0022-3565/82/2211-0206$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 221, No. 1

Printed in U.S.A.

A Drug Discrimination Analysis of Lysergic Acid Diethylamide (LSD): *In Vivo* Agonist and Antagonist Effects of Purported 5-Hydroxytryptamine Antagonists and of Pirenperone, A LSD-Antagonist[1]

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Accepted for publication January 11, 1982

ABSTRACT

Colpaert, Francis C., Carlos J. E. Niemegeers and Paul A. J. Janssen: *A drug discrimination analysis of lysergic acid diethylamide (LSD): In vivo agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, a LSD-antagonist.* J. Pharmacol. Exp. Ther. **221:** 206–214, 1982.

An *in vivo* analysis was conducted of the pharmacological interactions of the putative 5-hydroxytryptamine (5-HT) receptor blocking agents metergoline, methysergide, 2-bromo- lysergic acid diethylamide (LSD), mianserin, pizotifen, cyproheptadine, metitepine and cinanserin, with LSD. Rats were trained to discriminate i.p. injections of 0.16 mg/kg of LSD from saline injections in a two-lever procedure in which food served as the reinforcer. Potential antagonists were given as a s.c. pretreatment (t = 60 min) before the injection (t = 15 min) of either LSD or saline to test for antagonist and LSD-like agonist activity, respectively. The 5-HT antagonists produced only a partial antagonism of LSD; their dose-response curve in antagonizing LSD was invariably curvilinear, reached a ceiling level at 29 (methysergide and metitepine) to 71% (pizotifen) of effect, and assumed a biphasic shape with methysergide, mianserin and cyproheptadine. In addition, the 5-HT antagonists mimicked LSD; the maximum LSD-like agonist effect ranged from 14 (cinanserin) to 86% (methysergide and mianserin). A dose of 40 mg/kg of methysergide or mianserin produced effects which were similar to those of 40 mg/kg of mescaline and the peak agonist activity of methysergide, mianserin and cyproheptadine exceeded their peak antagonist effects. Stimulus generalization of these agents with 0.16 mg/kg of LSD typically was a linear function of dose. LSD-like agonist and antagonist effects of moderate intensity coexisted at the same dose of the same agent, but strong agonist activity was clearly incompatible with strong antagonist activity and *vice versa*. Only pirenperone, a newly synthesized compound, effectively antagonized LSD. This antagonism of LSD was unique in that it occurred at low (0.01–0.16 mg/kg) s.c. and oral doses, proceeded along a linear and steep gradient, reached the 100% level of effect and was not associated with any agonist activity. It is suggested 1) that LSDs discriminative stimulus properties in the rat are contingent upon agonist effects at 5-HT receptor sites in the brain, 2) that the putative 5-HT antagonists mentioned above act complexly as partial and mixed agonists/antagonists at these sites and 3) that pirenperone is a pure antagonist of LSD.

LSD is among the most powerful agents known to date in producing psychotropic effects (*e.g.*, hallucinations and psychoses) in man. Prominent among the pharmacological and biochemical properties of LSD is its ability to interact with the receptor sites of 5-HT in the brain. Much of the stereospecific binding of *d*-LSD to brain tissue occurs on 5-HT receptors (Bennett and Aghajanian, 1974; Peroutka and Snyder, 1979). Like 5-HT, LSD inhibits the firing rate of neurons in the raphe nuclei (Aghajanian *et al.*, 1970, 1973) and in areas receiving identified 5-HT terminals (Aghajanian *et al.*, 1972; Haigler and Aghajanian, 1974a). LSD also blocks the excitatory effects of 5-HT in areas in which 5-HT terminals have a sparse distribution (Boakes *et al.*, 1970).

Research into the mechanism of action of LSD in producing psychotropic effects has been greatly hampered by the paucity of psychophysiological methods through which these physiological effects and binding properties can be related to the behavioral action of the drug (Brawley and Duffield, 1972). One particularly interesting development, therefore, is that of the drug discrimination technique. Drug discrimination recently

Received for publication January 21, 1981.
[1]This work was supported by a grant from the Instituut voor Wetenschappelijk Onderzoek in Nijverheid en Landbouw. Parts of the present data were presented at the 12th Collegium Internationale Psychopharmacologium Congress, Göteborg, June 22–26, 1980 (Abstract No. 130) and at the 8th International Congress of Pharmacology, Tokyo, July 19–24, 1981 (Abstract No. 1224).

ABBREVIATIONS: LSD, lysergic acid diethylamine; 5-HT, 5-hydroxytryptamine; DS, discriminative stimulus; DL, drug lever; SL, saline lever; FR, fixed-ratio; FRF, first reinforcement.

has evolved as a method to determine and quantify stimulus properties of drugs which are not directly accessible to an outside observer (Colpaert and Rosecrans, 1978; Ho *et al.*, 1978; Lal, 1977). The technique typically involves training laboratory animals to discriminate the perceived stimulus effects of a given drug from those of its vehicle, and there is considerable evidence to suggest that the DS properties of at least some drugs in animals are closely related to their subjectively perceived effects in humans (for review, see: Colpaert, 1978; Weissman, 1978).

That rats can discriminate LSD from saline was originally reported by Hirschhorn and Winter (1971). Subsequent drug discrimination work on LSD has reached two important conclusions. First, studies of cross-generalization involving LSD (Cameron and Appel, 1973; Kuhn *et al.*, 1978; Schechter and Rosecrans, 1972; White *et al.*, 1977, 1979; Winter, 1979) have indicated that stimulus generalization with LSD in the rat represents a necessary, albeit perhaps not sufficient, condition for prediction of hallucinogenic activity of the indole/phenethylamine type in man (Winter, 1975a, 1980; Kuhn *et al.*, 1977). A second conclusion is that the DS properties of LSD are contingent upon agonist effects of the drug at 5-HT receptor sites in the brain. The evidence in support of this conclusion consists mainly of data showing that purported 5-HT receptor blocking agents antagonize the DS produced by LSD (Kuhn *et al.*, 1978; Winter, 1978) as well as that of mescaline (Browne and Ho, 1975; Winter, 1975b, 1978) and quipazine (White *et al.*, 1977, 1979; Winter, 1979).

The following considerations have led us to examine in some detail the interactions of purported 5-HT receptor blocking agents with the DS produced by LSD. First, an effective antagonism of the LSD-DS by cinanserin, metitepine, methysergide and some related compounds would be difficult to reconcile with the hypothesis that LSD acts by mimicking 5-HT at 5-HT receptor sites in the brain. This is because these agents essentially fail to block the inhibition of firing rate of brain neurons induced by 5-HT (Haigler and Aghajanian, 1974b). The same agents do antagonize 5-HT at sites in which it produces excitation, but LSD also blocks rather than mimicks this 5-HT excitation (Boakes *et al.*, 1970). Second, some of these agents (*i.e.*, cyproheptadine, methysergide and mianserin) have been shown (Colpaert *et al.*, 1979) to exert LSD-like *in vivo* agonist activity in addition to their reported antagonist effects. This finding suggests that the interactions of these purported 5-HT antagonists with LSD may be more complex than is apparent from the available data.

This study presents an extensive analysis of the effects of the putative 5-HT receptor blocking agents metergoline (Beretta *et al.*, 1965), methysergide (Fanchamps *et al.*, 1960), 2-bromo-LSD (Cerletti and Doepfner, 1958), mianserin (Vargaftig *et al.*, 1971), pizotifen (Sicuteri *et al.*, 1966), cyproheptadine (Stone *et al.*, 1961), metitepine (Pelz *et al.*, 1968) and cinanserin (Rubin *et al.*, 1964) in rats trained to discriminate i.p. injections of 0.16 mg/kg of *d*-LSD from saline. Over a wide range of doses, each drug was examined in terms of its ability both to mimic and to antagonize LSD. We also report on the discovery of pirenperone, a newly synthesized compound which the present data identify as a unique LSD-antagonist.

Materials and Methods

Animals. Male Wistar rats weighing 220 ± 10 g at the beginning of the experiment were used. The animals were housed in individual living cages, stored in a continuously illuminated and air-conditioned room (21 ± 1°C; relative humidity 65 ± 5%). Tap water was available freely. Access to dry powdered standard laboratory food was limited to 2 hr a day, as specified below.

A total of 93 rats were used in the experiments described here. The sample of animals participating in any given experiment was selected from the pool of trained rats which were available at that point of time during the 37-month period it took to conduct the study.

Apparatus. Six identical small animal test cages were used as experimental chambers. They were programed by solid-state logic programing equipment and fitted with a house light and two levers. Between the two levers, a food pellet receptacle was mounted 2 cm above the cage floor.

Discrimination training procedure. The drug discrimination procedure used here has been described in detail elsewhere (Colpaert *et al.*, 1976). Daily discrimination training started after habituation to the experimental conditions and initial acquisition of the lever press response. Fifteen minutes before it was placed in the test cage, the rat was injected i.p. with either the 0.16 mg/kg training dose of *d*-LSD tartrate or physiological saline. The injection volume of saline, LSD or any other drug solution was 1 ml/100 g b. wt. Depending on whether the rat was injected with LSD or saline, it obtained food by pressing either the DL or the SL, respectively. After every 10th lever press (FR 10) on the correct lever, a 45-mg food pellet was delivered by a food dispenser. Responses on the incorrect lever (*i.e.*, the SL after LSD and the DL after saline) had no consequences. The lever assignments were DL: left, SL: right in about half of the animals and SL: left, DL: right in the other half. These assignments remained unchanged throughout the study. The number of responses made on either lever before the FRF was obtained (and, thus, when 10 responses were made on the correct lever) was recorded. Fifteen minutes after the rat was put in the experimental chamber, the session was terminated and all correct and incorrect responses made in the course of the 15-min session were recorded. After the session, the animal was returned to its living cage. One hour later it was allowed to feed freely for 2 hr. On week-end days no sessions were run and the animals were given free access to food between 10 A.M. and 12 noon.

Every week, each rat was run once a day on 5 consecutive days. Daily training drug (D) or saline (S) injections were given according to two monthly alternating sequences, *i.e.*, 1) D-S-S-D-S, S-D-D-S-S, S-D-S-D-D, D-S-D-S-D and 2) S-D-D-S-S, D-S-D-S-D, D-S-S-D-D, S-D-S-D-S. Rats whose sequential numbers were odd were run according to one sequence, whereas even-numbered animals were run according to the alternative sequence. This was to control for the olfactory cues which may be deposited by one animal and which may serve as the DS for those animals which are subsequently tested in the same cage (A. Goudie and K. Extance, personal communication).

Discrimination training proceeded for at least 80 sessions (40 D and 40 S sessions) in every rat. Those animals which had always selected the correct liver with FRF ≤ 12 during the last 10 of these 80 sessions were then used in the generalization experiments. In the other animals, training was continued until they reached the same 10 day (FRF ≤ 12) criterion. This criterion was instituted to ensure that, at the outset of the generalization experiments, the apparent response control exerted by the training drug would be as complete as seems reasonably possible.

Stimulus generalization test procedure. Test sessions were run on Wednesdays and Fridays only and the training procedure was continued on the remaining days. On test days, the animal was given the test treatment being studied and was put in the operant chamber at a specified time after the treatment. It was then noted on which of the two levers the animal first totaled 10 responses. This lever is referred to as the "selected lever." Once this choice was established, the rat obtained a first food pellet and subsequent reinforcement was made contingent upon pressing (FR 10) the selected lever.

Testing was postponed to the next scheduled test day if FRF exceeded 16 on either of the 2 most recent training days. Also, test data were discarded and the test condition later retested if the test session was followed by a training session in which FRF exceeded 16. This correction procedure was applied to further increase the reliability of individual test data; incorrect lever selections in trained rats appeared

to typically occur in bursts of one to three sessions so that the correction procedure assisted in avoiding the contamination of test data which may occur during these bursts.

Discrimination control data. Due to the use of a correction procedure in the tests for stimulus generalization, discriminative performance on standard training sessions provided less than perfectly adequate control data against which the test data were to be evaluated. The saline and 0.16 mg/kg LSD (i.p., t = 15 min) training conditions were therefore also tested in the manner described above. These tests were carried out in 70 rats.

LSD, quipazine and mescaline. In order to facilitate quantitative comparisons of the present data with those obtained in previous studies, comparative dose-response studies were carried out on the stimulus generalization with the 0.16 mg/kg training dose of LSD induced by 0.02, 0.04, 0.08 and 0.31 mg/kg of LSD, 0.31 to 5 mg/kg of quipazine maleate and 10 to 40 mg/kg of mescaline sulfate. Selection of doses was based on previous data (Colpaert *et al.*, 1979) obtained in rats which were trained to discriminate 0.16 mg/kg of LSD from saline in the same procedure. The doses used in these and all subsequent experiments belong to the geometrical series 0.0025, 0.005, ..., 10 and 40 mg/kg. Ten rats were used at each dose of all three compounds.

Purported 5-HT antagonists. The purported 5-HT receptor blocking agents being studied were the ergoline derivatives metergoline, methysergide maleate and 2-bromo-LSD, the cinnamyl derivative cinanserin hydrochloride, cyproheptadine hydrochloride and the cyproheptadine-like tricyclic compounds metitepine maleate, mianserin hydrochloride and pizotifen maleate. These eight agents were examined in two different sets of experiments.

To determine their antagonist effects, the antagonists were injected s.c. 60 min before test; this s.c. injection was followed by an i.p. injection of 0.16 mg/kg of LSD at 15 min before test. The doses of antagonists being tested (see figs. 3–5) were selected on the basis of preliminary experimentation in a separate pool of trained rats. Each range extends from doses that were inactive up to doses that decreased response rate by about 40 to 50%. Each dose was tested in seven animals.

For the purpose of determining their agonist (*i.e.*, LSD-mimicking) effects, the antagonists were again injected s.c. at 60 min before test, but the animals were then given an i.p. injection of saline rather than LSD at 15 min before test. The dose ranges being tested here were similar to those tested in the experiments on antagonism, except that cyproheptadine was additionally tested at the 10 mg/kg dose. All doses were again tested in seven rats each.

Before being used for these experiments, the animals were given 1 week of habituation to a double treatment condition; that is, before every daily saline or 0.16 mg/kg LSD injection (i.p., t = 15 min), the animals were always given an additional injection of saline (s.c., t = 60 min). The double treatment condition on training days was continued for the duration of these experiments. Rats were then randomly selected to participate in the tests on agonist or antagonist drug effects.

Pirenperone. Preliminary experimentation with a number of newly synthesized compounds revealed pirenperone (WHO proposed international nonpropriety name of R 47 465) to be a uniquely effective LSD-antagonist; 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2-methyl-4*H*-pyrido [1,2-a] pyrimidin-4-1 has a molecular weight of 393.46 and is shown in figure 1.

Pirenperone was tested in the same manner as described for the purported 5-HT antagonists. The s.c. dose ranges being investigated were 0.0025 to 0.16 mg/kg in the experiments on antagonist effects and 0.01 to 0.63 mg/kg in the experiments on agonist effects. In addition, a third series of experiments was conducted to determine the antagonist effects of orally administered doses (0.0025–0.16 mg/kg) of the compound. This series was carried out in animals which were exposed to a double treatment condition whereby saline at t = 60 min was administered by gavage rather than s.c. The number of rats was seven for each test condition.

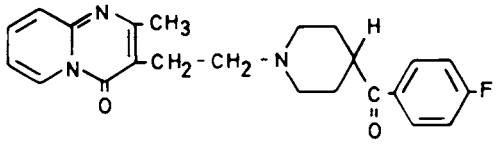

Fig. 1. Chemical structure of pirenperone (molecular formula: $C_{23}H_{24}FN_3O_2$)

Antagonist specificity. A number of other purported receptor blocking agents was also tested in an attempt to delineate the nature of the process(es) through which the LSD-DS might be blocked. The agents being used were the dopamine antagonist haloperidol (0.08 and 0.31 mg/kg), spiperone (0.04 and 0.16 mg/kg) which blocks both dopamine and 5-HT_2 receptors, the adrenergic antagonists phenoxybenzamine hydrochloride (10 and 40 mg/kg) and *dl*-propranolol hydrochloride (2.5 and 10 mg/kg), the histamine antagonist pyrilamine hydrochloride (10 and 40 mg/kg) and the opiate antagonist naloxone hydrochloride (0.63 and 2.5 mg/kg). All six compounds were injected s.c.; tests for possible antagonist effects proceeded as is described above for the purported 5-HT antagonists. Eight animals were used per dose.

Statistical analysis. Response rate data were at all times compared with rates of responding on the most recently preceding saline (training) session; the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956) was used throughout in comparisons of test rates with these control rates. The probability level for rejection of the null hypothesis was .05.

Because the control data indicated correct lever selection to exceed the 0.95 level with both (saline and 0.16 mg/kg LSD) control conditions, no statistical analysis was carried out on lever selection test data.

Results

Discrimination control data. Testing the two standard treatments yielded 95.7 and 97.1% (n = 70) treatment-appropriate lever selections after injection with 0.16 mg/kg of LSD (DL) and saline (SL), respectively. The rate of responding after saline varied between about 1200 and 2000 per 15-min session among different animals. Response rate after 0.16 mg/kg of LSD averaged 97.9% (S.E.M.: 2.8) of the rates observed after saline; this difference was not significant ($P > .05$).

LSD, quipazine and mescaline. After injection of 0.02 mg/kg of LSD, all rats selected the SL, but higher doses induced a dose-dependent increase in the number of rats which generalized the test treatment with the 0.16 mg/kg training dose (fig. 2). The regression line computed for the data plotted in log-linear coordinates intersected with the 50% level of effect at 0.055 mg/kg (ED_{50} value). LSD failed to appreciably affect response rate at any dose lower than 0.16 mg/kg, but did depress rate ($P < .05$) at 0.31 mg/kg.

Quipazine produced dose-dependent stimulus generalization with LSD at doses from 0.63 to 2.5 mg/kg (ED_{50}: 0.84 mg/kg). However, the lowest dose (*i.e.*, 2.5 mg/kg) at which quipazine produced 100% DL selection was associated with a significant rate depressant effect, and rate depression amounted to 43% at 2 times this dose (fig. 2).

Mescaline (10 to 40 mg/kg) similarly engendered dose-dependent generalization with LSD, the ED_{50} being 17.2 mg/kg. One of 10 animals did not respond at 40 mg/kg and rate depression at this dose averaged 60%.

Thus, on a molar basis, LSD was about 12 and 275 times more potent than quipazine and mescaline (expressed as the base), respectively. Note that only LSD produced discriminative control to its full extent in the absence of any detectable effect on response rate.

Purported 5-HT antagonists. Metergoline antagonized (fig. 3, bottom) LSD in a dose-dependent manner at doses ranging from 0.01 to 0.16 mg/kg. At doses in excess of 0.16 mg/

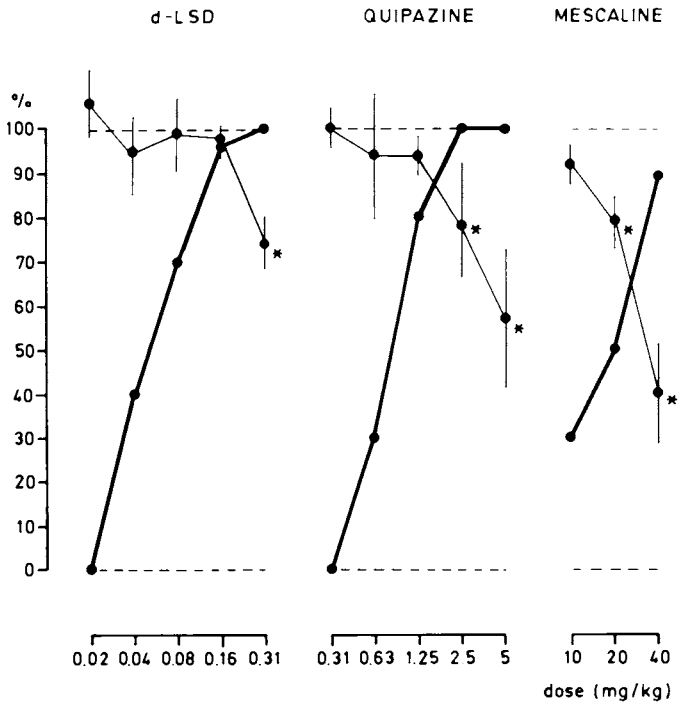

Fig. 2. Agonist effects of LSD, quipazine and mescaline in rats trained to discriminate 0.16 mg/kg of LSD from saline. All drugs were injected i.p. 15 min before test. Each data point is based on 10 observations, except for the 0.16 mg/kg dose of LSD (n = 70). Abscissa: dose of drugs in milligrams per kilogram. The ordinate expresses percentage of rats selecting the DL (bold line) and of response rate (lean). Animals not selecting the DL selected the SL. Response rate (mean ±1 S.E.M.) expresses the number of responses made on a test session as a percentage of responses on the most recently preceding saline session. The asterisk indicates $P < .05$ (two-tailed, Wilcoxon test; Siegel, 1956).

kg, LSD antagonism by metergoline failed to further increase and remained at an apparent ceiling level of approximately 43%. Little LSD-like agonist effects (fig. 3, top) were obtained with metergoline doses up to 0.63 mg/kg; but dose-dependent stimulus generalization did become apparent at 2.5 and 10 mg/kg. Note that metergoline's dose-response curve in antagonizing LSD levels off at doses well below the 2.5 mg/kg dose required to demonstrate agonist activity. In both experiments, reliable rate-depressant effects were obtained at 2.5 and 10 mg/kg, but rate depression with 10 mg/kg was more marked ($P < .05$) in the metergoline-LSD condition than in the metergoline-saline condition. Thus, at this dose, LSD increased metergoline's rate-depressant effects.

The dose-response relationship of 2-bromo-LSD in antagonizing LSD (fig. 3) resembled that of metergoline; pizotifen, cinanserin and metitepine also produced similar effects (fig. 4). Thus, at lower doses, each of these agents antagonized LSD according to a dose-dependent but relatively shallow curve; a ceiling was then reached at higher doses. LSD-like agonist activity was apparent at the highest dose tested of 2-bromo-LSD, pizotifen and cinanserin. LSD increased the rate-depressant effects of 2-bromo-LSD (10 mg/kg), pizotifen (2.5 and 10 mg) and cinanserin (40 mg/kg).

Methysergide failed to exert any LSD antagonist effect at doses from 0.01 to 0.16 mg/kg; 0.63 and 2.5 mg/kg did in part antagonize LSD, but still higher doses again exerted no effect at all (fig. 5). Cyproheptadine and mianserin generated a similar biphasic curve consisting of an initial rise and a subsequent decline of the antagonist effect. Methysergide, cyproheptadine and mianserin also produced dose-dependent stimulus generalization, the ED_{50} value being 6.06, 1.67 and 10.9 mg/kg,

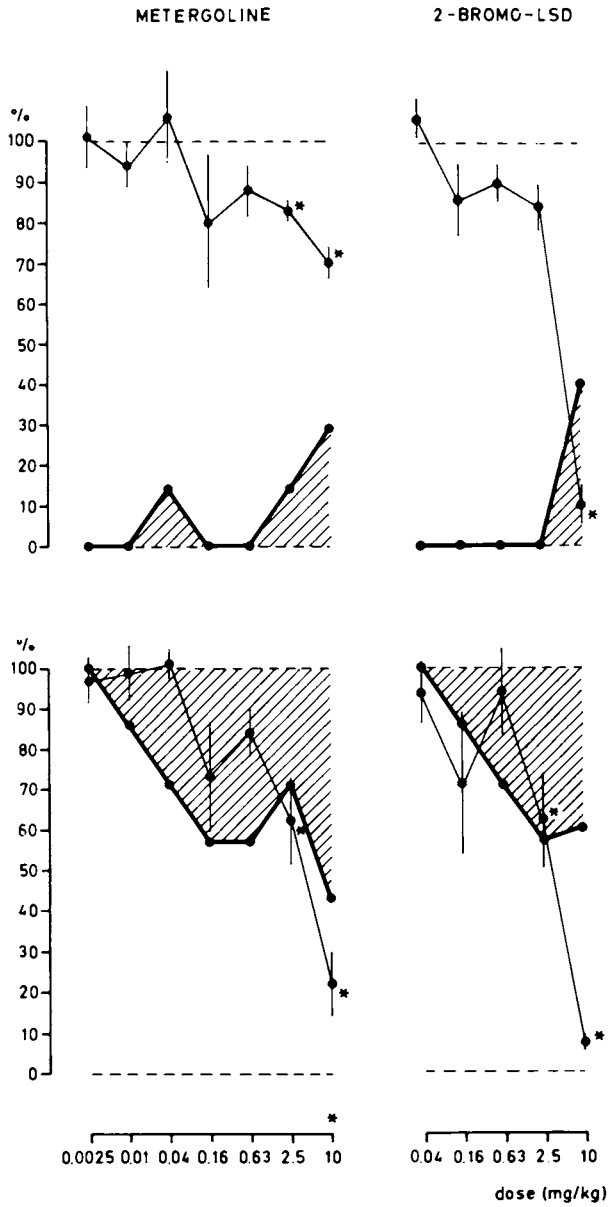

Fig. 3. Agonist and antagonist effects of metergoline and of 2-bromo-LSD in rats trained to discriminate 0.16 mg/kg of LSD from saline. Upper panel: agonist effects of metergoline and 2-bromo-LSD as determined by their ability to produce stimulus generalization with LSD. Lower panel: antagonist effects of the same compounds as determined by their ability to block the DS produced by LSD. In both experiments, the antagonists were injected s.c. 60 min before test and 45 min before the i.p. injection of saline (in agonist experiments, upper panel) or 0.16 mg/kg of LSD (in antagonist experiments, lower panel). N = seven per dose in both experiments. Abscissae: dose of antagonist in milligrams per kilogram. The ordinate expresses the percentage of rats selecting the DL (bold line) and of response rate (lean). Animals not selecting the DL selected the SL. Response rate (mean ±1 S.E.M.) expresses the number of responses made on a test session as a percentage of responses on the most recently preceding saline session; the asterisk indicates $P < .05$ (two-tailed, Wilcoxon test; Siegel, 1956) for the difference between the test result and the most recently preceding saline session. Asterisks just above the abscissa indicate $P < .05$ for the difference in rate between the agonist and antagonist test data.

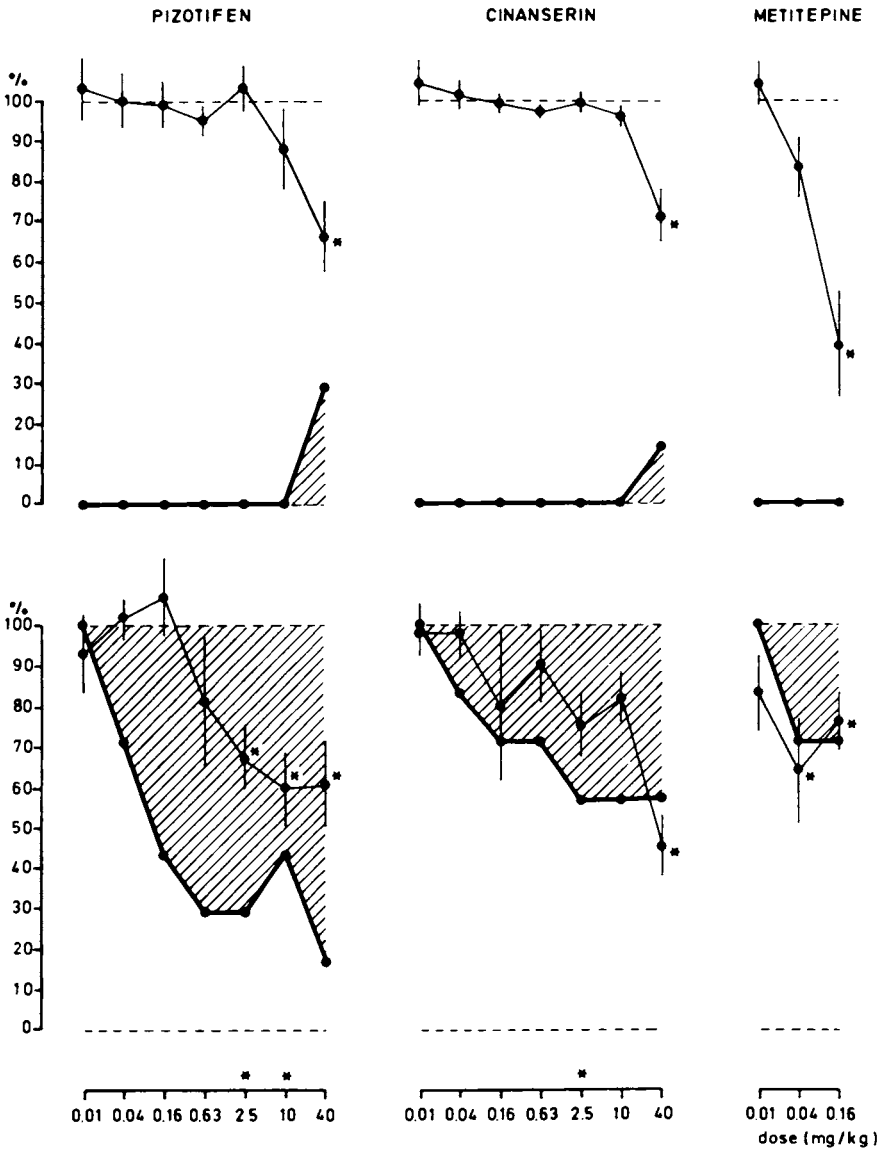

Fig. 4. Agonist and antagonist effects of pizotifen, cinanserin and metitepine in rats trained to discriminate 0.16 mg/kg of LSD from saline. Legend as in figure 3.

respectively. As with metergoline, 2-bromo-LSD, pizotifen and cinanserin, the initial agonist effect of mianserin occurred at a dose which was at ceiling for the compound's antagonist activity. With methysergide and cyproheptadine, however, the lowest dose producing an agonist effect was the same as the lowest dose producing an antagonist effect, and both effects increased in magnitude as the dose was increased. The two effects could thus coexist until agonist activity increased beyond a level of about 43%; at this point, the antagonist dose-response curve of methysergide and cyproheptadine as well as of mianserin suddenly dropped to the 0% level of effect (fig. 5). Finally, 0.16 mg/kg of LSD appeared to increase the rate-depressant effects of methysergide (40 mg/kg) and of mianserin (0.63 mg/kg).

It may be of interest to point out here three general features of the present data. First, no systematic relation is apparent between the LSD-mimicking or blocking activity of drugs and their effects on response rate, suggesting that these two types of effects may be caused by mutually independent drug actions. Second, LSD either increased (methergoline, methysergide, mianserin, pizotifen and cinanserin) or failed to affect (2-bromo-LSD, cyproheptadine and metitepine) the rate-depressant effects of the potential antagonists being studied; however, at no point did LSD reverse rate depression. Third, response rate in the drug discrimination procedure being used here proved to be a useful variable in monitoring the depressant effects of drugs on operant responding (Colpaert *et al.*, 1976; Herling *et al.*, 1980) and in delineating the range of doses within which drugs can be meaningfully investigated.

Pirenperone. After s.c. injection, pirenperone exerted no antagonist effect at 0.0025 mg/kg, but did antagonize LSD in a dose-dependent manner at higher doses (fig. 6). A complete block of the DS produced by 0.16 mg/kg of LSD occurred at 0.16 mg/kg of pirenperone (ED_{50}: 0.02 mg/kg). Pirenperone produced no LSD-like agonist activity whatsoever at the doses (*i.e.*, 0.01 to 0.16 mg/kg) required for LSD antagonism or at a dose 4 times higher than the one at which a complete block of LSD was obtained. Although pirenperone did itself produce reliable rate-depressant effects at doses of 0.04 to 0.63 mg/kg, no rate depression occurred with 0.0025 to 0.16 mg/kg when the pirenperone injection was followed by 0.16 mg/kg of LSD. Thus, 0.16 mg/kg of LSD significantly ($P < .05$) reversed the rate depression of 0.16 mg/kg of pirenperone and the difference fell only short of significance ($P = .085$) at the 0.04 mg/kg dose.

Oral administration of pirenperone before the injection of

0.16 mg/kg of LSD similarly produced dose-dependent antagonism, the ED_{50} being 0.0125 mg/kg. The data on response rate were more erratic than those obtained with s.c. injection but similarly failed to demonstrate any reliable rate-depressant effect (fig. 6, right panel).

Slope of dose-response curves. For the three series of experiments reported above, attempts were made to fit the data points of all compounds by a linear function in log-linear coordinates. This was done by computing the regression equation for these sets of data (method of least sum of squares); the ED_{50} values reported above do in fact indicate the dose at which the regression function intersected the 50% level of agonist or antagonist effect.

The agents whose dose-response data points in mimicking LSD reached at least 50% and could be fitted (χ^2 test; $P > .05$; Siegel, 1956) by a linear function were: LSD, quipazine and mescaline and also mianserin, methysergide and cyproheptadine (fig. 7). The regression line of LSD had a slope of 105.5 and similar slopes were obtained for the generalization gradient of quipazine and mescaline. In contrast, all three purported 5-HT antagonists whose data points conformed to a linear function had slopes that seemed to be shallower than that of LSD. Interestingly, the rank order of the slope of these three drugs was proportional to the magnitude of their antagonist effects at these doses. That is, at doses producing greater than 14% of agonist effect (>1 of seven rats tested), the cumulative antagonist effect was zero with mianserin, but amounted to 29% and 72% with methysergide and cyproheptadine, respectively.

Of the potential antagonists studied here, only the dose-response data points of pirenperone in antagonizing LSD could be fitted by a linear function; all other agents yielded significant ($P < .05$) χ^2 values. The slope of the antagonist function of pirenperone was −56.8 after s.c. injection and −71.4 after oral administration (fig. 7).

Antagonist specificity. Of the six other potential antagonists tested, only haloperidol and spiperone afforded a partial antagonism of 0.16 mg/kg of LSD (data not shown). The antagonism amounted to no more than 25% with spiperone and in the case of both neuroleptics was associated with severe rate-depressant effects. Spiperone and haloperidol also produced a marked attenuation of the percentage of responding on the selected lever. This reduction is characteristic of neuroleptic agents and may reflect a drug-induced loss of the response control by primary reinforcement; this control comes into play

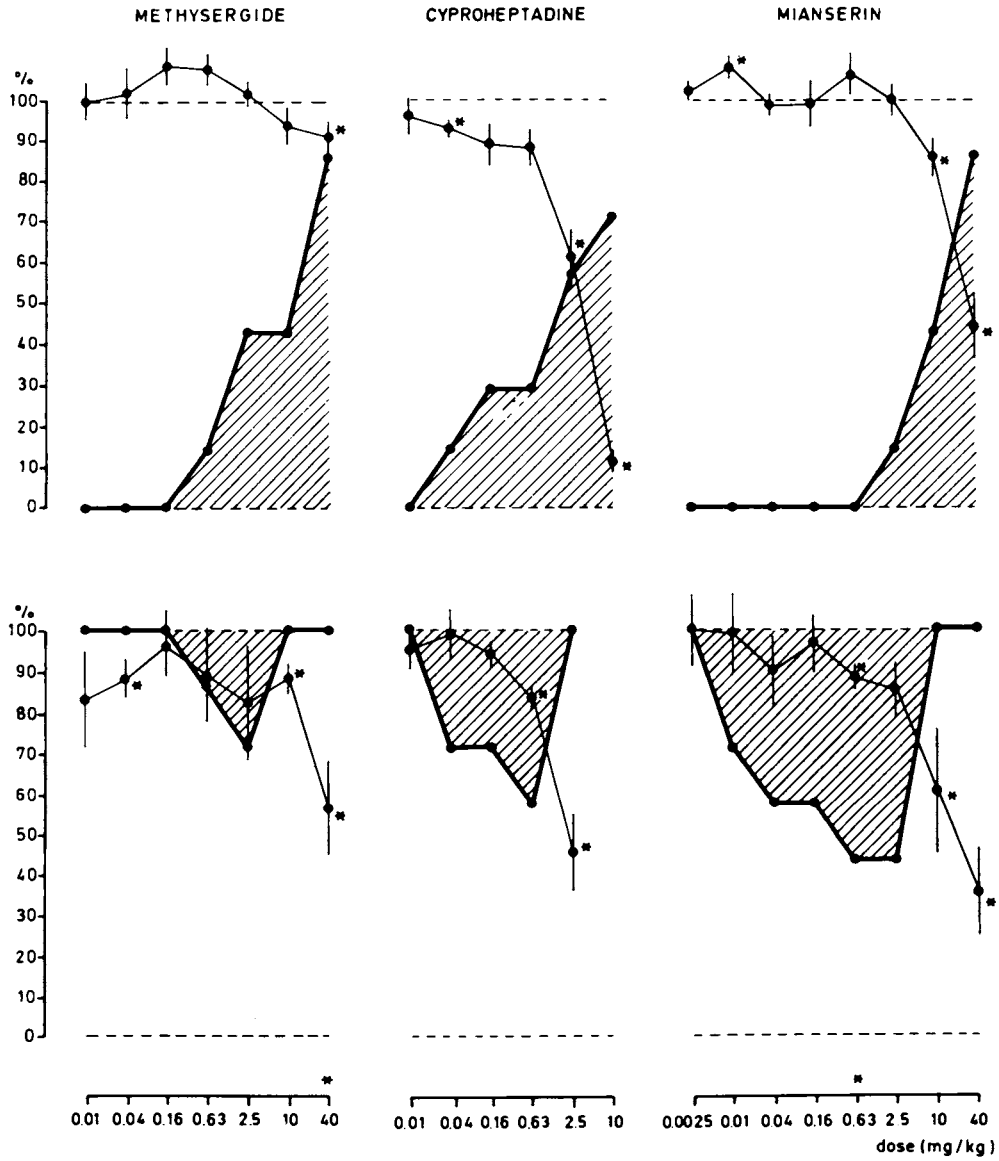

Fig. 5. Agonist and antagonist effects of methysergide, cyproheptadine and mianserin in rats trained to discriminate 0.16 mg/kg of LSD from saline. Legend as in figure 3.

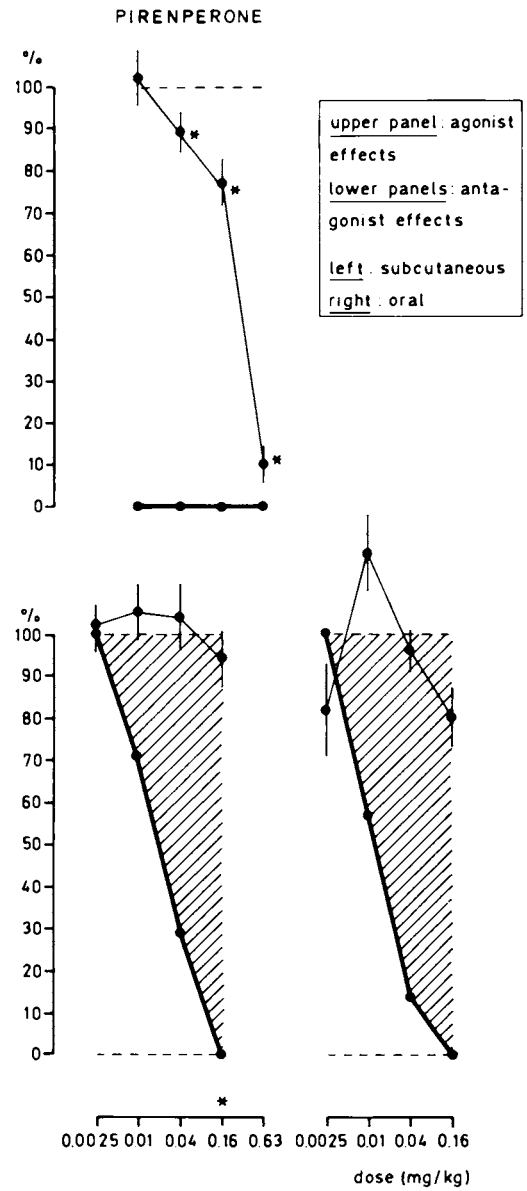

Fig. 6. Agonist and antagonist effects of pirenperone in rats trained to discriminate 0.16 mg/kg of LSD from saline. Legend: see insert and legend to figure 3.

after the first completion of the FR 10 schedule (Colpaert *et al.*, 1977).

Discussion

The main purpose of the experiments reported here was to analyze *in vivo* the pharmacological interactions of putative 5-HT receptor blocking agents with LSD in rats trained to discriminate i.p. injections of 0.16 mg/kg of LSD from saline.

The present findings indicate that metergoline, methysergide, 2-bromo-LSD, mianserin, pizotifen, cyproheptadine, metitepine and cinanserin antagonize the DS properties of LSD; their lowest effective dose varied from only 0.01 to 0.63 mg/kg. However, the LSD antagonism afforded by these agents was only partial in that maximum antagonist activity amounted to no more than 29 to 71%. The dose-response curve of these agents in antagonizing LSD is nonlinear; it typically consists of an initial rise and a subsequent ceiling at a level inferior to 100%. This ceiling differs among agents in its amplitude, in the lowest dose at which it is reached and in the range of doses over which it extends. With methysergide, mianserin and cyproheptadine, the ceiling was followed by a sharp decrease to the zero level of effect, so that their dose-response curves were biphasic. These findings, then, confirm and extend earlier evidence (Kuhn *et al.*, 1978; Winter, 1978) that putative 5-HT antagonists may antagonize the DS properties of LSD. They further indicate, however, that this antagonism is only partial, that the dose-response curve of these agents in antagonizing LSD is invariably curvilinear and that this curve in some cases has a biphasic shape.

The present experiments also provide strong evidence that the purported 5-HT antagonists exert LSD-like agonist activity in addition to their LSD-antagonist effects. That is, these agents mimicked the DS properties of LSD, the extent of stimulus generalization ranging from 14% with cinanserin to as much as 87% with methysergide and mianserin. In fact, 40 mg/kg of the latter two agents exerted as much agonist effect as 40 mg/kg of the known hallucinogen mescaline, and the peak agonist activity of methysergide, mianserin and cyproheptadine was more prominent than their peak antagonist effect. Only metitepine produced no apparent generalization, but the severe behavioral depressant effects of this compound (Pelz *et al.*, 1968) very much limited the range of doses over which it could be examined. The agonist dose-response curves of mianserin, methysergide and cyproheptadine could be fitted by linear functions, but the slope of their curves seemed to be shallower than that of LSD (fig. 7). This phenomenon is characteristic of mixed agonist/antagonist activity (Colpaert, 1978; Holtzman *et al.*, 1977), and may thus constitute additional indirect evidence of antagonist effects.

The following features of the data specify the relation that appears to exist between the antagonist and agonist effects of the purported 5-HT antagonists. First, peak agonist effects invariably occurred at doses in excess of those producing peak antagonist effects. Second, antagonist and agonist effects can coexist at the same dose with all agents, but pronounced (≥43%) agonist activity appears incompatible with pronounced antagonist activity. Third, the slope of the agonist curves of mianserin, methysergide and cyproheptadine was proportional to the magnitude of their antagonist effects. The data also suggest that the inflexion of the antagonist curve which occurred with all eight agents is related to the occurrence of agonist activity; the agonist activity may be subthreshold for generalization at the initial ceiling doses, but did in all cases become directly apparent at higher doses.

Because of the significance of LSD as a psychotropic drug, the failure of the purported 5-HT antagonists and of other receptor blocking agents to effectively antagonize the DS properties of LSD has prompted us to search for a compound that would act as an LSD antagonist under the present experimental conditions. Among the drugs examined here, the LSD-antagonist properties of the newly synthesized compound pirenperone are unique in their combining the following features (fig. 6). The antagonism was produced at low (0.01–0.16 mg/kg) s.c. and oral doses, reached the 100% level of effect and proceeded along a linear and relatively steep gradient. The antagonism occurred in the absence of significant rate depression and pirenperone is the only compound studied here whose intrinsic rate-depression effects were reversed by LSD. Pirenperone was found to produce no LSD-like agonist effects at all, also at doses 4 times in excess of the dose which antagonized LSD by 100%. Effects

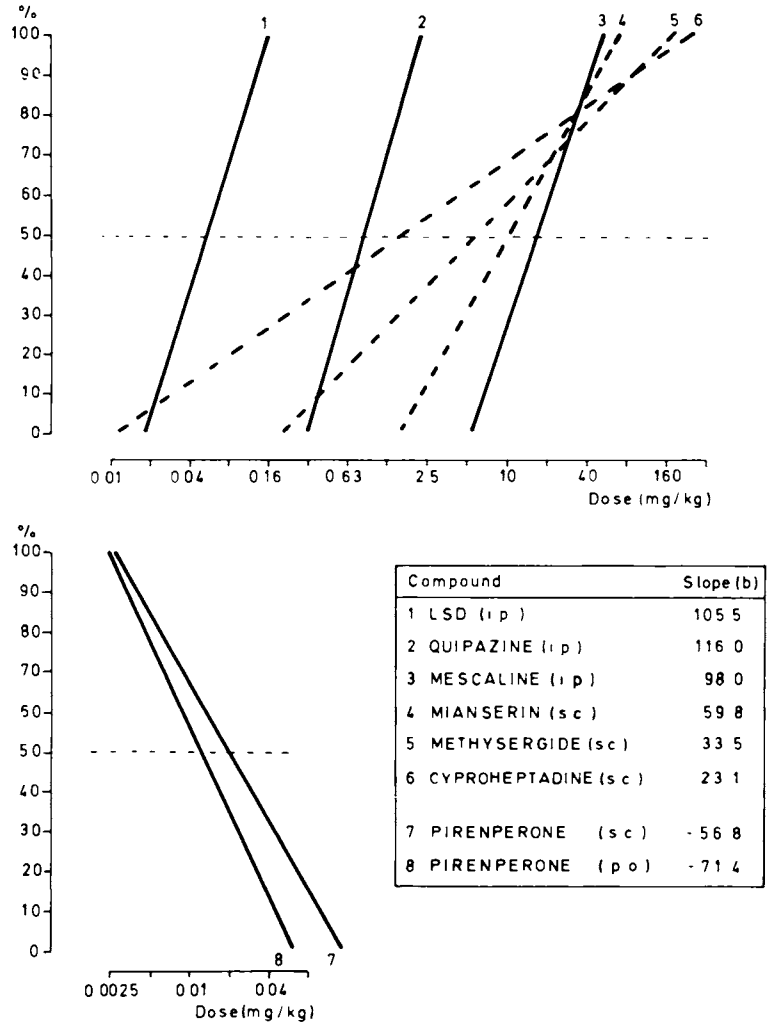

Fig. 7. Regression lines fitting dose-response data points of drugs in mimicking (upper panel) or antagonizing (lower panel) the DS properties of 0.16 mg/kg of LSD in rats trained to discriminate i.p. injections of 0.16 mg/kg of LSD from saline injections; b is the parameter b in the regression equation $y = a + bx$. The functions presented here were computed from data appearing in figures 2 to 6.

suggestive of subthreshold agonist activity were also lacking in that this compound's dose-response curve in antagonizing LSD failed to show the departure from linearity which occurred with the putative 5-HT antagonists.

These findings are consistent with the following theory: 1) the DS properties of 0.16 mg/kg of LSD in the rat are contingent upon agonist effects of LSD at 5-HT receptor sites in the central nervous system; 2) the purported 5-HT receptor blocking agents examined here act complexly as partial and mixed agonists/antagonists at these sites; and 3) pirenperone acts as a pure antagonist at these sites. These assertions possibly can be reconciled with available electrophysiological evidence (Aghajanian, 1976; Haigler and Aghajanian, 1977) by assuming 4) that the neurons on which 0.16 mg/kg of LSD acts to produce a DS are among those in which LSD mimicks the inhibitory neurotransmitter function, but not the excitatory effects of 5-HT in the brain.

The theory would seem to have several implications. First, because of the relation (see opening of the article) between the DS properties of LSD in the rat and its hallucinogenic effects in man, the theory implies that the purported 5-HT antagonists examined here, particularly methysergide, mianserin and cyproheptadine, may produce hallucinations of the indole/phenethylamine type in man. Although street use of some of these agents as substitutes for LSD is commonly known, little systematic evidence to this effect is available. Nonetheless, methysergide has been reported to potentiate the subjective effects of dimethyltryptamine (Sai-Halasz, 1962) and, more significantly, to produce LSD-like hallucinations in man (Abramson and Rolo, 1967; Persyko, 1972). Second, the complex agonist/antagonist interactions of the purported 5-HT antagonists with LSD and, presumably, with 5-HT, would seem to compromise the wide use (Garattini and Samanin, 1978) of these agents as neuropharmacological tools in studies on the behavioral and physiological role of 5-HT in the central nervous system. A third implication is that pirenperone may present a unique tool in investigating the mechanism of action of LSD and, perhaps, the role of 5-HT in central nervous system processes.

Finally, it is important to note that all data reported here were obtained from rats trained to discriminate LSD from saline at an i.p. dose of 0.16 mg/kg given 15 min before test. The route and time of administration and particularly the training dose of LSD of course determined the magnitude of the LSD effect that was being investigated in this study. Training dose largely determines the sensitivity of the drug discrimination paradigm to agonist (Colpaert *et al.*, 1980a, 1980b; Järbe and Rollenhagen, 1978; Shannon and Holtzman, 1979) and antagonist drug effects (Colpaert *et al.*, 1980b; Järbe and Rollenhagen, 1978). It remains to be determined, therefore, to what extent the LSD-like agonist and antagonist effects reported here extend to experiments using LSD training doses other than 0.16 mg/kg.

Acknowledgments

The authors gratefully acknowledge the expert technical assistance of Mr. G. Deneve. Drs. H. Friedli and H. Weidmann (Sandoz AG, Basle, Switzerland) kindly supplied samples of LSD and of 2-bromo-LSD.

References

ABRAMSON, H. A. AND ROLO, A.: Comparison of LSD with methysergide and psilocybin on test subjects. *In* The Use of LSD in Psychotherapy and Alcoholism, ed. by H. A. Abramson, pp. 53–73, Bobbs-Merrill Co. Inc., New York, 1967.

AGHAJANIAN, G. K.: LSD and 2-bromo-LSD: Comparison of effects on serotonergic neurons and on neurons in two serotonergic projection areas, the ventral lateral geniculate and amygdala. Neuropharmacology **15:** 521–528, 1976.

AGHAJANIAN, G. K., FOOTE, W. E. AND SHEARD, M. H.: Action of psychotogenic drugs on single raphe neurons. J. Pharmacol. Exp. Ther. **171:** 178–187, 1970.

AGHAJANIAN, G. K., FOOTE, W. E. AND SHEARD, M. H.: Lysergic acid diethylamine: Sensitive neuronal units in the midbrain raphe. Science (Wash. DC) **161:** 706–708, 1973.

AGHAJANIAN, G. K., HAIGLER, H. J. AND BLOOM, F. E.: Lysergic acid diethylamide and serotonin: Direct actions on serotonin-containing neurons. Life Sci. **17:** 615–622, 1972.

BENNETT, J. L. AND AGHAJANIAN, G. K.: d-LSD binding to brain homogenates: Possible relationship to serotonin receptors. Life Sci. **15:** 1935–1944, 1974.

BERETTA, C., GLÄSSER, A. H., NOBILI, M. B. AND SILVESTRI, R.: Antagonism of 5-hydroxytryptamine-induced bronchospasm in guinea pigs by 8β-carbobenzyloxyaminomethyl-1-dimethyl-10-ergoline. J. Pharm. Pharmacol. **17:** 423–428, 1965.

BOAKES, R. J., BRADLEY, P. B., BRIGGS, I. AND DRAY, A.: Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: A possible neuronal basis for the actions of LSD 25. Br. J. Pharmacol. **40:** 202–218, 1970.

BRAWLEY, P. AND DUFFIELD, J. C.: The pharmacology of hallucinogens. Pharmacol. Rev. **24:** 31–66, 1972.

BROWNE, R. G. AND HO, B. T.: Role of serotonin in the discriminative stimulus properties of mescaline. Pharmacol. Biochem. Behav. **3:** 429–435, 1975.

CAMERON, O. G. AND APPEL, J. B.: A behavioral and pharmacological analysis of some discriminable properties of d-LSD in rats. Psychopharmacologia **33:** 117–134, 1973.

CERLETTI, A. AND DOEPFNER, W.: Comparative study on the serotonin antagonism of amide derivatives of lysergic acid and of ergot alkaloids. J. Pharmacol. Exp. Ther. **122:** 124–136, 1958.

COLPAERT, F. C.: Discriminative stimulus properties of narcotic analgesic drugs. Pharmacol. Biochem. Behav. **9:** 863–887, 1978.

COLPAERT, F. C., NIEMEGEERS, C. J. E. AND JANSSEN, P. A. J.: On the ability of narcotic antagonists to produce the narcotic cue. J. Pharmacol. Exp. Ther. **197:** 180–187, 1976.

COLPAERT, F. C., NIEMEGEERS, C. J. E. AND JANSSEN, P. A. J.: Differential haloperidol effect on two indices of fentanyl-saline discrimination. Psychopharmacology **53:** 169–173, 1977.

COLPAERT, F. C., NIEMEGEERS, C. J. E. AND JANSSEN, P. A. J.: *In vivo* evidence of partial agonist activity exerted by purported 5-hydroxytryptamine antagonists. Eur. J. Pharmacol. **58:** 505–509, 1979.

COLPAERT, F. C., NIEMEGEERS, C. J. E. AND JANSSEN, P. A. J.: Factors regulating drug cue sensitivity: Limits of discriminability and the role of a progressively decreasing training dose in fentanyl-saline discrimination. J. Pharmacol. Exp. Ther. **212:** 474–480, 1980a.

COLPAERT, F. C., NIEMEGEERS, C. J. E. AND JANSSEN, P. A. J.: Factors regulating drug cue sensitivity: The effect of training dose in fentanyl-saline discrimination. Neuropharmacology **19:** 705–713, 1980b.

COLPAERT, F. C. AND ROSECRANS, J. A. (EDITORS): Stimulus Properties of Drugs: Ten Years of Progress, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978.

FANCHAMPS, A., DOEPFNER, W., WEIDMANN, H. AND CERLETTI, A.: Pharmakologische Charakterisierung von Deseril (R), einem Serotonin-Antagonisten. Schweiz. Med. Wochenschr. **90:** 1040–1046, 1960.

GARATTINI, S. AND SAMANIN, R.: Drugs affecting serotonin: A survey. *In* Physiological Regulation and Pharmacological Action, Serotonin in Health and Disease, ed. by W. B. Essman, vol. 2, pp. 247–293, Spectrum Publications, Inc., New York, 1978.

HAIGLER, H. J. AND AGHAJANIAN, G. K.: Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving a serotonergic input. J. Pharmacol. Exp. Ther. **188:** 688–699, 1974a.

HAIGLER, H. J. AND AGHAJANIAN, G. K.: Peripheral serotonin antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotonergic input. J. Neural. Transm. **35:** 257–273, 1974b.

HAIGLER, H. J. AND AGHAJANIAN, G. K.: Serotonin receptors in the brain. Fed. Proc. **36:** 2159–2164, 1977.

HERLING, S., COALE, E. H., VALENTINO, R. J., HEIN, D. W. AND WOODS, J. H.: Narcotic discrimination in pigeons. J. Pharmacol. Exp. Ther. **214:** 139–146, 1980.

HIRSCHHORN, I. D. AND WINTER, J. C.: Mescaline and lysergic acid diethylamide (LSD) as discriminative stimuli. Psychopharmacologia **22:** 64–71, 1971.

HO, B. T., RICHARDS, D. W. III AND CHUTE, D. L. (EDITORS): Drug Discrimination and State Dependent Learning, Academic Press, New York, 1978.

HOLTZMAN, S. G., SHANNON, H. E. AND SCHAEFER, G. J.: Discriminative properties of narcotic antagonists. *In* Discriminative Stimulus Properties of Drugs, ed. by H. Lal, pp. 47–72, Plenum Press, New York, 1977.

JÄRBE, T. U. C. AND ROLLENHAGEN, C.: Morphine as a discriminative cue in gerbils: Drug generalization and antagonism. Psychopharmacology **58:** 271–275, 1978.

KUHN, D. M., WHITE, F. J. AND APPEL, J. B.: Discriminative stimulus properties of hallucinogens: Behavioral assay of drug action. *In* Discriminative Stimulus Properties of Drugs, ed. by H. Lal, pp. 137–154, Plenum Press, New York, 1977.

KUHN, D. M., WHITE, F. J. AND APPEL, J. B.: The discriminative stimulus properties of LSD; Mechanisms of action. Neuropharmacology **17:** 257–263, 1978.

LAL, H. (EDITOR): Discriminative Stimulus Properties of Drugs, Plenum Press, New York, 1977.

PELZ, K., JIRKOWSKY, I., ADLEROVÁ, E., METYŠOVÁ, J. AND PROTIVA, M.: Neurotrope und psychotrope Substanzen XXV. Über die in 8-Stellung durch die Methyl-, tert- buty- methoxy- methylthio- und methansulfonyl-gruppe substituierten 10-(4-methylpiperazine) 10, 11-dihydrobenzo [b, f] thiepin-derivate. Collect. Czech. Chem. Commun. **33:** 1895–1910, 1968.

PEROUTKA, S. J. AND SNYDER, S. H.: Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. Mol. Pharmacol. **16:** 687–699, 1979.

PERSYKO, I.: Psychiatric adverse reactions to methysergide. J. Nerv. Ment. Dis. **154:** 299–301, 1972.

RUBIN, B. J., PIALA, J., BURKE, J. C. AND CRAVER, B. N.: A new and specific serotonin inhibitor (SQ 10,643), 2'(3-dimethylamino-propylthio) cinnamanlide hydrochloride: Antiserotonin activity on uterus and on gastrointestinal, vascular and respiratory systems of animals. Arch. Int. Pharmacodyn. Ther. **152:** 132–143, 1964.

SAI-HALASZ, A.: The effect of antiserotonin on the experimental psychosis induced by dimethyltryptamine. Experientia **18:** 137–138, 1962.

SCHECHTER, M. D. AND ROSECRANS, J. A.: Lysergic acid diethylamide (LSD) as a discriminative cue: Drugs with similar stimulus properties. Psychopharmacologia **26:** 313–316, 1972.

SHANNON, H. AND HOLTZMAN, S. G.: Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rat. Psychopharmacology **61:** 239–244, 1979.

SICUTERI, F., FRANCHI, G., DEL BIANCO, P. L. AND ANSELMI, B.: Antagonism sulla 5-HT istamina e sulla aceticoline di un antiacentinila nuovo. Bull. Soc. Ital. Biol. **47:** 1097–1100, 1966.

SIEGEL, S.: Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill Book Company, New York, 1956.

STONE, S. A., WENGER, H. C., LUDDEN, C. T., STAVORSKI, J. M. AND ROSS, C. A.: Antiserotonin-antihistaminic properties of cyproheptadine. J. Pharmacol. Exp. Ther. **131:** 73–84, 1961.

VARGAFTIG, B. B., COIGNET, J. L., DE VOS, C. J., GRYSEN, H. AND BONTA, I. L.: Mianserin hydrochloride: Peripheral and central effects in relation to antagonism against 5-hydroxytryptamine and tryptamine. Eur. J. Pharmacol. **16:** 336–346, 1971.

WEISSMAN, A.: Generalization of the discriminative stimulus properties of Δ^9-tetrahydrocannabinol to cannabinoids with therapeutic potential. *In* Stimulus Properties of Drugs: Ten Years of Progress, ed. by F. C. Colpaert and J. A. Rosecrans, pp. 99–122, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978.

WHITE, F. J., APPEL, J. B. AND KUHN, D. M.: Discriminative stimulus properties of quipazine: Direct serotonergic mediation. Neuropharmacology **18:** 143–151, 1979.

WHITE, F. J., KUHN, D. M. AND APPEL, J. B.: Discriminative stimulus properties of quipazine. Neuropharmacology **16:** 827–832, 1977.

WINTER, J. C.: The effects of 2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethoxy-4-ethylamphetamine (DOET), d-amphetamine, and cocaine in rats trained with mescaline as a discriminative stimulus. Psychopharmacologia **44:** 29–32, 1975a.

WINTER, J. C.: Blockade of the stimulus properties of mescaline by a serotonin antagonist. Arch. Int. Pharmacodyn. Ther. **214:** 250–253, 1975b.

WINTER, J. C.: Stimulus properties of phenethylamine hallucinogens and lysergic acid diethylamide: The role of 5-hydroxytryptamine. J. Pharmacol. Exp. Ther. **204:** 416–423, 1978.

WINTER, J. C.: Quipazine-induced stimulus control in the rat. Psychopharmacology **60:** 265–269, 1979.

WINTER, J. C.: Effects of phenethylamine derivatives, BL-3912, fenfluramine, and Sch-12679, in rats trained with LSD as a discriminative stimulus. Psychopharmacology **68:** 159–162, 1980.

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