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Behaviorally Inactive Doses of Mianserin Antagonize the Suppressant Effect of Lysergic Acid Diethylamide on a Fixed-Ratio Operant[1]

LINDA P. DWOSKIN and SHELDON B. SPARBER
Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota
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ABSTRACT

Male Sprague-Dawley rats were trained to respond for food reinforcement on a fixed-ratio 15 schedule. Low, behaviorally inactive, doses of mianserin (0.1–1.0 mg/kg) administered 30 min before the operant session antagonized the behavioral suppression induced by lysergic acid diethylamide (LSD; 50 μg/kg) administered immediately before the session. Mianserin (0.5–1.0 mg/kg) also partially antagonized the behavioral suppressant effects of higher doses of LSD. At a dose which suppressed behavior to 70% of control when administered alone, mianserin (10 mg/kg) did not antagonize the behavioral suppression induced by LSD (100–200 μg/kg). The data suggest that the suppression of operant behavior produced by mianserin may be the result of nonselective or multiple actions, or possibly the emergence of an LSD-type partial agonist property. Specificity of drug action as an antagonist apparently occurs at the behaviorally inactive, low doses of mianserin which may act through a selective blockade of serotonin receptors stimulated by LSD.

Controversy exists concerning the action of mianserin, a tetracyclic antidepressant (Fell *et al.*, 1973), because it is devoid of the ability to block the uptake of NE or 5-HT into rat brain tissue or synaptosomes (Kafoe and Leonard, 1973; Zis and Goodwin, 1979). Mianserin also does not exhibit a typical pattern for antidepressants in central nervous system screening tests performed in animals including: amphetamine-induced gnawing (Fell *et al.*, 1973); potentiation of barbiturate sleep time (Vargaftig *et al.*, 1971); and antagonism of reserpine-induced hypothermia (Goodlet and Sugrue, 1974). A number of biochemical (Kafoe and Leonard, 1973; Fludder and Leonard, 1979; Sugrue, 1981), electrophysiological (Engberg and Svensson, 1980) and behavioral (Robson *et al.*, 1978) studies indicated that mianserin may act through a yohimbine-like mechanism. However, we found that although the suppressant effect of a low dose of clonidine (*i.e.*, 15 μg/kg) on operant behavior was completely blocked by behaviorally inactive, low doses of the *alpha*-2 antagonist yohimbine, mianserin (1.0–35.0 mg/kg) was inactive as an antagonist of the suppression induced by clonidine on operant behavior (L. P. Dwoskin and S. B. Sparber, manuscript in preparation). These results indicated that mianserin was not acting as a yohimbine-like drug in these experiments. Furthermore, high doses of mianserin (*e.g.*, 10 mg/kg and higher) were observed to suppress operant behavior.

Evidence from several behavior studies (Vargaftig *et al.*, 1971; Van Riezen, 1972; Maj *et al.*, 1978) indicates that mianserin may act as an antagonist at 5-HT receptors in the central nervous system. More recently, mianserin was reported to have a high affinity for the 5-HT_2-site in binding studies (Leysen *et al.*, 1981; Peroutka and Snyder, 1981). In order to verify that the operant behavior assay was capable of demonstrating a pharmacological action of mianserin at 5-HT receptors, we investigated the interaction of mianserin and a 5-HT agonist, LSD, upon the same schedule-controlled operant behavior. We also reasoned that the selectivity of the action of mianserin as an antagonist would be evident if the doses of mianserin, which were previously demonstrated to be incapable of antagonizing the behavioral suppressant effects of clonidine, were able to antagonize the behavioral suppressant effects of LSD.

Evidence from binding (Burt *et al.*, 1976; Green *et al.*, 1978), biochemical (Pieri *et al.*, 1974; Green *et al.*, 1977) and behavioral (da Prada *et al.*, 1975; Persson, 1978) studies indicate that LSD may also affect dopaminergic and histaminergic systems. However, LSD was chosen to study the involvement of 5-HT in the action of mianserin because the preponderance of evidence, including biochemical (Rosecrans *et al.*, 1967; Anden *et al.*, 1968; Randic and Padjen, 1971), binding (Bennett and Snyder,

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ABBREVIATIONS: NE, norepinephrine; 5-HT, serotonin; LSD, lysergic acid diethylamide; FR, fixed-ratio; ANOVA, analysis of variance; 5-HTP, 5-hydroxytryptophan.

1976; Peroutka and Snyder, 1979) and electrophysiologic (Aghajanian *et al.*, 1972; Haigler and Aghajanian, 1974), as well as brain perfusion data (Tilson and Sparber, 1972), support a role for 5-HT receptors in producing the action of LSD, especially at low doses. Additionally, LSD was chosen because it too suppressed FR behavior at reasonably low doses (*e.g.*, less than 200 μg/kg) (Appel and Freedman, 1965; Sparber and Tilson, 1971).

Methods

Subjects. Ten adult male Sprague-Dawley rats weighing between 350 to 420 g (free-feeding) were obtained from Colony 4 of Holtzman Company (Madison, WI). Upon arrival they were individually housed in a temperature (21 ± 1°C) and humidity (40–50%) controlled environment under a 12-hr light-dark cycle (lights on at 7:00 A.M.). Body weights were gradually reduced to and maintained at 80% of the free-feeding weight by a modified feeding schedule. Water was freely available in their home cages throughout the experiment. Animals were drug naive and were approximately 9 months of age when experimental sessions began.

Apparatus. Behavioral sessions were conducted in standard operant conditioning chambers (model no. 143-22; BRS/LVE, Beltsville, MD). Each chamber was equipped with a single lever which could be depressed by a weight of at least 29 g, a dispenser for the delivery of 45-mg food pellets (formulation T101, Bio Serv, Frenchtown, NJ) and a speaker for the introduction of white masking noise. External sound and light attenuation were provided by enclosing the chamber in a custom made insulated and ventilated outer chamber which was equipped with a closed-circuit video system (Nielsen *et al.*, 1980).

The house light and cue light above the lever provided a low level of illumination during training and experimental sessions. All experimental events and contingencies were automatically controlled and recorded by a Nova-2/10 mini-computer (Data General Corp., Southboro, MA), coupled with an Interact System interfacing (BRS/LVE). Upon termination of the behavioral session, the computer printed the number of responses emitted by the animal during each minute of the session. Continuous records of behavior were also recorded on cumulative recorders (Ralph Gerbrands, Arlington, MA).

Training procedure. Food pellets were delivered noncontingently every 30 sec during 30 min sessions on each of 2 days in order to magazine train the subjects. They were then placed in the experimental chamber for three 30-min sessions in which each lever press resulted in food delivery (Ferster and Skinner, 1957). During the next 30-min session, each animal was placed on a FR 2 schedule of reinforcement, in which 2 lever presses resulted in food pellet delivery. On subsequent daily sessions, the ratio was slowly increased until the number of responses necessary for reinforcement was 15 (FR 15), after which stable responding during the daily 30-min behavioral sessions was attained. Stable responding was defined as variation of not more than ±10% from the mean during three consecutive sessions. Stable baseline response rates were obtained for the group in approximately 2 weeks after being placed on the FR 15 schedule.

After initial training, rats were habituated for 3 days to two i.p. injections (1 ml/kg) of water and saline administered 30 min before and immediately before, respectively, the operant behavioral session. Thirty minutes after the daily session, supplemental food (Purina Lab Chow) was supplied, if necessary, to individual animals for the purpose of maintaining the 80% of free-feeding body weight.

Behavioral interaction of mianserin and LSD. The behavioral effects of mianserin (0.1–10.0 mg/kg) and the interaction of mianserin with one dose of LSD (200 μg/kg) were first studied simultaneously using a Latin Square design ($N = 10$). Unfortunately, it was necessary to sacrifice one of the animals before completion of this initial experiment and its data were not included in the analysis.

After administration of 200 μg of LSD per kg, most of the rats did not resume responding for the remainder of the session (*i.e.*, a ceiling effect). Therefore, it was felt that only a conservative estimate of the effect of LSD and of the antagonistic properties of mianserin was determined. To characterize the antagonistic properties of mianserin more completely, the interaction of mianserin and two lower doses of LSD was subsequently examined. Upon completion of the initial experiment, the animals were randomly divided into two groups, such that the average response rates for the groups were not different. Five of the animals were used to study the interaction of LSD (100 μg/kg) with mianserin (0.1–10.0 mg/kg) and four were used to study the interaction of LSD (50 μg/kg) with mianserin (0.1–1.0 mg/kg) using Latin Square designs. At least 72 hr separated drug sessions, each of which was preceded by a control session the day before in which vehicle was administered i.p. 30 min before and immediately before the operant session.

Data analyses. The number of reinforcers received during the period (30 min) in the operant chamber directly reflects the number of responses emitted and is therefore a reasonable approximation of the rate of responding in FR schedules. The average rate of responding for the group of animals ($N = 9$) was 170.6 ± 6.4 responses/min (mean ± S.E.M.) and the range was 132.5 to 199.0 responses per minute. Because FR operant behavior is characterized by a constant, high rate of responding which remains stable over an extended period of time, individual data may be expressed as the number of reinforcers received after drug as a percentage of those received after vehicle on the previous day. In this manner, variability due to individual differences in response rate is eliminated.

In addition to using the number of reinforcers the rats received during the session as an approximation of the responding rate, an additional measure of the effect of LSD upon FR 15 responding and of the ability of mianserin to antagonize this effect was utilized. After the administration of hallucinogenic drugs, FR behavior is abruptly disrupted and after varying periods of time it abruptly returns to responding rates which often are close to control (Appel and Freedman, 1965). The latency to onset of this disruption and the duration of the pause in responding are dose-dependent and amenable to quantitation (Sparber and Tilson, 1971). In the experiments reported herein, the duration of the pause in responding was defined as the amount of time (minutes) taken to receive five reinforcers (*i.e.*, depress the lever 75 times) after a period of 2 min in which no reinforcers were received. In those instances in which responding did not resume (*i.e.*, <5 reinforcers received) for the remainder of the behavioral session, the period of the pause in responding was used as the duration. For example, if a subject ceased responding 4 min into a 30-min session and did not resume responding before the end of the session, the value used for statistical analysis was 24 min (4 min of responding + 2 min criterion for a pause in responding, subtracted from the session length).

The data were analyzed using repeated measures ANOVA (Winer, 1971). Subsequent individual comparisons were made using Duncan's New Multiple Range test for differences between pairs of treatment means (Beyer, 1968) and Dunnett's test to compare a single control mean with multiple treatment means (Winer, 1971), where appropriate. Data were analyzed using two-tailed statistical tests.

Although separate experiments on the ability of mianserin to antagonize the effects of three different doses of LSD were carried out, the results of these experiments have been depicted together in most of the figures. This was done for efficient use of space and ease of comparison.

Drugs. Mianserin HCl was a gift from Organon (Oss, Holland) and was freshly dissolved in distilled water. Drug doses of mianserin pertain to the salt. LSD was a gift from the National Institute on Drug Abuse (Bethesda, MD) and was supplied as *d*-LSD tartrate. A stock of LSD (400 μg/ml) was dissolved in 0.9% saline. Dilutions of LSD from stock solutions stored at 4°C were made daily and the same stock solution was not used for more than 2 weeks. Doses of LSD pertain to the base. All drug injections were administered i.p. in a volume of 1 ml/kg.

Results

Figure 1 illustrates that behaviorally inactive doses of mianserin antagonized the suppression of responding induced by

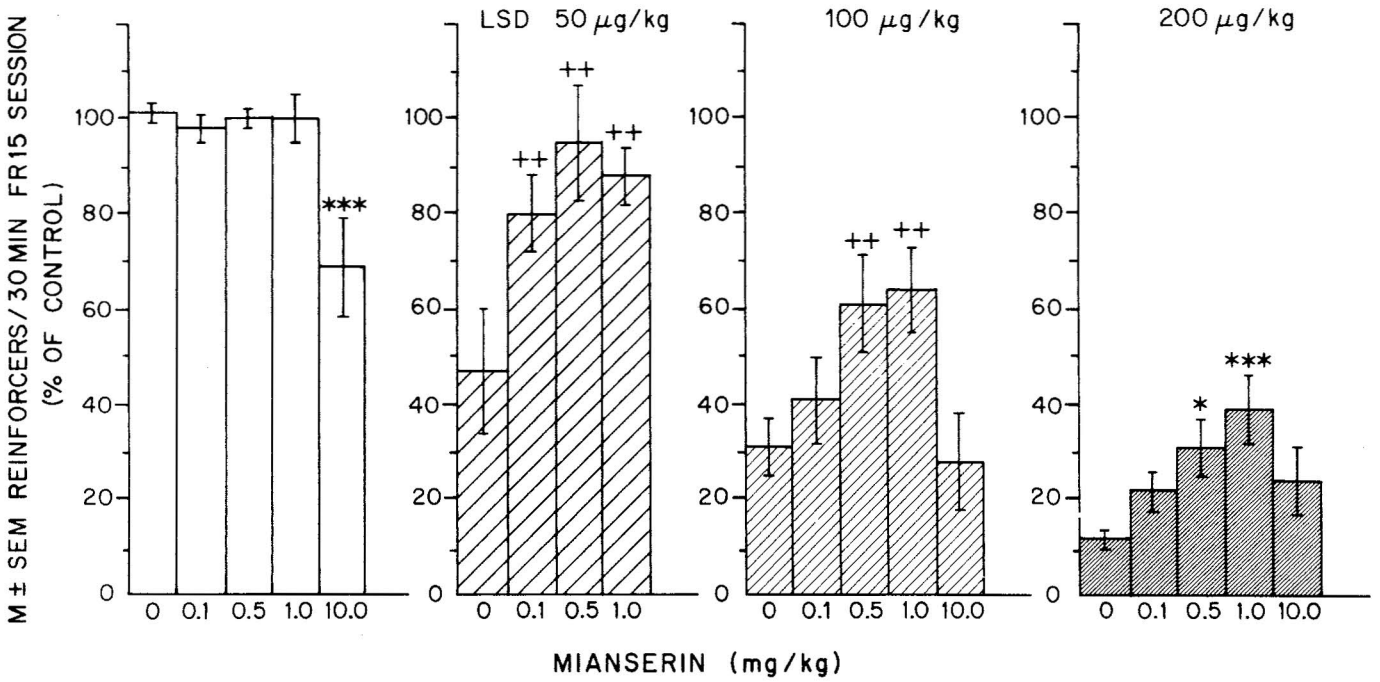

Fig. 1. Antagonism of the behavioral suppressant effect of LSD by behaviorally inactive doses of mianserin. Data are expressed as the number of reinforcers earned as a percentage of the control day before drug administration in which water and saline were administered. Clear bars illustrate the effect of mianserin (0.1–10.0 mg/kg) administered 30 min before the 30 min FR 15 behavioral session. Striped bars illustrate the behavioral effect of LSD (50–200 μg/kg) administered immediately before the operant session and the antagonism of the LSD-induced behavioral effect obtained by pretreatment with behaviorally inactive doses of mianserin (0.1–1.0 mg/kg) administered 30 min before the operant session. The effect of various doses of mianserin alone and the interaction of mianserin with 200 μg of LSD per kg were examined simultaneously as a single experiment. Therefore, in this case Duncan's test comparing pairs of treatment means was used to determine whether mianserin produced a significant behavioral suppression compared with vehicle, as well as to determine whether mianserin significantly antagonized the suppressant effect of LSD. $^{++}P < .01$ by Dunnett's analysis; $^{*}P < .05$; $^{***}P < .001$ by Duncan's analysis. M, mean.

LSD. The initial experiment examined the effects of mianserin alone on behavior, as well as its ability to antagonize the behavioral effect of 200 μg of LSD per kg. Statistical analysis using repeated measures ANOVA revealed a significant overall main effect due to treatment [$F(9,72) = 60.473$, $P < .001$]. Subsequent comparisons using Duncan's analysis indicated that 10 mg of mianserin per kg alone produced a statistically reliable reduction in the number of reinforcers received to about 70% of control. Pretreatment with 0.5 to 1.0 mg of mianserin per kg significantly increased the number of reinforcers received from an average of 12% of control after 200 μg of LSD per kg alone to about 40% of control. Statistical analysis of the antagonism of the behavioral effect of 100 μg of LSD per kg revealed a significant overall main effect of treatment [repeated measures ANOVA, $F(4,16) = 6.189$, $P < .005$]. Subsequent comparisons using Dunnett's test indicated that pretreatment with 0.5 to 1.0 mg of mianserin per kg significantly antagonized the behavioral effect of 100 μg of LSD per kg from an average of 31% of control after LSD alone to about 60% of control. Analysis of the interaction of mianserin and the lowest dose of LSD (50 μg/kg) also revealed a significant overall main effect due to treatment [repeated measures ANOVA, $F(3,9) = 20.469$, $P < .001$]. Subsequent comparisons using Dunnett's test indicated that all three doses of mianserin significantly antagonized the behavioral effect of the lowest dose of LSD, which alone reduced the number of reinforcers received to an average of 47% of control. Whereas 0.1 and 1.0 mg of mianserin per kg restored behavior to about 80 and 90% of control responding, respectively, 0.5 mg of mianserin per kg resulted in responding at approximately control rates.

Figure 2 illustrates the duration of the behavior disruption or prolonged pause in responding as a function of the dose of LSD and the antagonism of the pause in responding produced by the behaviorally inactive doses of mianserin. The antagonism of the extended pause in responding induced by 200 μg of LSD per kg was analyzed by repeated measures ANOVA, which revealed a significant main effect due to treatment [$F(4,32) = 8.251$, $P < .001$]. Subsequent comparisons using Dunnett's test indicated that pretreatment with 0.5 to 10.0 mg of mianserin per kg significantly reduced the duration of the pause from an average of 22 min to 9 min. After administration of 100 μg of LSD per kg, the duration of the pause in responding was about 15 min. Statistical analysis of the antagonism by mianserin revealed a significant overall main effect due to treatment [repeated measures ANOVA, $F(4,16) = 3.832$, $P < .05$] and subsequent analysis using Dunnett's test indicated that pretreatment with 0.5 or 1.0 mg of mianserin per kg significantly shortened the duration of the pause in responding to about 2 to 3 min. Repeated measures ANOVA revealed that the average length of the pause in responding (10 ± 5 min; mean ± S.E.M.) after administration of 50 μg of LSD per kg was not significant. After this dose of LSD, only two of the four animals behavior were disrupted, according to our criterion. After 0.5 mg of mianserin per kg and 50 μg of LSD per kg, a pause in responding was observed in only one of these two animals. After pretreatment with 1.0 mg of mianserin per kg, behavior was not disrupted in any animals. Administration of mianserin alone did not result in an extended period of no responding (*i.e.* > 2 min without reinforcement), even though 10 mg/kg decreased the number of reinforcers received to about 70% of control.

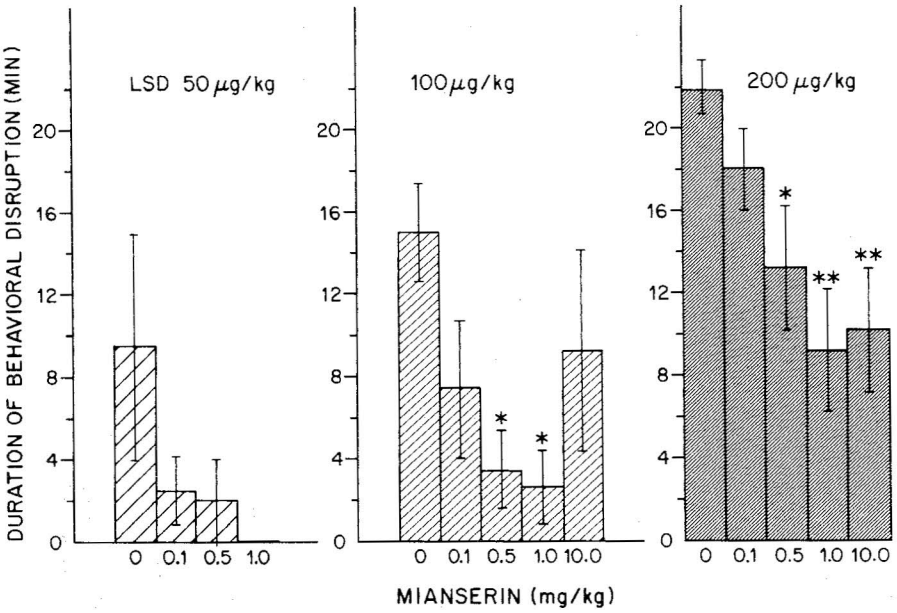

Fig. 2. The duration of the disruption or pause in responding as a function of the dose of LSD and its antagonism by mianserin. LSD (50–200 μg/kg) was administered immediately before the FR 15 behavioral session and mianserin (0.1–10.0 mg/kg) was administered 30 min before the session. The measure of behavioral disruption is the number of minutes taken to earn five reinforcers after a period of at least 2 min during which no reinforcers were earned. *P < .05; **P < .01 by Dunnett's analysis.

During the pause in responding, after administration of the higher doses of LSD alone, the animals appeared to remain relatively still, with their ventral body surface on the grid of the operant chamber. With mianserin pretreatment, they were more active during this period, although rearing and obvious exploratory types of behavior were not emitted.

Examples of the qualitative nature of the behavioral effects of mianserin, LSD and mianserin plus LSD are depicted in figures 3 and 4. Figure 3 shows cumulative response records for rat 43 after administration of vehicle 10 mg of mianserin per kg, 200 μg of LSD per kg and 200 μg of LSD per kg 30 min after pretreatment with 0.1 to 10.0 mg of mianserin per kg. Although administration of 10 mg of mianserin per kg did not result in an extended pause, it's behavioral suppressant action is obvious; rat 43 received about 75% of the reinforcers received on the previous control day. Inspection of this cumulative record reveals that the suppression of responding induced by 10 mg of mianserin per kg is apparently due to a slowing of the overall response rate, without the prolonged pauses seen after higher doses of LSD. When 200 μg of LSD per kg was administered immediately before the behavioral session, 2 min after the start of the session, rat 43 ceased responding and did not resume responding during the remainder of the session. Administration of 0.1 and 0.5 mg of mianserin per kg 30 min before the LSD (200 μg/kg) injection reduced the period of no responding from greater than 26 min to 19 and 10 min, respectively. Administration of 1.0 mg of mianserin per kg completely antagonized the prolonged pause induced by 200 μg of LSD per kg (*i.e.*, no disruption > 2 min). Inspection of this record reveals that early in this session the rate of responding was not different from that under control conditions but, approximately 6 min after the start of the session, the frequency of short postreinforcement pauses increased and the overall rate of responding slowed. A pause in responding (8 min) occurred during the session in which 10 mg of mianserin per kg was administered 30 min before 200 μg of LSD per kg. Examination of this cumulative record reveals the pause in responding and a slowed rate of responding. Generally, inspection of the records (not shown) after administration of mianserin before 100 μg of LSD per kg revealed a similar pattern. The duration of the pause in responding induced by LSD (100 μg/kg) was reduced in a dose-dependent manner, but the pause reappeared after 10.0 mg of mianserin per kg and this dose of LSD.

Figure 4 illustrates the cumulative response records for rat 44 after administration of vehicle, 50 μg of LSD per kg and 50 μg of LSD per kg 30 min after pretreatment with 0.1 to 1.0 mg of mianserin per kg and demonstrates the qualitative nature of the effects of LSD in a subject which did not show an extended pause in responding after the low dose of LSD. Under control conditions, rat 44 responded with a steady, high response rate. When 50 μg of LSD per kg was administered immediately before the 30-min behavioral session, there was no pause (> 2 min without reinforcement); however, the number of reinforcers received during this session was 65% of that received during the control session. Inspection of the cumulative record reveals that approximately 8 min after the start of the session, the frequency of short postreinforcement pauses increased and the overall slope was reduced. Complete antagonism of the behavioral effect induced by 50 μg of LSD per kg was obtained when 0.5 mg of mianserin per kg was administered 30 min before the session. With this dose of mianserin, rat 44 received about 110% of the number of reinforcers received under control conditions. Pretreatment with 0.1 and 1.0 mg of mianserin per kg also resulted in significant antagonism of the behavioral effect of LSD (50 μg/kg) and rat 44 received 80 and 95%, respectively, of the reinforcers received during the control sessions.

Figure 5 summarizes the effects of LSD alone and in combination with various doses of mianserin (data reorganized from that shown in figs. 1 and 2). The relative extent of the antagonism of the behavioral effects of LSD by behaviorally inactive doses of mianserin (0.1–1.0 mg/kg) can be seen by the progressive shift of the LSD dose-response curve.

Discussion

The behavioral interaction of mianserin and LSD was examined to test the hypothesis that mianserin acts as an antagonist of a 5-HT receptor upon which LSD appears to have an agonist effect. The dose of LSD (200 μg/kg) which was used in

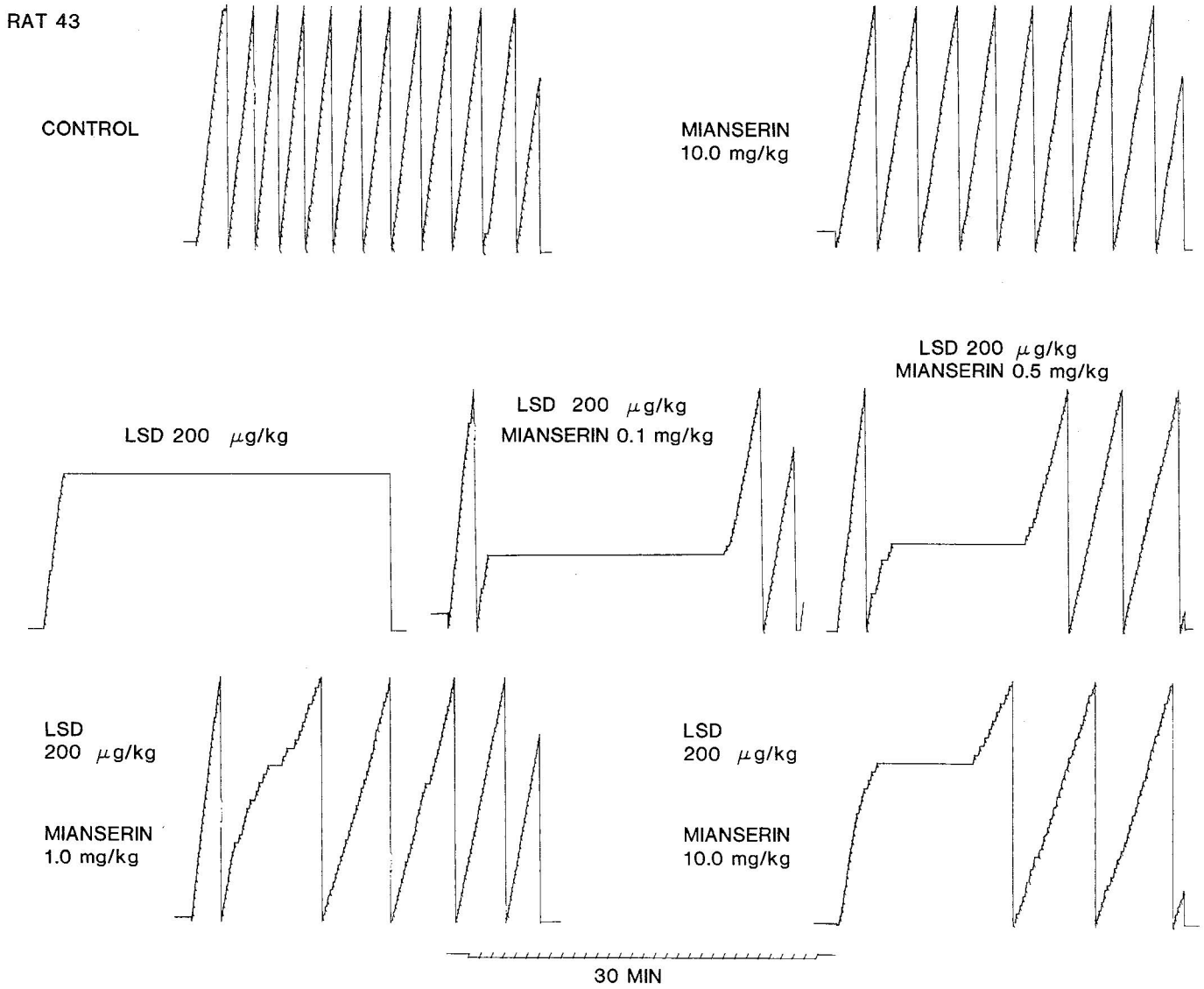

Fig. 3. Representative cumulative records for rat 43 illustrating the effect of mianserin (10.0 mg/kg), LSD (200 μg/kg) and the antagonism of the LSD-induced effect by pretreatment with mianserin (0.1–10.0 mg/kg). The control record illustrates the pattern of responding after water and saline were administered in lieu of mianserin and LSD, respectively. The length the stepping pen traversed before reset represents 400 responses on the lever.

the initial drug interaction experiment was chosen because our previous experience indicated that it should induce the typical disruption of FR behavior (Sparber and Tilson, 1971) and because this dose of LSD was also reported to be capable of significantly affecting the release of radiolabeled 5-HT into perfusate from push-pull cannulas implanted in the brains of rats (Gallager and Aghajanian, 1975). Consistent with previous studies (Appel and Freedman, 1965; Sparber and Tilson, 1971), LSD produced a dose-dependent suppression of the average FR response rate and, at certain doses, an abrupt cessation of responding. Antagonism of the LSD-induced effects by behaviorally inactive, low doses of mianserin was clearly evident for both of the behavioral measurements. Furthermore, mianserin completely antagonized the effects of 50 μg of LSD per kg, such that responding was indistinguishable from that under control conditions. The results of the present study, therefore, support the hypothesis that mianserin acts as a pharmacologic antagonist at 5-HT receptors, at doses (0.1–1.0 mg/kg) which are behaviorally inactive and which previously have been shown to be incapable of antagonizing the behavioral effect of clonidine (L. P. Dwoskin and S. B. Sparber, manuscript in preparation).

The results of the present study are compatible with binding studies which report that mianserin has a high affinity for the 5-HT_2 site (Leysen *et al.*, 1981; Peroutka and Snyder, 1981). Therefore, low doses of mianserin may act through a selective blockade of the 5-HT_2 receptor to antagonize the LSD-induced suppression of conditioned behavior. Our results also agree with previous behavioral studies reporting that mianserin inhibits the 5-HTP-elicited stereotyped head twitches in mice (Van Riezen, 1972) and rats (Maj *et al.*, 1978) and the tryptamine-induced stereotyped slow contraction of the digits and paws in rats (Vargaftig *et al.*, 1971). However, the interpretation of the studies using 5-HTP and tryptamine as 5-HT agonists is problematic, because 5-HTP is decarboxylated in all monoaminergic neurons and tryptamine may act either as a false transmitter or through the tryptaminergic neuronal system. Another problem with this model for 5-HT stimulation is that it measures behaviors which ordinarily do not occur in the absence of

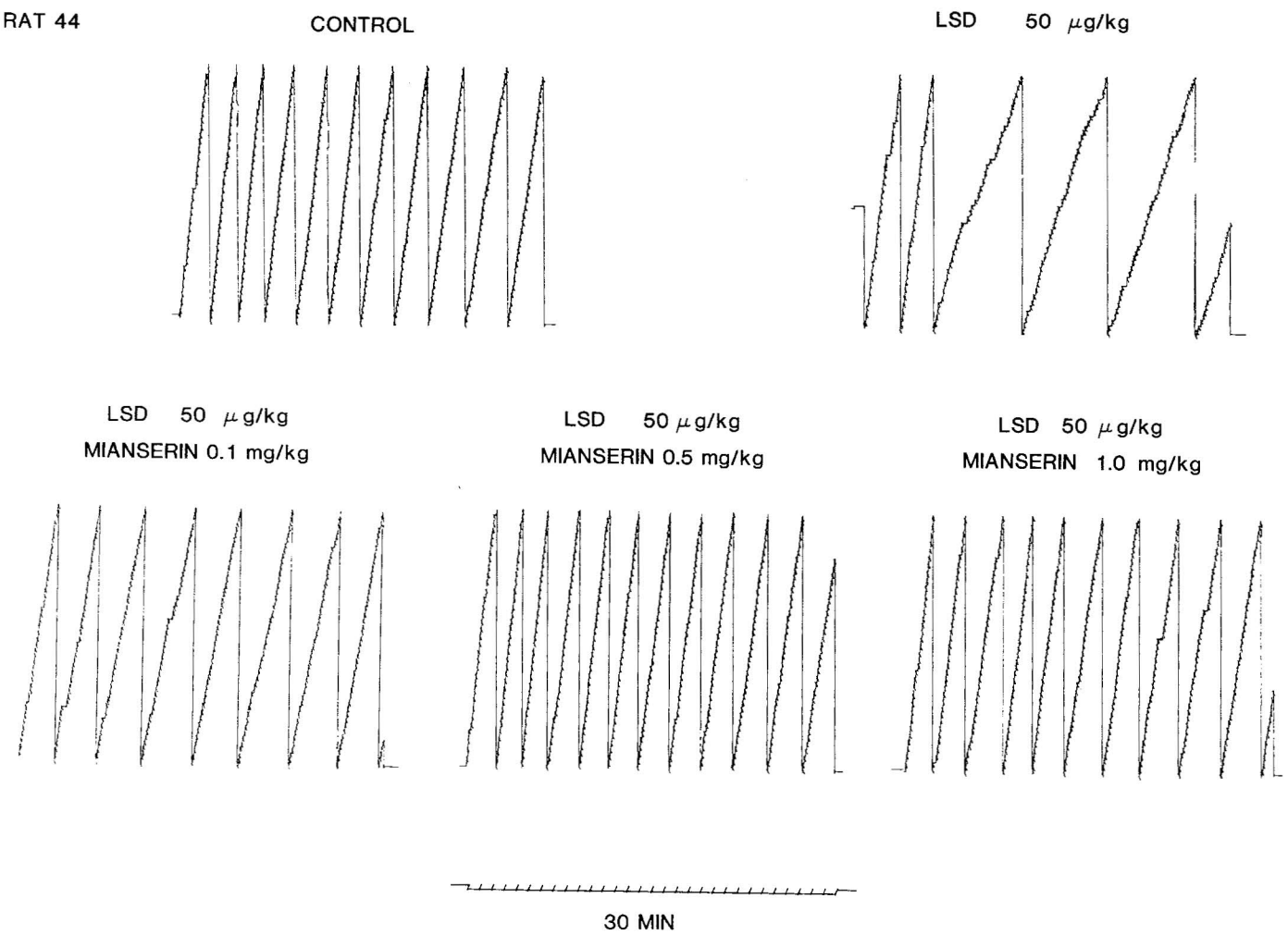

Fig. 4. Representative cumulative records for rat 44 illustrating the effect of LSD (50 μg/kg) and the antagonism of the LSD-induced effect by pretreatment with mianserin (0.1–1.0 mg/kg). Note that this dose of LSD did not induce behavioral disruption. The length the stepping pen traversed before reset represents 400 responses on the lever.

5-HTP treatment. The antagonist alone may produce effects (*e.g.*, general motor depression) which are incompatible with the expression of the syndrome. Therefore, the antagonist would be construed as blocking the syndrome *via* a 5-HT receptor effect, while it actually may be acting nonselectively to suppress many behaviors.

The data reported herein also suggest that behavioral suppression induced by high doses of mianserin may be the result of nonselective actions, only partially or not at all related to its ability to act as an antagonist at sites responsible for the behavioral effect of LSD. The high dose of mianserin (10 mg/kg), when administered alone, produced a suppressed rate of responding, but did not result in a prolonged pause in responding, as did higher doses of LSD. This dose of mianserin did not antagonize the behavioral effects of LSD to as great a degree as the lower, behaviorally inactive doses. Actually, the high dose of mianserin produced a reappearance of the pause in responding when administered with the higher doses of LSD.

During preparation of this manuscript, a study (Colpaert *et al.*, 1982) appeared in which mianserin was reported to act complexly as a mixed agonist/antagonist when examined in a two-lever, drug-discrimination paradigm in which food served as the reinforcer. Rats were trained to discriminate 160 μg of LSD per kg from saline and subsequently various doses of drugs were administered alone or before the LSD to determine the extent of their agonist and/or antagonist action, respectively. In agreement with the study reported herein, mianserin partially blocked the discriminative stimulus properties of LSD at doses which appeared not to produce a rate-depressant effect. Interestingly, according to the interpretation of the authors, mianserin displayed agonist properties at doses which were behaviorally active, which, in part, might explain the reappearance of the pause in responding in the present study (fig. 3) when behaviorally active doses of mianserin were administered in conjunction with LSD.

Several biochemical, physiological and behavioral studies have suggested that mianserin may have *alpha*-2 antagonistic properties. Specifically, Kafoe and Leonard (1973) reported that mianserin increases the turnover of NE, measured by increased incorporation of [^{3}H]tyrosine into [^{3}H]NE in whole rat brain and by an increased concentration of normetanephrine in the amygdala. Furthermore, mianserin was reported to antagonize the decrease in normetanephrine produced by clonidine (Fludder and Leonard, 1979). Sugrue (1981) reported that mianserin increases whole-brain levels of 3-methoxy-4-hydroxyphenylethyleneglycol sufate and antagonizes the suppression in 3-methoxy-4-hydroxyphenylethyleneglycol sulfate levels induced by clonidine. Engberg and Svensson (1980)

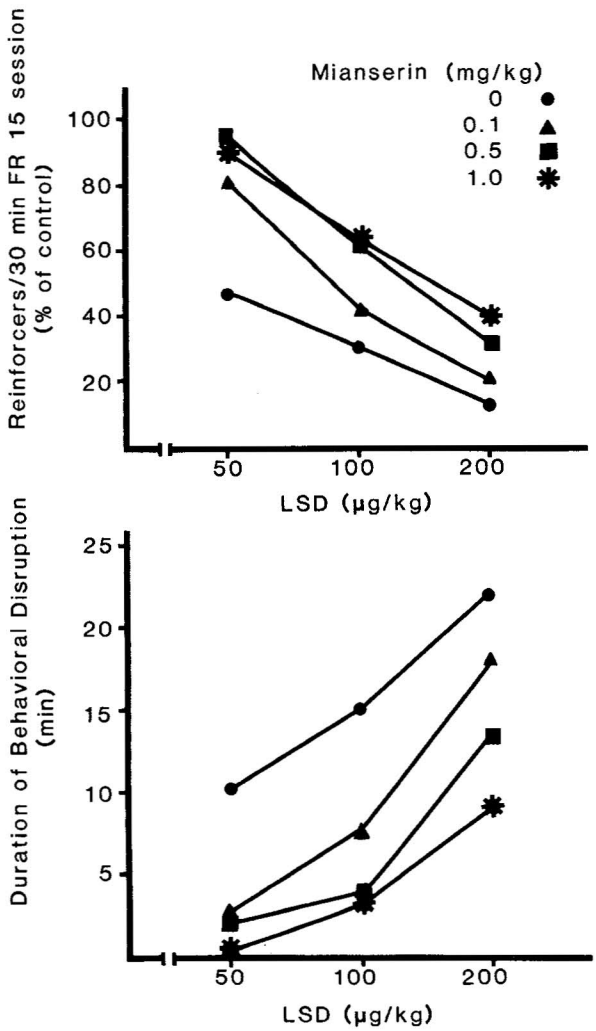

Fig. 5. Relative extent of antagonism of the behavioral effects of LSD by behaviorally inactive doses of mianserin (0.1–1.0 mg/kg) expressed as response rate (top) or as duration of disruption or pause in responding (bottom). Data were taken from figures 1 and 2.

reported that mianserin increases the firing of locus ceruleus neurons and antagonizes the suppression of firing of these neurons induced by clonidine. When high doses (100–300 μg/kg) of clonidine were used to increase avoidance failures in a behavioral study, mianserin was reported to antagonize this effect of clonidine (Robson *et al.*, 1978). These results are not compatible with our data. In all cases the dose(s) (8–30 mg/kg) of mianserin used were capable of altering FR behavior and, moreover, were 50 to 300 times higher than that which we report can significantly antagonize the effect of LSD upon behavior.

More recently, mianserin has been demonstrated to be incapable of antagonizing the suppression of hypothalamic self-stimulation behavior induced by clonidine, suggesting that mianserin is not a selective antagonist of the *alpha*-2 receptor (Hunt *et al.*, 1981). However, using the identical model, these investigators previously reported that yohimbine also was unable to antagonize the clonidine-induced suppression of self-stimulation behavior (Hunt *et al.*, 1978). Therefore, clonidine may not be producing the suppression of self-stimulation behavior by an *alpha*-2 receptor mediated action. Data from our laboratory generally do not support the hypothesis that mianserin is acting as an antagonist of the *alpha*-2 receptor, as yohimbine but not mianserin antagonized the operant behavioral suppressant action of clonidine (L. P. Dwoskin and S. B. Sparber, manuscript in preparation).

The results of our studies also suggest that low doses of LSD act *via* a single mechanism to suppress operant behavior. In this study, a low dose of mianserin (0.5 mg/kg) was able to antagonize completely the behavioral effect of 50 μg of LSD per kg. In a subsequent study, a higher, but still behaviorally inactive, dose of mianserin (2.5 mg/kg) completely antagonized the 70% suppression of behavior induced by 100 μg of LSD per kg, wherea methysergide (0.1–35.0 mg/kg), a classical 5-HT_1 antagonist, produced only partial antagonism (L. P. Dwoskin and S. B. Sparber, manuscript in preparation).

In summary, behaviorally inactive, low doses of mianserin antagonized, in a dose-related fashion, the behavioral suppressant effect of LSD, suggesting a specific antagonism at 5-HT receptors activated by LSD. The pharmacologic relevance of these behaviorally inactive doses of mianserin, which were unable to antagonize the behavioral suppressant effect of clonidine in an earlier study (L. P. Dwoskin and S. B. Sparber, manuscript in preparation) was thereby confirmed. A behaviorally active, higher dose of mianserin was not as effective in antagonizing the behavioral effect of LSD, suggesting that at higher doses mianserin is not a selective antagonist at 5-HT receptors, but that at these doses some other mechanism(s) of the action of mianserin comes into play. Finally, this additional action of mianserin is not *via* an action at *alpha*-2 receptors. It is suggested that pharmacological studies examining specific or selective effects of mianserin in rats use doses 1 to 2 orders of magnitude lower than heretofore utilized.

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Send reprint requests to: Dr. S. B. Sparber, Department of Pharmacology, University of Minnesota, 3-260 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455.