranolol HCl (Research Biochemicals Inc., Natick, NJ), and (+)MDMA HCl (NIDA, Rockville, MD) were dissolved in saline and injected subcutaneously in a volume of 1 ml/kg of body weight. Fluoxetine HCl (Lilly, Indianapolis, IN) was dissolved in saline for intraperitoneal injections in a volume of 2 ml/kg of body weight. Twenty-four rats received RU 24969 at doses of 0, 1.25, 2.5, or 5 mg/kg (0.0044, 0.0087, or 0.0174 mmol/kg). The 2.5 mg/kg dose, which increased locomotor activity significantly but submaximally, was used for assessments of drug interactions. Pretreatments with (+/−)propranolol (20 mg/kg or 0.0676 mmol/kg) and (+)propranolol (10 mg/kg or 0.0338 mmol/kg) were administered 30 minutes prior to an injection of RU 24969 or saline. Fluoxetine (10 mg/kg or 0.0289 mmol/kg) injections were given 50 minutes before RU 24969 or saline. All animals were placed in the testing chambers 10 minutes after the final drug treatment. Each experimental group consisted of six rats.

Data Analysis

Crossings, center entries, holepokes, and rearings were examined in 10-, 30-, or 60-minute intervals, as appropriate. Statistical comparisons were performed using repeated-measures analysis of variance (ANOVA) (Dixon 1988). Post-hoc comparisons were made using Tukey's Studentized Range Method on 30-minute blocks of data. The ***d*** statistic was analyzed by ANOVA on the first 60 minutes of data. Comparisons for *CV9* data were made using the Mann-Whitney U-test.

RESULTS

Dose Response

As summarized in Table 1, RU 24969 dramatically altered the behavioral activity profile of rats. A significant effect of RU 24969 dose on crossings was found for the 2-hour test session ($F[3,20] = 26.87$, $p < .0001$). As shown in Figure 1, horizontal locomotion was increased

Table 1. Dose-Dependent Effects of RU 24969 on Behavioral Measures

Dose (mg/kg) (*n*)	0.0 (6)	1.25 (6)	2.5 (6)	5.0 (6)
Crossings				
0–30 min	363.7 ± 44.2	658.7 ± 148.1	800.0 ± 62.7**	870.5 ± 75.4**
30–60 min	139.7 ± 17.0	385.0 ± 100.1	826.0 ± 75.0**	1021.8 ± 93.4**
60–90 min	97.5 ± 36.2	233.7 ± 73.3	805.0 ± 78.5**	1099.5 ± 129.9**
90–120 min	48.2 ± 17.7	233.5 ± 80.7	795.3 ± 79.1**	1051.3 ± 141.5**
Total holepokes				
0–30 min	133.5 ± 16.6	70.3 ± 13.9*	60.3 ± 18.6*	25.7 ± 12.7**
30–60 min	68.2 ± 16.8	80.8 ± 28.5	65.7 ± 25.9	30.7 ± 21.4
60–90 min	59.5 ± 21.7	81.3 ± 22.8	65.5 ± 19.0	27.8 ± 18.1
90–120 min	43.7 ± 19.9	64.2 ± 27.8	50.8 ± 15.6	26.8 ± 18.7
Total rearings				
0–30 min	95.3 ± 14.5	14.5 ± 12.3**	0.7 ± 0.7**	0.0 ± 0.0**
30–60 min	26.3 ± 7.0	4.2 ± 1.3**	0.0 ± 0.0**	0.2 ± 0.2**
60–90 min	13.5 ± 7.8	4.0 ± 2.1	1.5 ± 1.5	0.0 ± 0.0
90–120 min	6.2 ± 3.1	6.2 ± 3.9	3.0 ± 1.7	0.0 ± 0.0
Center entries to crossings ratio				
0–30 min	0.316 ± 0.023	0.168 ± 0.016	0.085 ± 0.027**	0.047 ± 0.024**
30–60 min	0.351 ± 0.053	0.149 ± 0.047*	0.085 ± 0.030**	0.051 ± 0.026**
60–90 min	0.325 ± 0.080	0.136 ± 0.068	0.062 ± 0.021*	0.036 ± 0.019**
90–120 min	0.257 ± 0.088	0.168 ± 0.072	0.068 ± 0.019	0.041 ± 0.023
Center entries				
0–30 min	117.3 ± 22.0	104.2 ± 24.0	60.3 ± 17.9	39.9 ± 18.7*
30–60 min	51.2 ± 12.8	55.2 ± 22.3	64.3 ± 23.5	48.2 ± 26.9
60–90 min	43.2 ± 17.8	24.0 ± 7.7	46.2 ± 15.2	31.5 ± 18.1
90–120 min	17.2 ± 5.8	35.2 ± 17.8	52.5 ± 14.9	32.3 ± 19.8
CV9 (medians)				
0–30 min	0.969	1.135*	1.216**	1.390**
30–60 min	1.100	1.451*	1.148	1.590*
60–90 min	1.018	2.164*	1.348*	1.729*
90–120 min	1.361	1.580	1.390	1.702

Data are expressed as group means ± SEM, with the exception of the CV9 statistic, which is expressed as median values.
* $p < 0.05$ relative to saline controls by Tukey's tests following significant ANOVA.
** $p < 0.01$ relative to saline controls by Tukey's tests following significant ANOVA.

dose dependently, with the largest effects being produced by the 2.5 and 5 mg/kg doses. Levels of activity in rats treated with high doses of RU 24969 remained elevated during the 2nd hour of testing, whereas the activity of control animals decreased across time. Hence, the interaction between time and drug was significant ($F[33,220] = 5.95$, $p < .0001$). The activity produced by 2.5 and 5 mg/kg doses of RU 24969 at the end of the 2-hour session was still greater than that of the control animals at any time.

In addition to increasing locomotor activity, RU 24969 induced changes in investigatory behavior. The effects of RU 24969 on total holepokes are detailed in Table 1. Despite the increases in activity produced by RU 24969, total holepokes were decreased significantly, especially by the higher doses. Repeated holepokes were decreased significantly by RU 24969 throughout the test session ($F[3,20] = 5.82$ $p < .005$). Although the total number of varied holepokes was not decreased by RU 24969, the interaction between time and drug was significant ($F[33,220] = 2.33$, $p < .0005$). Control animals had high levels of investigatory holepokes for the first 30 minutes of testing, which decreased as they habituated to the chambers. In contrast, rats at the 2.5 and 5 mg/kg doses of RU 24969 exhibited an initial decrease in total and varied holepokes, but the rate of varied holepokes did not decrease with time. As shown in Table 1, RU 24969 (1.25 to 5 mg/kg) caused a pronounced decrease in rearing behavior (for 0 to 120 minutes, $F[3,20] = 17.84$, $p < .0001$). At the two highest doses, virtually no rearing occurred during the 2-hour test session. Both repeated and varied rearings were decreased similarly by RU 24969.

The spatial patterns of locomotion were altered dramatically by RU 24969. At high doses (2.5 to 5 mg/kg), animals typically avoided the center of the testing chamber and walked or ran along the walls. This pattern is illustrated for a typical animal in Figure 2. For comparison purposes, the patterns exhibited by a representative control animal and an animal treated with 10 mg/kg

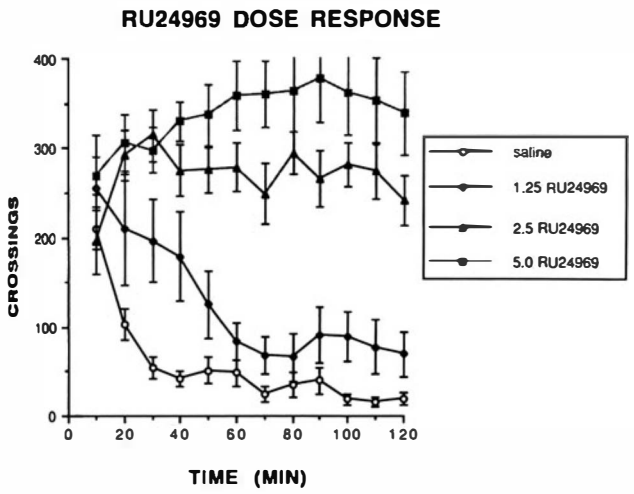


Figure 1. The effects of various doses (mg/kg) of RU 24969 on movements between 15 cm squares (crossings) are shown for successive 10-minute intervals after the animals were placed in the chamber. Data are shown as group means ± SEM.

MDMA are also shown in Figure 2. The detailed description of the behavioral profile and locomotor patterns induced by various doses of MDMA have been reported previously (Gold et al. 1988). The rotational pattern observed after RU 24969 was marked by frequent direction changes for a single wall-length or after several revolutions. Animals exhibiting few center entries changed directions less frequently than animals with less peripheral paths. The RU 24969-induced tendency to avoid the center region can be seen in a comparison of the ratio of center entries to crossings (Table 1). The thigmotaxic effect of RU 24969 was most pronounced during the first hour ($F[3,20] = 27.07$, $p < .0001$), when all three doses differed significantly from the control animals. Similarly, the absolute number of center entries was decreased by RU 24969 despite the concomitant increases in locomotor activity ($F[3,20] = 3.09$, $p < .05$) (Table 1). By the 2nd hour of the session, a significant drug effect on the ratio of center entries to crossings was still present (for 60 to 90 minutes: $F[3,20] = 5.79$, $p < .01$), with post-hoc analysis revealing only the 5 mg/kg dose to be significantly different from the control groups. The *CV9*, which assesses the degree to which animals repeat the same locomotor paths (Geyer 1990), increased dose dependently after the administration of RU 24969 (Table 1). During the initial 30 minutes, all doses differed significantly from controls (1.25 mg/kg, U = 26; 2.5 mg/kg, U = 23; 5 mg/kg, U = 21; all $p < .05$). These differences were generally maintained throughout the session. Thus, the spatial pattern of RU 24969-treated rats shows less diversity than the paths of control animals. In addition, the *d* statistic decreased with dose: mean (± SEM) = 1.582 (0.023), 1.482 (0.027), 1.294 (0.033), and 1.212 (0.034) for 0, 1.25, 2.5, and 5 mg/kg RU 24969, respectively. The ANOVA confirmed the reliability of these differences ($F[3,20] = 39.99$, $p < .0001$).

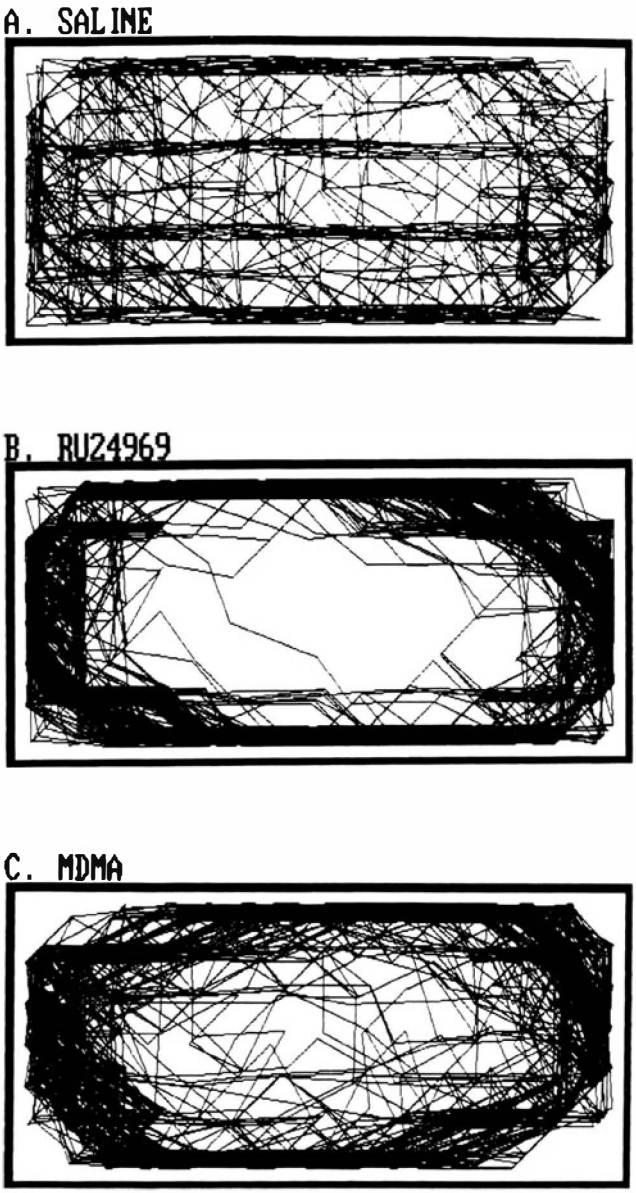
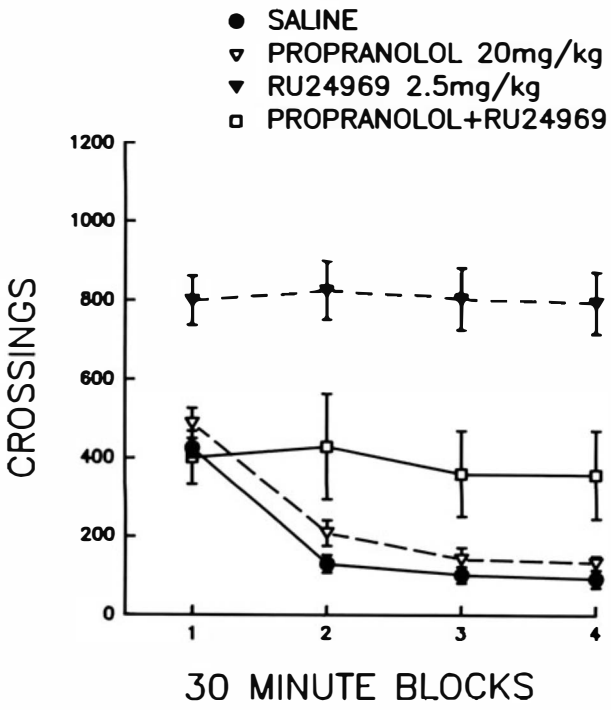


Figure 2. The locomotor patterns exhibited by animals injected with **A** saline, **B** 2.5 mg/kg RU 24969, or **C** 10 mg/kg MDMA are shown for the initial 60 minutes of activity in the BPM chamber. Each animal was selected to be representative of its group. Darker lines signify increased activity along a given path.

Propranolol and RU 24969 Interactions

Rats receiving 20 mg/kg (+/−)propranolol 30 minutes prior to 2.5 mg/kg of RU 24969 did not exhibit the same behavioral activity profile as rats treated only with RU 24969. As shown in Figure 3, racemic propranolol significantly reduced the increase in crossings produced



by RU 24969, resulting in a significant pretreatment-by-drug interaction across the entire session ($F[3,20] = 22.1$, $p < .0001$). Racemic propranolol alone had no effect on locomotor activity.

By contrast, there was no significant pretreatment-by-drug interaction between racemic propranolol and RU 24969 for the number of holepokes ($F[3,20] = 0$, ns) (Table 2). Likewise, racemic propranolol failed to antagonize the RU 24969-induced suppression of rearings (for 0 to 120 minutes: $F[3,20] = .6$, ns), although the interaction between time and drug was significant (for 0 to 120 minutes: $F[33,220] = 2.69$, $p < .005$) (Table 2).

In keeping with its ability to antagonize the locomotor activating effects of RU 24969, racemic propranolol antagonized the RU 24969-induced tendency to

Figure 3. Graph illustrating crossings after saline or 2.5 mg/kg RU 24969, with saline or 20 mg/kg (+/−)propranolol pretreatment are shown as group means ± SEM for successive 30-minute intervals. (+/−)propranolol significantly reduced the hyperactivity produced by RU 24969. This experiment was run concurrently with the RU 24969 dose-response study. Hence, the 2.5 mg/kg RU 24969 group was the same as that shown in Figure 1 and Table 1.

Table 2. Interaction Between (+/−)Propranolol and RU 24969

Pretreatment Treatment (*n*)	Saline Saline (6)	Saline RU 24969 (6)	Propranolol Saline (6)	Propranolol RU 24969 (6)
Crossings				
0–30 min	425.8 ± 24.8	800.0 ± 62.7**	488.0 ± 38.7	400.5 ± 68.0
30–60 min	130.0 ± 22.8	826.0 ± 74.9**	207.5 ± 33.5	429.0 ± 134.7
60–90 min	102.3 ± 21.0	805.0 ± 78.5**	142.3 ± 30.1	360.2 ± 109.6
90–120 min	93.2 ± 22.9	795.3 ± 79.1**	133.0 ± 18.2	358.2 ± 113.3
Total holepokes				
0–30 min	149.8 ± 20.1	60.3 ± 18.6*	105.3 ± 16.0	81.8 ± 23.1
30–60 min	67.0 ± 12.2	65.7 ± 25.9	84.5 ± 6.5	64.3 ± 19.1
60–90 min	79.5 ± 17.1	65.5 ± 19.0	98.5 ± 16.4	50.0 ± 13.6
90–120 min	77.5 ± 21.4	50.8 ± 15.6	94.2 ± 21.5	58.0 ± 21.6
Total rearings				
0–30 min	111.3 ± 23.1	0.7 ± 0.7**	89.5 ± 9.4	10.8 ± 9.1**
30–60 min	34.8 ± 6.6	0.0 ± 0.0**	29.2 ± 5.0	7.2 ± 4.6**
60–90 min	18.5 ± 5.3	1.5 ± 1.5	23.8 ± 6.7	3.7 ± 2.5
90–120 min	17.2 ± 5.7	3.0 ± 1.7	24.5 ± 6.6	1.5 ± 1.1
Center entries to crossings ratio				
0–30 min	0.278 ± 0.010	0.085 ± 0.026**	0.239 ± 0.027	0.185 ± 0.040
30–60 min	0.352 ± 0.068	0.085 ± 0.030**	0.239 ± 0.019	0.195 ± 0.032
60–90 min	0.383 ± 0.043	0.062 ± 0.021**	0.267 ± 0.027	0.135 ± 0.030**
90–120 min	0.366 ± 0.081	0.068 ± 0.019**	0.268 ± 0.051	0.101 ± 0.031**
Center entries				
0–30 min	117.3 ± 4.7	60.3 ± 17.9	120.7 ± 18.3	72.5 ± 19.2
30–60 min	45.8 ± 11.7	64.3 ± 23.5	49.8 ± 8.0	84.5 ± 25.4
60–90 min	41.3 ± 10.4	46.2 ± 15.2	41.7 ± 10.9	42.3 ± 9.6
90–120 min	35.5 ± 11.9	52.5 ± 14.9	39.2 ± 11.1	32.2 ± 12.5

Data are expressed as group means ± SEM.
* $p < 0.05$ relative to saline controls by Tukey's tests following significant ANOVA.
** $p < 0.01$ relative to saline controls by Tukey's tests following significant ANOVA.

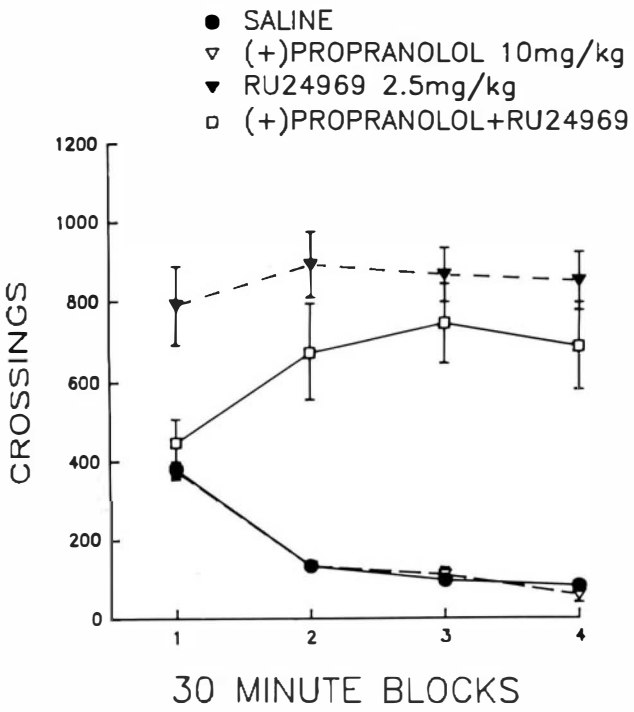


Figure 4. Graph illustrating crossings after saline or 2.5 mg/kg RU 24969, with saline or 10 mg/kg (+)propranolol pretreatment are shown as group means ± SEM for successive 30-minute intervals. (+)Propranolol had no significant effect on the hyperactivity produced by RU 24969.

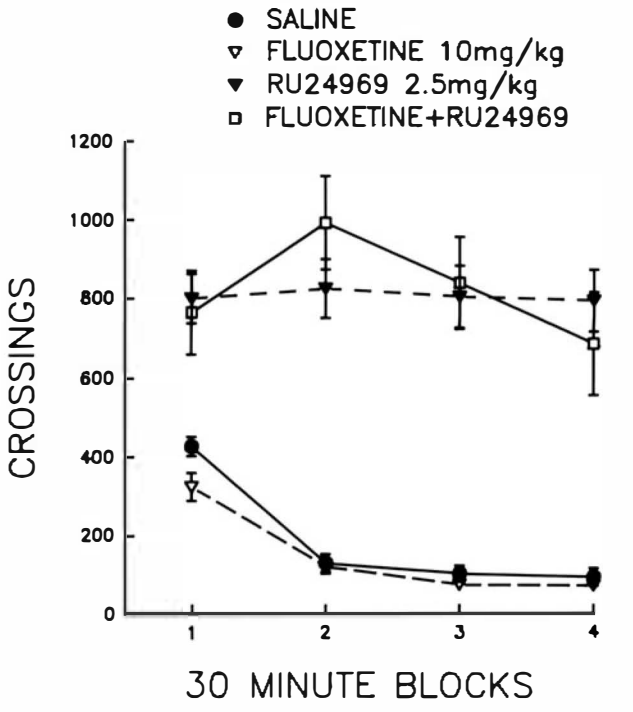


Figure 5. Graph illustrating crossings after saline or 2.5 mg/kg RU 24969, with saline or 10 mg/kg fluoxetine pretreatment are shown as group means ± SEM for successive 30-minute intervals. Fluoxetine had no significant effect on the hyperactivity produced by RU 24969. This experiment was run concurrently with the RU 24969 dose-response and (+/−)propranolol studies. Hence, the 2.5 mg/kg RU 24969 group was the same as that shown in Figure 1 and Table 1 and the saline/saline group was the same as that shown in Figure 3 and Table 2.

avoid the center of the chamber. The ratio of center entries to crossings showed a significant pretreatment-by-drug interaction for the first three 30-minute blocks (0 to 30 minutes: $F[3,20] = 6.06$; 30 to 60 minutes: $F[3,20] = 7.18$; 60 to 90 minutes: $F[3,20] = 9.07$; all $p < .05$).

Because only the (−)isomer of propranolol has appreciable affinity for 5-HT_1 sites (Middlemiss 1985), an additional study was done using a 10-mg/kg pretreatment with (+)propranolol. No significant interaction between RU 24969 and (+)propranolol was observed, as illustrated in Figure 4 (crossings: 0 to 60 minutes; $F[3,20] = 3.16$, ns).

Fluoxetine and RU 24969 Interaction

As reported previously (Callaway et al. 1990), fluoxetine pretreatment alone had no effect on the behaviors measured. Fluoxetine pretreatment did not affect RU 24969-induced behavioral activity. Figure 5 shows the failure of fluoxetine to antagonize the locomotor activation produced by RU 24969, despite the effectiveness of this dose of fluoxetine in blocking the effects of MDMA in this paradigm (Callaway et al. 1990). Similarly, no significant interactions between fluoxetine and RU 24969 were found for holepokes, rearings, center-to-crossings ratio, or center entries (Table 3).

DISCUSSION

The present findings demonstrate that the behavioral profile exhibited by rats treated with the 5-HT_{1B} agonist RU 24969 is similar to that produced by indirect 5-HT agonists such as MDMA. In addition to confirming previous reports of locomotor hyperactivity induced by RU 24969 (Tricklebank et al. 1986; Green et al. 1984; Oberlander et al. 1987), these experiments revealed that RU 24969 produces concomitant decreases in investigatory holepoking and rearing. Furthermore, detailed analyses of the spatial patterns of locomotion provided further evidence that the behavioral profile induced by RU 24969 qualitatively resembles that characteristic of MDMA and related drugs. Prominent locomotor hyperactivity in rats is produced by RU 24969 at doses of 2.5 mg/kg and above. Locomotor activity is increased by MDMA and other 5-HT-releasing drugs with a similar pattern of persistent forward running or walking around the perimeter of the chamber, interrupted by occasional changes of direction (Gold et al. 1988; Calla-

Table 3. Interaction Between Fluoxetine and RU 24969

Pretreatment Treatment (*n*)	Saline Saline (6)	Saline RU 24969 (6)	Fluoxetine Saline (6)	Fluoxetine RU 24969 (6)
Crossings				
0–30 min	425.8 ± 24.8	800.0 ± 62.7**	324.0 ± 35.7	764.6 ± 106.9**
30–60 min	130.0 ± 22.8	826.0 ± 75.0**	121.0 ± 17.5	922.8 ± 118.7**
60–90 min	102.3 ± 21.0	805.0 ± 78.5**	74.2 ± 10.6	840.2 ± 116.5**
90–120 min	93.2 ± 22.9	795.3 ± 79.1**	71.2 ± 10.7	685.7 ± 130.9**
Total holepokes				
0–30 min	149.8 ± 20.1	60.3 ± 18.6**	90.0 ± 20.7	21.5 ± 5.9**
30–60 min	67.0 ± 12.3	65.7 ± 25.9	76.0 ± 30.0	33.4 ± 13.3
60–90 min	79.5 ± 17.1	65.5 ± 19.0	67.3 ± 27.5	33.3 ± 7.5
90–120 min	77.5 ± 21.4	50.8 ± 15.6	52.0 ± 16.5	34.2 ± 12.2
Total rearings				
0–30 min	111.3 ± 23.1	0.7 ± 0.7**	72.8 ± 10.7	0.8 ± 0.8**
30–60 min	34.8 ± 6.6	0.0 ± 0.0**	17.2 ± 6.0	0.0 ± 0.0**
60–90 min	18.5 ± 5.3	1.5 ± 1.5*	12.2 ± 5.6	0.0 ± 0.0*
90–120 min	17.2 ± 5.7	3.0 ± 1.7	12.7 ± 5.1	0.2 ± 0.2*
Center entries to crossings ratio				
0–30 min	0.278 ± 0.010	0.085 ± 0.027**	0.242 ± 0.045	0.045 ± 0.024**
30–60 min	0.352 ± 0.068	0.085 ± 0.030**	0.413 ± 0.045	0.056 ± 0.024**
60–90 min	0.383 ± 0.043	0.062 ± 0.021**	0.420 ± 0.053	0.070 ± 0.025**
90–120 min	0.366 ± 0.081	0.068 ± 0.019**	0.522 ± 0.083	0.031 ± 0.014**
Center entries				
0–30 min	117.3 ± 4.7	60.3 ± 17.9	84.7 ± 21.6	41.5 ± 23.9
30–60 min	45.8 ± 11.7	64.3 ± 23.5	49.7 ± 9.4	51.8 ± 25.8
60–90 min	41.3 ± 10.4	46.2 ± 15.2	31.2 ± 5.8	60.2 ± 27.9
90–120 min	35.5 ± 11.9	52.5 ± 14.9	39.0 ± 8.9	24.2 ± 13.7

Data are expressed as group means ± SEM.
* $p < 0.05$ relative to saline controls by Tukey's tests following significant ANOVA.
** $p < 0.01$ relative to saline controls by Tukey's tests following significant ANOVA.

way et al. 1991b). The spatial patterns of locomotion typically exhibited by RU 24969- and MDMA-treated rats are virtually indistinguishable, and unlike the movement patterns of saline-treated animals. Avoidance of the center is observed in most but not all animals treated with RU 24969 or MDMA. Sustained increases in horizontal locomotion are observed with both drugs, whereas control animals decrease locomotor activity after the first 30 minutes of chamber exploration.

In addition to the qualitative similarities between the locomotor activity patterns produced by RU 24969 and indirect 5-HT agonists, both similarly decrease investigatory behaviors, including both holepokes and rearings. Treatment with MDMA depresses initial levels of total holepokes, which then rise or remain the same across a 120-minute testing session, unlike control animals that exhibit fewer holepokes over time (Gold et al. 1988). The level of total holepokes following RU 24969 administration has the same pattern of initially decreased levels that remain constant across time. The effect of high doses of MDMA on rearing is to decrease rearing initially with a subsequent although variable increase over time (Gold et al. 1988). Similarly, RU 24969 depresses the initial number of rearings but to a greater extent than MDMA, and no increase in rearings is observed during a 120-minute test session.

As discussed previously with regard to MDMA (Gold et al. 1988), the profile of behavioral changes following RU 24969 is distinct from that produced by other stimulant drugs. Throughout a wide range of doses, amphetamine, which induces behavioral activation via DA release in the mesolimbic pathway (Kelly et al. 1975), produces highly varied patterns of locomotor and investigatory behavior, characterized by concomitant increases in locomotion, holepokes, and rearings (Geyer et al. 1987). The hyperactivity induced by MDMA and RU 24969 is accompanied by decreases in investigatory responses. Whereas the activity engendered by MDMA and RU 24969 is primarily along the walls with directional changes typically occurring only when a corner is encountered, amphetamine produces increased locomotion that is distributed throughout all regions of the chamber and includes frequent directional changes (Geyer et al. 1987). Hence, RU 24969 and MDMA increase and amphetamine decreases the *CV9* measure of the predictability of the pattern (Geyer et al. 1986; Gold et al. 1988). In addition, both RU 24969 and MDMA produce relatively straight paths and there-

fore decrease the **d** statistic (Paulus and Geyer 1992); however, amphetamine does not affect this measure except at high doses when **d** is increased (Paulus and Geyer 1991). Thus, although manipulations of central DA systems have been shown to affect the response to RU 24969 (Green et al. 1984; Tricklebank et al. 1986; Uchitomi and Yamawaki 1988), it is probable that RU 24969 activation is not a direct result of DA release. Similar conclusions have been reached by Oberlander et al. (1986). Like MDMA and RU 24969, the direct DA agonist apomorphine decreases holepokes and rearings, but produces only weak and variable stimulation of locomotor activity characterized by unidirectional circling patterns (Geyer et al. 1987). The cholinergic antagonist scopolamine also produces a form of hyperactivity dominated by movements around the perimeter of the chamber, but it increases investigatory responding at the same time (Geyer et al. 1986). Caffeine also increases investigatory responding together with increases in locomotor activity (Geyer et al. 1986).

The qualitative similarity in locomotor patterns and activity between MDMA and RU 24969 suggests that these drugs exert their behavioral effects by means of closely related mechanisms, presumably within the 5-HT system. The locomotor hyperactivity induced by MDMA and related drugs such as parachloroamphetamine, MBDB, and methylenedioxyamphetamine is due to release of presynaptic 5-HT (Callaway et al. 1990; Callaway et al. 1991a). Accordingly, the locomotor activating effects of these indirect 5-HT agonists are prevented by pretreatment with the 5-HT synthesis inhibitor parachlorophenylalanine or by administration of 5-HT uptake inhibitors such as fluoxetine, zimelidine, or sertraline (Callaway et al. 1990, 1991a). By contrast, the effects of RU 24969 are insensitive to parachlorophenylalanine (Green et al. 1984; Uchitomi and Yamawaki 1988) or fluoxetine (present study). These results are consistent with the hypothesis that the effects of RU 24969 are mediated by postsynaptic rather than presynaptic serotonergic mechanisms. This hypothesis is further corroborated by evidence indicating that the effects of RU 24969 are not reduced and are typically enhanced by neurotoxic lesions of 5-HT neurons induced by 5,7-dihydroxytryptamine (Nisbet and Marsden 1984; Oberlander et al. 1986, 1987; Tricklebank et al. 1986). Thus, it appears that RU 24969 produces hyperactivity by acting via a postsynaptic 5-HT receptor, possibly the same 5-HT receptor upon which the endogenous 5-HT released by MDMA must act.

As summarized above, direct agonists at 5-HT_{1A} or 5-$HT_{1C/2}$ receptors suppress rather than increase locomotor activity in this paradigm (Mittman and Geyer 1989; Wing et al. 1990). Hence, the hypothesis tested in these studies was that the 5-HT_{1B} receptor might mediate the activating effects of the indirect 5-HT agonists. Although considerable biochemical evidence suggests that the 5-HT_{1B} receptor contributes importantly to the effects of RU 24969 (Sills et al. 1984; Doods et al. 1985) pharmacologic evidence for the selective effects of RU 24969 at the 5-HT_{1B} site is somewhat inconsistent (Tricklebank et al. 1986). In the present studies, the racemic form of propranolol antagonized the locomotor activation of RU 24969 but had minimal effects by itself and did not influence the ability of RU 24969 to decrease investigatory behavior. These results are consistent with the similar influences of racemic propranolol on the effects of MDMA (Callaway et al. 1992). Thus, the present results with racemic propranolol provide additional evidence for a commonality in the mechanisms of action of RU 24969 and the indirect 5-HT agonists. However, in previous studies, both the (+) and (−)isomers of propranolol have been found to suppress RU 24969-induced hyperactivity (Tricklebank et al. 1986), a result that is inconsistent with evidence for selective antagonism of 5-HT_1 sites by (−)propranolol (Middlemiss 1985). A preliminary report of apparently comparable studies provides some evidence for a stereoselective antagonism of the effects of RU 24969 by propranolol (Mylecharane et al. 1991). The isomer (−)propranolol but not (+)propranolol inhibits the behavioral responses produced by other drugs acting at 5-HT_1 receptors (Green and Grahame-Smith 1976). Preliminary studies with (+)propranolol in the present experimental paradigm failed to show a significant antagonism of the hyperactivity produced by RU 24969, suggesting that the 5-HT_{1B} receptor may be the site at which locomotor activity is increased by RU 24969. Nevertheless, further studies using converging measures will be needed to clarify the somewhat enigmatic interactions between RU 24969 and the isomers of propranolol.

There is evidence that the DA system plays a secondary role in the expression of behavioral activation induced by RU 24969 (Green et al. 1984; Tricklebank et al. 1986; Uchitomi and Yamawaki 1988). Although RU 24969 has negligible affinity for catecholamine receptors, evidence that hyperactivity is eliminated by reserpine but not parachlorophenylalanine or 5,7-dihydroxytryptamine suggests that intact catecholamine systems are required for the full expression of RU 24969-induced hyperactivity (Tricklebank et al. 1986). The DA antagonist haloperidol partially inhibits the increased horizontal locomotion induced by RU 24969 (Oberlander and Boissier 1981; Green et al. 1984; Kennett et al. 1987). However, recent evidence has demonstrated that lesions of the globus pallidus, which disrupt the output of dopaminergic systems and decrease the effect of amphetamine, potentiate the locomotor hyperactivity induced by RU 24969 and preclude the ability of even high doses of haloperidol to antagonize RU 24969-induced hyperactivity (Oberlander et al. 1986). These results strongly suggest that the hyperactivity produced

by RU 24969 is not mediated directly via dopaminergic neurons. Instead, Oberlander et al. (1986) concluded that RU 24969 acts postsynaptically at a 5-HT receptor to activate the motor system via an influence that is parallel to, rather than sequential with, the DA system.

In summary, the present studies demonstrate both qualitative and pharmacologic similarities in the behavioral profiles induced by MDMA and RU 24969. Both the 5-HT_{1B} agonist and the 5-HT-releasing agents produce marked increases in repetitive patterns of forward locomotion together with dose-related decreases in investigatory holepokes and rearings. In both cases, the hyperactivity but not the decreased investigatory behavior is blocked by racemic propranolol. These results are consistent with the hypothesis that the hyperactivity associated with indirect 5-HT agonists is mediated by the activation of postsynaptic 5-HT_{1B} receptors.

ACKNOWLEDGMENT

This work was supported by grants from the National Institute on Drug Abuse (DA02925 and DA06325). Mark A. Geyer was supported by a Research Scientist Development Award from the National Institute of Mental Health (MH00188). We thank Virginia Masten, Richard Sharp, and Diana Martinez for their assistance in the conduct of these studies and Dr. Martin Paulus for helpful discussions of the results.

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