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Mixed D_2/5-HT_2 Antagonism Differentially Affects Apomorphine- and Amphetamine-Induced Stereotyped Behavior

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FELDMAN, D. J., R. A. FRANK, J. H. KEHNE, R. FLANNERY, D. BROWN, S. SONI, G. BYRD, AND S. SHAH. *Mixed D_2/5-HT_2 antagonism differentially affects apomorphine- and amphetamine-induced stereotyped behavior.* PHARMACOL BIOCHEM BEHAV **58**(2) 565–572, 1997—Evidence supports the hypothesis that psychostimulant stereotypy is mediated through postsynaptic dopamine receptors. Given the recent findings of behavioral, neurochemical and electrophysiological studies showing 5-HT_2 modulation of dopamine systems, a series of experiments were undertaken to assess the ability of D_2 and 5-HT_2 antagonists to reverse apomorphine and amphetamine stereotypy in the rat. Haloperidol reduced stereotyped behavior induced by d-amphetamine (50% reduction with 0.162 mg/kg) and apomorphine (50% reduction with 0.112 mg/kg) MDL 28,133A, a mixed D_2/5-HT_2 antagonist, also reduced stereotypy in the apomorphine group (50% reduction with 3.89 mg/kg) but was much less effective in antagonizing the effects of d-amphetamine (not even a 25% reduction with 9.0 mg/kg). MDL 100,907, a selective 5-HT_2 antagonist, was ineffective at reducing stereotyped behavior induced by either stimulant. Thus, 5-HT_2 modulation of dopaminergic activity was not demonstrated in the case of psychostimulant stereotypy. Furthermore, 5-HT_2 antagonism did not induce stereotypy, as has been proposed in some models. These findings provide further support for the hypothesis that antipsychotic medications with high affinity for 5-HT_2 receptors do not interfere with the regulation of the nigrostriatal dopaminergic system and, therefore, would be less likely to produce extrapyramidal side effects.

Stereotypy D_2 receptor 5-HT_2 receptor Amphetamine Apomorphine MDL 100,907 MDL 28,133A Haloperidol Atypical neuroleptics Extrapyramidal side effects Rat

NEUROLEPTIC management of psychotic symptoms allowed the deinstitutionalization of patients with schizophrenia in the 1970s and 1980s to occur. Many people who were once perceived as uncontrollable were able to move to more independent living situations if they medically managed their symptoms. Antipsychotic potency of "typical" neuroleptics was found to be related to their affinity for the dopamine D_2 receptor (5). However, D_2 antagonism also correlated highly with the risk for extrapyramidal side effects (EPS) such as tardive dyskinesia and parkinsonism (4).

Extrapyramidal side effects of neuroleptics have contributed to patient noncompliance with antipsychotic medication and have decreased their socializability. New, "atypical" antipsychotic drugs, which have reduced EPS liability, are in various stages of development (19). Clozapine, for example, often is more effective than other neuroleptic drugs at reducing positive symptoms, ameliorates negative and disorganized symptoms of schizophrenia but rarely produces EPS (16). Differing from the standard neuroleptic drugs such as haloperidol and chlorpromazine, pharmacological studies have shown that clozapine and another atypical neuroleptic drug, risperidone, have a much greater affinity for serotonergic than for dopaminergic receptors (19).

Recent efforts have been directed at developing serotonin antagonists that are potent antipsychotics and free of EPS. Very selective serotonin antagonists have been developed

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which, in addition to their clinical utility, are valuable in exploring mechanisms involved in psychosis and other dopamine-related behaviors. For example, Nash (22), using in vivo microdialysis, showed that a serotonin 5-HT_2 receptor blocker, ketanserin, could slow the dopamine (DA) release stimulated by administration of the amphetamine analogue, 3,4-methylenedioxymethamphetamine (MDMA) in rats. Based on work using a more selective 5-HT_2 antagonist, MDL 100,907, Schmidt et al. (26) went on to speculate that the tonic activation of 5-HT_2 receptors is necessary for increased DA synthesis needed in states of accelerated DA release. The implication of this type of interaction is that compounds that block 5-HT_2 receptors should prevent new DA synthesis from keeping up with increased release induced by stimulant administration.

Further studies with MDL 100,907 have elucidated regional differences in 5-HT_2 modulation of DA in the brain (20,23). Chronic treatment with 5-HT_2 antagonists produces a decline in neural firing of the mesocorticolimbic but not of the nigrostriatal DA system. Furthermore, under normal conditions, MDL 100,907 does not alter DA or its metabolite levels, the release of DA, or the firing rate of DA neurons, once again supporting the theory that 5-HT_2 receptors influence DA systems only in high states of activation.

Given this relationship, one functional outcome of 5-HT_2 blockade might be to reduce the behavioral effects of a stimulant drug. Some behavioral studies using new, highly selective 5-HT compounds have been consistent with this prediction, whereas others have not. Sorenson et al. (29) blocked amphetamine-induced locomotion in mice with MDL 100,907 but were unsuccessful in affecting apomorphine-induced climbing behavior or stereotypy in rats.

Moser et al. (20) demonstrated an effect of MDL 100,907 on amphetamine-induced locomotion and disruption of latent inhibition, both measures of mesocorticolimbic DA systems. In contrast, brain stimulation reward studies have failed to find significant effects of serotonergic modulation of DA, even though these studies also are believed to evaluate activity in the mesocorticolimbic DA tract (15,30). A dissociation of neurochemical control of different behavioral responses to psychostimulants is suggested by these researchers to explain these conflicting results. In other words, 5-HT_2 receptors are proposed to regulate only some of the behavioral properties of stimulants and not others (30).

MDL 100,907 failed to attenuate the facilitating effect of d-amphetamine on intracranial self-stimulation (30). However, MDL 28,133A, a mixed 5-HT_2/D_2 antagonist, did reduce amphetamine-induced facilitation of brain stimulation reward (8). The effect of MDL 28,133A may be due to D_2 receptor antagonism; MDL 28,133A is about fourfold less potent at the D_2 site relative to the 5-HT_2 site, whereas MDL 100,907 shows at least a 1000-fold separation in affinity for these two receptors (12).

The potential for atypical neuroleptics to produce EPS exists because of the evidence provided above. In summary, there are data to suggest that the 5-HT_2 system modulates some DA function and that many atypical neuroleptic drugs antagonize 5-HT_2 receptors. Assessing the effects of atypical neuroleptics on the nigrostriatal pathway is, therefore, crucial to drug development (11). Stereotyped behaviors induced by psychostimulants are mediated by postsynaptic nigrostriatal DA systems (3,14). Through exploring differences among binding characteristics of antipsychotics and respective differences in attenuation of stereotyped behavior, characterization of the influence of 5-HT systems on stimulant-induced behavior also may be possible.

The present study was designed to evaluate the ability of MDL 100,907 and MDL 28,133A to reduce psychostimulant stereotypy. Such an attenuation would suggest that (a) the compound might have a liability for producing EPS and (b) 5-HT_2 antagonism modulates DA function in the nigrostriatal pathway. Many studies have shown that haloperidol, a typical neuroleptic drug, reduces stereotyped behavior induced by DA agonists (17). This DA antagonist was used as a standard of comparison for the effects of the serotonergic compounds. The receptor binding characteristics of these drugs are as follows: MDL 28,133A displaces 50% of bound [^{3}K]ketanserin (5-HT_2 standard) at 59.8 nM (K_i) and displaces 50% of bound [^{3}H]spiroperidol (D_2 standard) at 240 nM [K_i (12)]; MDL 100,907 has a K_i for [^{3}H]ketanserin of 0.85 nm but fails to displace [^{3}H]spiroperidol at concentrations greater than 1000 nM (25); haloperidol has a K_i for [^{3}H]ketanserin of 41.6 nM (25) and a K_i for [^{3}H]spiroperidol of 3.6 nM (2).

The actions of these antagonists were evaluated using d-amphetamine and apomorphine to induce stereotyped behavior (abbreviated as AmIS and ApIS, respectively). Based on its affinity for the D_2 receptor, haloperidol was expected to block both ApIS and AmIS. MDL 28,133A also was expected to reduce stereotypy induced by both stimulants based on its ability to antagonize postsynaptic D_2 receptors. Haloperidol should be more potent than MDL 28,133A given its higher affinity for the D_2 receptor. It was predicted that MDL 100,907 would have no effect on ApIS or AmIS, based on the previous stereotypy study evaluating this compound (29) and the evidence that serotonin antagonists, such as clozapine, have a limited EPS liability (19).

METHOD

Subjects

Three hundred twelve male Sprague-Dawley rats (Zivic-Miller Labs, Pittsburgh, PA) were housed in hanging stainless steel wire cages on a 12-h light/dark cycle (600–1800, lights on) at 21°C with unrestricted access to Purina rat chow and water. Rats were housed one to three per cage. After at least 4 days of acclimatization to the colony room, each rat was habituated to the testing field. Their mean weight at the time of testing was 261 g $\pm$ 31 g.

Apparatus

A Plexiglas® box surrounded each 30.5 cm $\times$ 30.5 cm $\times$ 30.5 cm stereotypy field. Only the front and top panels of each box were left clear so that views of other rats in the experiment were prohibited. The environment for stereotypy testing included a metronome sounding a tone every 3 s. Trained research assistants viewed and scored the stereotypy sessions. At least two scorers observed each rat and judged their behavior according to a checklist derived from reviews of the stereotypy literature and from pilot observations of rats in the laboratory. One set of scores per rat provided data for the study; the additional observer served as a reliability check for the measurement technique.

Procedure

Within 72 h prior to testing, the rats were placed in the stereotypy field for 20 min to allow for habituation, with a metronome beeping in the background just as in the test conditions. On the day of testing, each rat was placed in the stereotypy field for another 20-min habituation period, during which the rats were weighed. They were then injected with the ran-

domly selected antagonist dose (MDL 100,907: 0.66, 1.33, 2.0, 2.66 mg/kg; or MDL 28,133A: 1 .5, 3.0, 6.0, 9.0 mg/kg; or haloperidol: 0.05, 0.1, 0.18, 0.25 mg/kg; or saline control) and stimulant (apomorphine 0.4 mg/kg, or d-amphetamine 4.0 mg/kg or saline control) and returned to the stereotypy field for a 65-min measurement period. The antagonist injection was given intraperitoneally 20 min before the stimulant injection was administered subcutaneously on the back of the neck. The scorers were blind to the drug conditions on all occasions.

Ten rats each were randomly assigned to each stimulant–antagonist combination (24 combinations) or stimulant–saline condition (2 groups). In the groups without a stimulant, it was predicted that little, if any, stereotypy would be observed, and pilot data supported this notion. Based on this prediction, only four rats each were randomly assigned to the antagonist-saline condition (12 groups) or saline-only control (1 group).

Five minutes following stimulant injection and placement of the rat into the stereotypy field, the scorers began their observations. These measurements continued for 90-s observation periods, during which the rat's behavior was noted once every 3 s for a total of 30 observations. Any head or body movement that spatially repeated the previous movement was recorded as a repetitive behavior (e.g., repetitive head movements, sniffing downward, licking the floor or wall, gnawing, grooming, sniffing upward, repetitive rearing, repetitive locomotion along the same path, repetitive climbing and foot shuffling).

To measure most efficiently the stereotyped behavior, typical stereotypies (e.g., repetitive head movements and sniffing downward) were primarily observed when they occurred. Repetitive head movements were given priority in scoring over larger body behaviors (which were typically nonstereotyped) unless the animal reared three times in a row or completed a locomotive circle. If the animal reared three times from the same location while moving its head repetitively, the behavior was scored at the third rearing as "repetitive rearing." For every locomotive circle the rat completed while moving its head, "repetitive locomotion on the same path" was scored. If the animal's head was not moving repetitively, each 3-s period in which the animal either reared from the same location or continued walking along the same path was scored as repetitive rearing and repetitive locomotion, respectively. Gnawing, sniffing upward, and grooming were eliminated from the final list of stereotyped behaviors because of their frequent expression in no-drug control animals.

Behaviors that were scored one point for stereotypy were sniffing downward, repetitive head movements, foot shuffling, licking the floor or wall, repetitive locomotion along same path and repetitive climbing. Nonstereotyped behavior, which scored no points, included gnawing, standing still, lying down, walking, rearing, sniffing upward and grooming (10,24). The maximum possible score for each 90-s measurement period was 30 because there were 30 3-s observations in the period. These 90-s observational periods occurred every 15 min on the following schedule: 5, 20, 35, 50, and 65 min poststimulant injection.

Data analyses and other statistics were generated by SAS statistical software (version 6.04), using the raw scores and an arcsine transformation of the scores (21). Because the raw scores are actually percentages of time that an animal engaged in stereotypy multiplied by 30, standard errors were expected to be smaller near the extremes of the scale. The arcsine transformation spreads these extreme scores and their errors so that Type 1 error is deflated. A multivariate analysis of variance (MANOVA) across antagonists and doses was calculated for the no-stimulant conditions to explore possible stereotypy-enhancing effects of the antagonists. A repeated measures analysis of variance (ANOVA) was performed to examine time-dependent changes for the no-stimulant groups. Multiple ANOVAs were calculated across the doses of each antagonist. Nonparametric Kruskal-Wallis χ^2 approximations also were performed because the data violated the assumption for homogeniety of variance. Linear regression of the group means was used to calculate a dose–response curve for each antagonist–stimulant condition.

Drugs

MDL 28,133A (1-(4-fluorophenyl)-2-[4[(4-methanesulfoamido-phenyl)-carbonyl]-1-piperidinyl]-ethanone hydrochloride) and MDL 100,907 (R(+)-α(2,3-dimethoxyphenyl)-1-[2(4-fluorophenylethyl)]-4-piperidinemethanol) were synthesized at and provided by Höechst Marion Roussel, Incorporated (Cincinnati, OH). D-amphetamine sulfate was provided courtesy of the National Institute on Drug Abuse and Research Triangle Institute, North Carolina. Apomorphine hydrochloride and haloperidol were purchased from Sigma Chemical Company (St. Louis, MO). All drugs were dissolved in saline at temperatures no greater than 70°C and were administered in a volume of 1 ml per kg of body weight.

RESULTS

One rat accidentally received the wrong antagonist injection, which was discovered after the study ended, leaving one antagonist-stimulant condition [d-amphetamine (4.0 mg/kg); MDL 28,133A (6.0 mg/kg)] with only nine usable scores for the analysis. On 70 occasions of 1555 observation periods, the number of scored behaviors did not equal 30, due primarily to the vigilance demand required of the scorers. Only seven of these inappropriately scored sessions had fewer than 28 or more than 31 behaviors scored. For five of these seven errors, the reliability scorers took closer to 30 measurements relative to the primary scorer; thus, the reliability scorer's data were used for those 90-s scoring periods. For any session without exactly 30 behaviors scored, a percentage of stereotyped behavior relative to total observed behavior was calculated and multiplied by 30 to get an estimated raw score.

Interrater reliability of the scoring protocol proved to be very high. Pearson product-moment correlations among the seven raters' stereotypy scores ranged from 0.842 to 0.997, with an overall correlation of 0.951. All correlations were significant ($p < 0.001$).

Baseline Conditions

Fewer than 4.0% of scored behaviors in the control (saline–saline) condition were classified as stereotyped, and these were repetitive head (2.3%) and sniffing down (1.5%) behaviors. The majority of the time (96.0%), control animals were lying still in a corner of the scoring field (89.3%) or moving their head randomly (6.7%).

When only antagonist drugs were given, the number of stereotyped movements increased (some raw scores as high as 30), particularly with MDL 100,907 (9.2%) and MDL 28,133A (12.3%). However, in evaluating each of the five stereotypy scores per rat as a dependent variable, these increases were not significant across antagonists or doses using a MANOVA (Wilks' $\Lambda_{100907\ (20,\ 37.4)} = 0.12$, $0.05 < p < 0.10$; Wilks' $\Lambda_{28133A\ (20,\ 37.4)} = 0.41$, $p > 0.10$).

Repeated measures ANOVA revealed no evidence for time-dependent changes in stereotyped behavior for the saline-only or antagonist-only conditions ($p > 0.10$). Similarly, the dose of the antagonists in conditions without a stimulant injection did not affect the amount of stereotyped behavior ($p > 0.10$). Overall, when no-stimulant conditions were collapsed across time and dose of antagonist, stereotypy scores were low, averaging 2.46 with a standard error of 0.34.

Rats injected with only apomorphine responded to the drug within 5 min and all observed behavior was stereotyped. At the first observation point (5 min), 82.3% of the rat's behavior was scored as sniffing down, with 10.7% repetitive head movements and 6.7% licking the floor and sides of the stereotypy field. By 20 min, the intensity of ApIS began to decrease, returning to baseline levels between 35 and 50 min. When only d-amphetamine was administered, rats began responding with almost all stereotyped behavior within 20 min. The resulting behavior consisted of repetitive head movements (85.5%), followed by repetitive locomotion (2.0%), repetitive rearing (1.4%), sniffing down (1.0%) and nonstereotyped movements (11.1%). AmIS remained at peak levels in these animals for at least 65 min. The time courses of stereotypy induced by apomorphine (0.4 mg/kg) and d-amphetamine (4.0 mg/kg) are compared in Fig. 1, which depicts the difference in peak and duration of stereotyped behavior induced by the two stimulants. Based on their time course over 65 min, it was determined that the intervals over which the effects of the stimulants were submaximal were 20–35 min for ApIS and 5–20 min for AmIS. To minimize the possible contribution of ceiling effects, data from these observational periods were summed for the analyses of the antagonist's effects on stereotypy. Thus, stereotypy scores could range from 0 to 60. An arcsine transformation of these data was then applied, which spreads scores near the ceiling or floor of the data range (21). The same pattern of significant results was obtained from transformed and untransformed data.

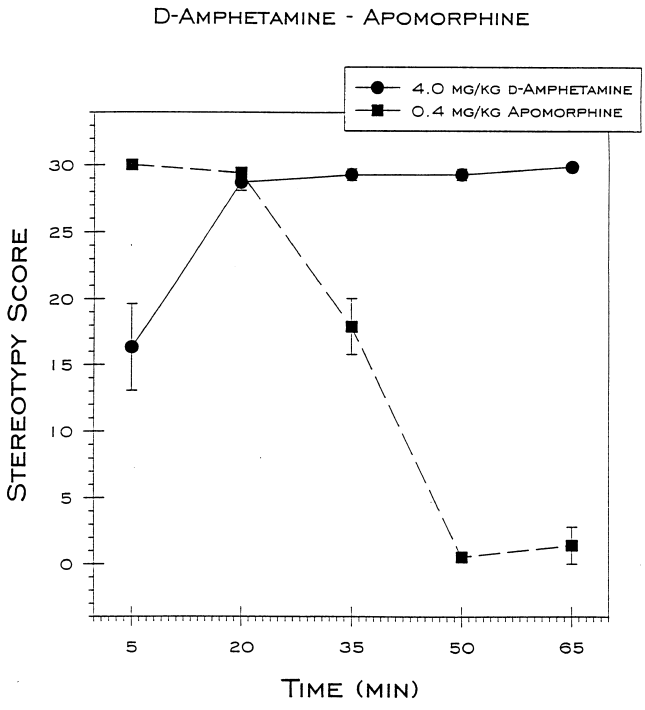

FIG. 1. Time–response curves for 0.4 mg/kg apomorphine and 4.0 mg/kg d-amphetamine. No drugs were administered other than the stimulant.

Because the data violated the assumption of homogeneity of variance, even after transformation, a nonparametric test of the raw scores also were calculated (Kruskal-Wallis χ^2 approximations). Both nonparametric tests of raw data and ANOVAs of transformed and untransformed data provided the same pattern of significant results. Only the analyses of the untransformed data are reported.

Apomorphine Conditions

Table 1 lists the means and standard errors for each condition. Haloperidol and MDL 28,133A blocked ApIS in a dose-dependent manner [$F(4, 45) = 17.34$, $p < 0.001$; $F(4, 45) = 23.5$, $p < 0.001$, respectively]. MDL 100,907 had an unpredicted effect on ApIS, appearing to slightly potentiate its effects [$F(4, 45) = 3.22$, $p < 0.03$]. However, this did not occur in a dose-dependent manner.

A dose–response curve for haloperidol's effects on ApIS is plotted in Fig. 2. Saline level across these two observation periods (20 and 35 min), or 100% reduction of stereotypy, is taken to be 4.92 (the collapsed no-stimulant score of 2.46 multiplied by two). Baseline stereotypy, or the average stereotypy score for rats injected with only apomorphine, was 47.30. A line was fit to all the untransformed data ($r^2 = 0.59$; $p < 0.001$), and the haloperidol dose needed to reduce ApIS by 50% (ED_{50}) was calculated to be 0.112 mg/kg. Similarly, a dose–response curve was established for MDL 28,133A. This is shown in Fig. 3, and like haloperidol, a linear equation fit to the data is significant ($r^2 = 0.69$; $p < 0.001$). The ED_{50} of MDL 28,133A is 3.89 mg/kg. Haloperidol and MDL 28,133A were able to block completely ApIS within the range of doses tested in the experiment, as illustrated by the linear regressions (Fig. 2 and 3).

Amphetamine Conditions

Statistics were calculated on raw and transformed scores summed from observations taken at 5 and 20 min following d-amphetamine administration. The group means and standard errors are listed in Table 1. Haloperidol significantly reduced AmIS [$F(4, 45) = 7.73$, $p < 0.001$], and like its effect on ApIS, increasing doses of haloperidol had greater attenuating effects (Fig. 2). As seen from this linear regression, haloperidol was not able to eliminate AmIS completely to saline levels. As shown in Table 1, neither MDL 28,133A nor MDL 100,907 was able to antagonize AmIS [$F(4, 44) = 1.33$, $p > 0.10$; $F(4, 45) = 0.16$, $p > 0.10$, respectively].

A dose–response curve was calculated for haloperidol's effects on AmIS and is shown in Fig. 2 ($r^2 = 0.45$; p 0.001). The ED_{50} for haloperidol against AmIS was 0.162 mg/kg, which is similar to the potency of this drug against ApIS (0.112 mg/kg). Although MDL 28,133A does not significantly reduce AmIS scores, a significant dose–response curve illustrates a weak effect on AmIS ($r = 0.080$; $p < 0.05$; Fig. 3). An ED_{50} could not be calculated because the highest dose of MDL 28,133A did not reduce AmIS by even 25%.

Comparison of Stimulants and Antagonists

Based on the binding data presented earlier in this paper (2,13), MDL 28,133A and haloperidol have a 240:3.6 nM (or

TABLE 1

MEAN STEREOTYPY SCORES AND STANDARD ERRORS FOR EXPERIMENTAL CONDITIONS

		Stimulant			
		Apomorphine 0.4 mg/kg (20–35 min)		d-amphetamine 4.0 mg/kg (5–20 min)	
Antagonist	Dose (mg/kg)	Mean (SE)	*p*	Mean (SE)	*p*
Saline		47.3 (2.03)		45.0 (3.57)	
MDL 100,907	0.66	45.0 (2.69)	<0.03	47.0 (4.79)	>0.10
	1.33	55.6 (1.40)		45.9 (2.37)	
	2.00	49.9 (3.22)		47.6 (3.26)	
	2.66	53.0 (2.36)		47.0 (3.24)	
MDL 28,133A	1.5	35.5 (2.60)	<0.001	43.7 (3.18)	>0.10
	3.0	30.7 (4.00)		39.0 (3.24)	
	6.0	11.4 (4.63)		41.6 (2.49)	
	9.0	5.4 (2.38)		35.2 (4.42)	
Haloperidol	0.05	42.0 (5.83)	<0.001	45.2 (3.68)	<0.001
	0.10	24.8 (5.42)		32.1 (5.01)	
	0.18	6.7 (3.27)		19.8 (5.80)	
	0.25	6.4 (3.92)		14.9 (3.43)	

Mean stereotypy scores and standard errors for experimental conditions. Ten rats provided data for each condition except for MDL 28,133A (6.0 mg/kg)–d-amphetamine (4.0 mg/kg) condition, in which only nine rats were used. The *p* values given are from the ANOVAs on the untransformed data. For the analysis of MDL 100,907–apomorphine conditions, the group means are significantly increased relative to saline levels.

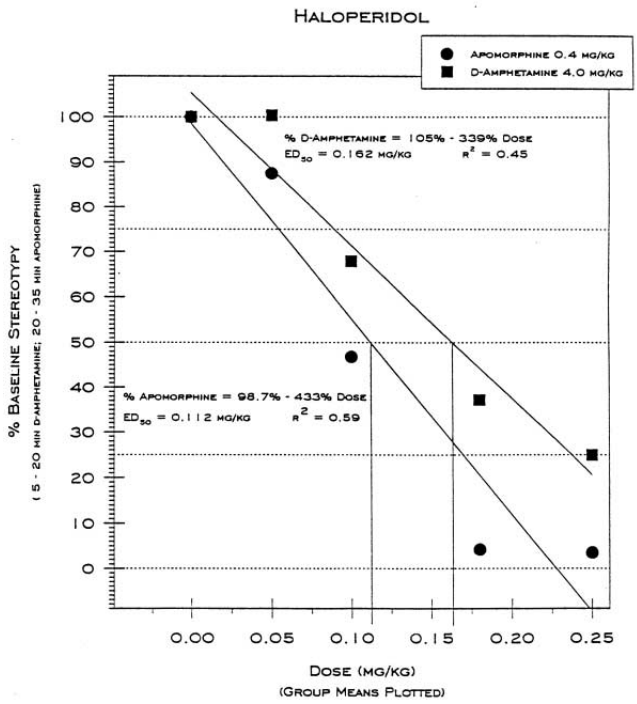

FIG. 2. Dose–response curves for the effects of haloperidol on 0.4 mg/kg apomorphine and 4.0 mg/kg d-amphetamine. Group means are plotted with regression lines for each stimulant. The means represent two summed observation periods: 5- and 20-min periods for d-amphetamine and 20- and 35-min periods for apomorphine.

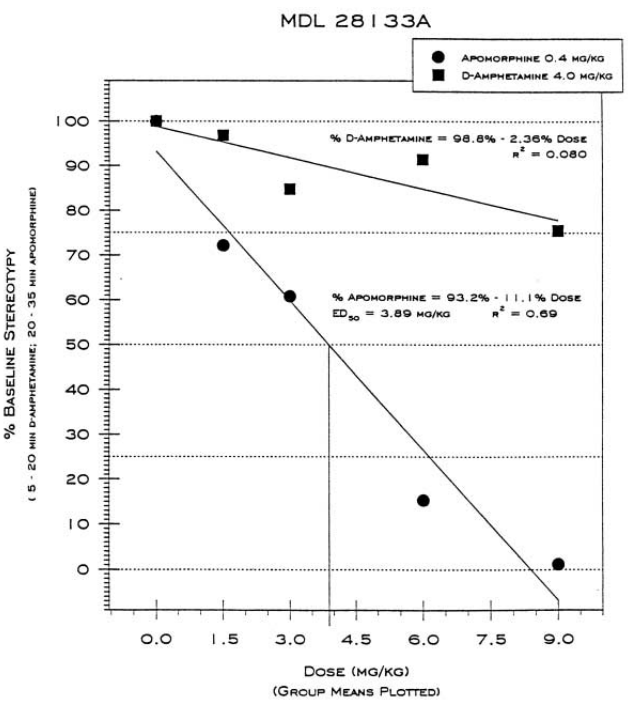

FIG. 3. Dose–response curves for the effects of MDL 28,133A 0.4 mg/kg apomorphine and 4.0 mg/kg d-amphetamine. Group means are plotted with regression lines for each stimulant. The means represent two summed observation periods: 5- and 20-min periods for d-amphetamine and 20- and 35-min periods for apomorphine.

66.7:1) dose ratio in terms of their affinity for D_2 receptor sites. The ratio of ED_{50}s for MDL 28,133A and haloperidol against ApIS was 35:1. For AmIS, however, quite a different effect was observed. Because MDL 28,133A was not strong enough to reduce AmIS by 50%, an effective dose could not be calculated. However, if the linear equation is used to extrapolate an effective dose for MDL 28,133A, the ED_{50}, would be 20.7 mg/kg, more than twice the highest dose employed. The hypothetical ratio of ED_{50}s for these antagonists against AmIS is 120:1. Because MDL 100,907 demonstrated no capacity to attenuate ApIS or AmIS, ED_{50} values could not be approximated.

DISCUSSION

As expected, haloperidol significantly reduced the stereotyped behavior produced by either stimulant, reflecting its ability to antagonize the postsynaptic D_2 receptor involved in the mediation of stereotyped responses (3). Likewise, the ability of MDL 28,133A to reduce ApIS is most likely due to its demonstrated affinity for the D_2 receptor (13). The ratio of the dose of haloperidol to the dose of MDL 28,133A required to displace 50% of radiolabeled spiroperidol is 66.7:1 (2,13), illustrating their difference in affinity. This difference is also reflected in a potency ratio for blocking ApIS (35:1). As hypothesized, MDL 100,907, which does not compete for D_2 receptor sites, was ineffective in reducing ApIS.

Several recent studies have demonstrated that 5-HT_2 antagonists can modulate the activity of DA neurons, and these drugs may have potential as antipsychotic medications (13,25,26,29,31). From this perspective, the 5-HT_2 antagonists MDL 28,133A and MDL 100,907 might be expected to reduce amphetamine stereotypy due to amphetamine's presynaptic mechanism of action. However, given the lack of EPS liability for mixed 5-HT_2 antagonists such as clozapine and risperidone and previous evidence showing that MDL 100,907 has no effect on stereotyped behavior (29), no effect of 5-HT_2 antagonists on AmIS would be predicted. The results of the present experiment clearly indicate that 5-HT_2 antagonism does not reduce AmIS, either alone or in combination with D_2 antagonism. However, a comparison of the effects of MDL 28,133A on AmIS and ApIS indicates that D_2 antagonism alone cannot account for the experimental outcomes. If it could, the doses of haloperidol and MDL 28, 133A, which produced the same attenuation of ApIS, should have had similar effects on AmIS. Perhaps 5-HT_2 receptors play some indirect role in the neurochemical mediation of amphetamine-induced stereotypy.

In reviewing 5-HT_2 influences on stereotyped behavior, MDL 100,907 appeared to potentiate ApIS ($p < 0.03$). Although the opportunity for Type I error was inflated due to the heterogeneous variance among the samples, ANOVA is typically robust for violations of this assumption. In addition, the arcsine transformation of the data and the use of submaximal scoring intervals reduced the differences in variance among the groups. It is surprising, given this finding, that MDL 100,907 did not significantly increase AmIS, possibly due to the ceiling effect of the d-amphetamine dose, and there is evidence to support this explanation. First, haloperidol was not able to completely block AmIS within the dose range chosen for the experiment. The dose of d-amphetamine, 4.0 mg/kg, may be too high, intensifying AmIS so that it could not be antagonized as easily as ApIS. Second, the MDL 100,907 group means were all higher than baseline AmIS, suggesting a trend toward potentiation of stereotypy. The hypothesis that 5-HT_2 antagonism potentiates stimulant-induced stereotyped responses should be evaluated in future experiments using smaller doses of d-amphetamine and a measure that allows for increases in stereotypy beyond the ceiling values in this experiment. If this effect is real, adding a 5-HT_2 antagonistic component to a neuroleptic compound might prevent the expression of EPS.

Evidence supporting a role of the 5-HT_2 receptor in the reduction of ApIS or AmIS was not obtained, suggesting that, in terms of DA systems responsible for the expression of stereotyped motor behavior, 5-HT_2 receptors do not regulate nigrostriatal DA production following d-amphetamine administration. This finding is inconsistent with the results of the microdialysis research, which suggests that 5-HT_2 antagonism prevents new dopamine synthesis during high states of activation (26). Several models proposing an inhibitory role of serotonin on nigrostriatal DA systems (6,7,9,18,27,28,31) would be consistent with the pattern of results found in this experiment. MDL 100,907 appeared to potentiate stereotyped responses in the presence of a stimulant, and MDL 28,133A, instead of being more effective in reducing AmIS, appeared to be less potent. The 5-HT_2 antagonism of MDL 28,133A might have actually worked against its antagonism of D_2 receptors following d-amphetamine administration.

Studies using intracranial self-stimulation (8,30) are in agreement with the results of this experiment: 5-HT_2 antagonism does not appear to attenuate the effects of DA stimulants, but D_2 blockers are capable of reducing some stimulant effects. Whereas psychostimulant effects on self-stimulation presumably involve the mesocorticolimbic (A10) system, stereotypy involves the nigrostriatal DA pathway (A9) (3,14). Moser et al. (20) noted that MDL 100,907 can attenuate locomotor activity, the disruption of latent inhibition and DA release following d-amphetamine or MDMA administration, but it has no effect on amphetamine facilitation of self-stimulation and amphetamine drug-discrimination tasks. They suggest that the behavioral effects of stimulant drugs, particularly those of d-amphetamine, are dissociatively influenced by serotonergic mechanisms.

Studies showing a decrease in stimulant-induced locomotion (20) following serotonin antagonist administration may not be incompatible with the finding of stereotypy enhancement by serotonin antagonists for reasons related to the Lyon-Robbins theory of stereotyped behavior (24). Stereotypy is a result of a competition among behaviors that potentially may be expressed. At 4.0 mg/kg of d-amphetamine, rats typically produce one of three responses: locomotion, rearing (stereotyped) or conditioned responding (stereotyped) (24). By decreasing the locomotor activity of rats through 5-HT_2 antagonism, one increases the potential expression of stereotyped movements (repetitive rearing and repetitive conditioned responding). Even though there is some evidence that locomotion and stereotyped behavior are not complementary (28), this model provides a nonphysiological framework from which the pattern of results of this study may be understood. Until further experiments are designed, this can be only speculation.

Another complexity in comparing the results of the apomorphine and d-amphetamine experiments is the difference in behavioral response to these stimulants. Apomorphine produces a predominance of sniffing down, whereas d-amphetamine produces repetitive head movements. Obviously, these drugs do not produce the same neurochemical effects; d-amphetamine affects a variety of neurotransmitter systems, including 5-HT and norepinephrine (24). The action of this stimulant on these other systems may modulate the effects of

d-amphetamine on DA terminals. In addition, d-amphetamine, being a presynaptic DA releaser, may be more susceptible to alterations of stereotyped behavior based on individual differences (24).

Janssen et al. (11) initially proposed that the particular ability of a compound to block ApIS is more predictive of motor side-effect potential than neuroleptic efficacy. The failure to find reduction of AmIS and ApIS by MDL 100,907 would imply that it has reduced liability for producing EPS in human populations. In contrast, MDL 28,133A has the potential to block ApIS in a way similar to haloperidol, a neuroleptic with well-documented EPS potential (1). However, its reduced potency in attenuating AmIS suggests that it has an atypical profile that requires further exploration. Perhaps the additional 5-HT_2 antagonist component of neuroleptics protect them from producing some types of motor side effects. This notion, too, requires further evaluation.

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