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Interactions Between Serotonergic Agonists and Antagonists in Rats Trained With LSD as a Discriminative Stimulus

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WINTER, J. C. AND R. A. RABIN. *Interactions between serotonergic agonists and antagonists in rats trained with LSD as a discriminative stimulus.* PHARMACOL BIOCHEM BEHAV **30**(3) 617–624, 1988.—Drugs purported to have selective affinities for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors were tested in rats trained with 0.1 mg LSD versus saline. Included were 5-methoxy-dimethyltryptamine (MDMT), 2,5-dimethoxy-4-methyl-amphetamine (DOM), 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), *m*-trifluoromethylphenyl-piperazine (TFMPP), and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU-24969). Tests were then repeated in the presence of either pizotyline or pirenperone. DOM substituted for LSD and both were blocked by pizotyline and pirenperone. MDMT, 8-OH-DPAT, TFMPP, and RU-24969 substituted less completely and were variably affected by the antagonists. An unexpected result was potentiation of the stimulus or disruptive effects of certain doses of 8-OH-DPAT and TFMPP by pizotyline and pirenperone. The present findings suggest more complex interactions between these drugs than has previously been assumed.

Stimulus control LSD 8-OH-DPAT TFMPP RU-24969 5-Methoxy-DMT DOM Pizotyline Pirenperone

THE past two decades have seen drug-induced stimulus control emerge as an exceptionally powerful tool for the characterization of psychoactive drugs. Mescaline, a phenethylamine hallucinogen, and lysergic acid diethylamide (LSD), a hallucinogen of the indoleamine type, were first reported to function as discriminative stimuli in 1971 [21]. It was subsequently demonstrated that the stimulus properties of both classes of hallucinogen are blocked by serotonergic antagonists such as cinanserin, methysergide, cyproheptadine, and pizotyline [3, 25, 38, 49, 50]. Interesting complications of this simple picture of LSD and mescaline as serotonergic agonists were introduced by the finding that many of the drugs presumed to be pure antagonists in fact possess agonistic properties [4,5] and the demonstration that pizotyline, one of the more effective antagonists, can itself function as a discriminative stimulus [35].

Concurrent with the expansion of our knowledge of the stimulus properties of hallucinogens has been the recognition of multiple serotonergic receptors [31, 40, 41, 43]. The original division of serotonin receptors in the central nervous system into 5-HT_1 and 5-HT_2 subtypes by Peroutka and Snyder [41] has since been expanded to include a number of subtypes, two of the more extensively studied being 5-HT_{1A} and 5-HT_{1B}. Thus an opportunity has been provided for the behavioral investigation not just of serotonergic agonists and antagonists but of drugs purported on the basis of radioligand binding data to be specific for one or another receptor subtype.

The functional significance of the various serotonergic receptor subtypes is unclear. A part of this uncertainty is due to a lack of agreement as to the degree of selectivity of any given drug. Because assay temperature and concentration of the radioligand used as well as the ionic strength and composition of the assay buffer all influence the affinity of a drug for a receptor, identical assay conditions must be used to compare relative affinities of drugs at each subtype. The purpose of the present investigation was to assess a group of serotonergically active drugs in terms of (a) their discriminative stimulus properties and interactions with serotonergic antagonists in rats trained with LSD versus saline and (b) their affinities at the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 binding sites as determined under comparable assay conditions. In addition to LSD, the drugs examined were 2,5-dimethoxy-4-methylamphetamine (DOM), 5-methoxy-dimethyltryptamine (MDMT), *m*-trifluoro-methylphenylpiperazine (TFMPP), 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), the piperidinyl indole, RU-24969, and the antagonists, pizotyline and pirenperone.

METHOD

Animals

A group of 20 male Fischer 344 rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. They were housed in pairs under a natural light-dark cycle and allowed free access to water in the home cage.

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Subjects were maintained at 75–80% of their expected free-feeding weight by limiting access to food to 2 hours per day.

Apparatus

Three small animal test chambers (Coulbourn Instruments model E10-10) housed in larger light-proof sound-insulated boxes were used for all experiments. The box contained a house light and exhaust fan. The chamber contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper which delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Operant Procedure

After learning to drink from the dipper, subjects were trained to depress first one and then the other of the two levers. The number of responses for each reinforcement was gradually increased from one to ten and all subsequent training and testing employed a fixed ratio (FR10) schedule of reinforcement. Discrimination training was then begun. Each ten-minute session was preceded by the injection of either LSD (100 microgram/kg) or saline. Following the administration of LSD, every tenth response on the drug appropriate-lever was reinforced. Similarly, responses on the saline-appropriate lever were reinforced following the injection of saline. For half of the subjects, the left lever was designated as the LSD-appropriate lever. During discrimination training, drug and saline were alternated on a daily basis. LSD-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to delivery of the first reinforcer were on the appropriate lever.

After LSD-induced stimulus control was well established, cross tests (tests of generalization) were conducted with a range of doses of LSD, DOM, MDMT, 8-OH-DPAT, TFMPP, and RU-24969. In this way a dose response relationship was obtained for each drug. The same range of doses was then examined in the presence of either pizotyline or pirenperone. Cross tests were conducted once per week in each animal so long as performance during the remainder of the week did not fall below a criterion of 83% correct responding. In general, tests were equally divided between Thursday and Friday sessions. During cross tests, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the LSD-appropriate lever. Agonists and antagonists were administered 15 minutes and 60 minutes, respectively, before testing. All comparisons of data were by means of individual applications of Wilcoxon's signed ranks test. Differences were considered to be significant if they would be expected to arise by random sampling alone with a probablity less than 0.05.

5-HT Binding Assay

Rats were sacrificed by decapitation and the frontal cortices were rapidly removed. Tissue was homogenized (Brinkmann Polytron) in 50 mM Tris (pH 7.4), and the homogenate was centrifuged at 30,000×g for 15 minutes at 4°C. The resulting pellets were resuspended in the Tris buffer, and the samples were incubated at 37°C for 15 minutes to remove endogenous 5-HT [37]. The samples were then centrifuged at 30,000×g for 15 minutes. The resulting pellets

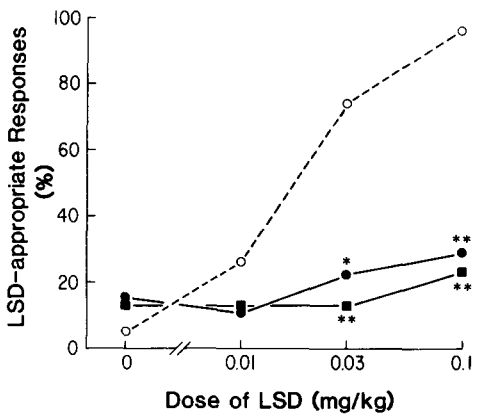

FIG. 1. The effects of LSD alone (open circles) and in the presence of either pizotyline (closed circles; 10 mg/kg) or pirenperone (closed squares; 0.16 mg/kg) in rats trained with LSD (0.1 mg/kg) as a discriminative stimulus. LSD and the antagonists were injected 15 min and 60 min, respectively, before testing. The values given at the zero dose level are the effects of saline, pizotyline, and pirenperone when given alone. Each is the mean of two determinations in each of 10 subjects. All other points represent the mean of one determination in each of 10 animals. *Ordinate:* Mean percentage of responses on the LSD-appropriate lever. *Abscissa:* Dose plotted on a log scale. Statistical comparisons are with the value for LSD alone; $*p<0.05$; $**p<0.01$.

were resuspended in the Tris buffer and again centrifuged at 30,000×g for 15 minutes. The final pellet was resuspended (40 mg wet weight of tissue/ml for (^{3}H)5-HT binding; 5.3 mg/ml for (^{3}H)ketanserin binding) in 50 mM Tris (pH 7.4) containing 2.5 mM $MgCl_2$, 5.7 mM ascorbate, and 10 micromolar pargyline.

(^{3}H)5-HT binding to frontal cortical membranes was measured by a modification of the method of Sills *et al.* [45]. Binding of (^{3}H)5-HT was carried out in a final volume of 450 microliter consisting of 50 mM Tris (pH 7.4), 2.5 mM $MgCl_2$, 5.7 mM ascorbate, 0.1 mM GTP, 10 micromolar pargyline, (^{3}H)5-HT (specific activity 19.2–21.8 Ci/mmole; New England Nuclear) and various concentrations of unlabeled drug. Incubations were started by the addition of 100 microliter of tissue and were carried out for 24 minutes at 30°C. (^{3}H)Ketanserin (61.8 Ci/mmole; New England Nuclear) binding to frontal cortical membranes was carried out under similar conditions except that the assay volume was 1 ml and the incubation, which was initiated with 750 microliter of tissue, was carried out for 30 min at 30°C. The incubations were terminated by the addition of 5 ml cold 50 mM Tris (pH 7.4), and the samples rapidly filtered by vacuum filtration through Whatman GF/B glass fiber filters. Filters were rinsed twice with 5 ml of cold 50 mM Tris buffer, and radioactivity was measured by liquid scintillation spectrophotometry (30% efficiency) after incubating the filters in Budget-Solve scintillation cocktail (RPI) overnight. Specific binding of [^{3}H]5-HT was defined as the difference in the amount of radioactivity bound in the absence and presence of 10 micromolar unlabeled 5-HT. Specific binding of (^{3}H)ketanserin was defined with 1 micromolar unlabeled methysergide. Data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier Biosoft). A partial F-test was used to determine whether a one site or two site model best fit the data [8,36].

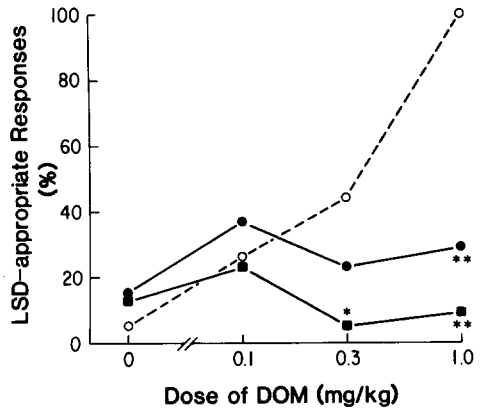

FIG. 2. The effects of DOM alone (open circles) and in the presence of either pizotyline (closed circles) or pirenperone (closed squares) in rats trained with LSD as a discriminative stimulus. DOM was injected 15 min before testing. All other details are as in Fig. 1.

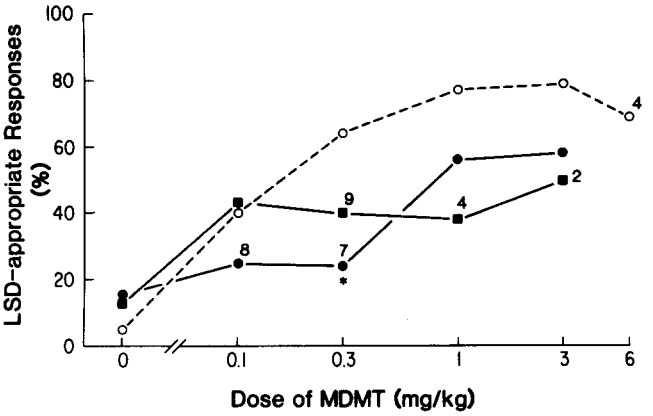

FIG. 3. The effects of MDMT alone (open circles) and in the presence of either pizotyline (closed circles) or pirenperone (closed squares) in rats trained with LSD as a discriminative stimulus. MDMT was injected 15 min before testing. Numbers adjacent to data points indicate the number of animals which completed the session. All other details are as in Fig. 1.

Drugs

(+)-Lysergic acid diethylamide (+)-tartrate (LSD) and racemic 2,5-dimethoxy-4-methylamphetamine (DOM) were provided by the National Institute on Drug Abuse, Rockville, MD. *m*-Trifluoromethylphenylpiperazine (TFMPP) was purchased from Aldrich Chemical Co., Milwaukee, WI. 5-Methoxy-N-dimethyltryptamine oxalate (MDMT) and racemic 8-hydroxy-2-(di-*n*-propylamino) tetralin HBr (8-OH-DPAT) were purchased from Research Biochemicals Inc., Wayland, MA. 5-Methoxy-3-(1,2,3,6-tetrahydro-4-pyrindinyl-1H-indole succinate (RU-24969) was generously provided by Roussel UCLAF, Romainville, France. Pizotyline maleate (BC-105, pizotifen) and pirenperone (R 47 465) were gifts from Sandoz Pharmaceuticals, East Hanover, NJ, and Janssen Pharmaceutica Research Laboratories, Beerse, Belgium, respectively. All drugs were dissolved in saline and injected IP in a constant volume of 1 ml/kg of body weight.

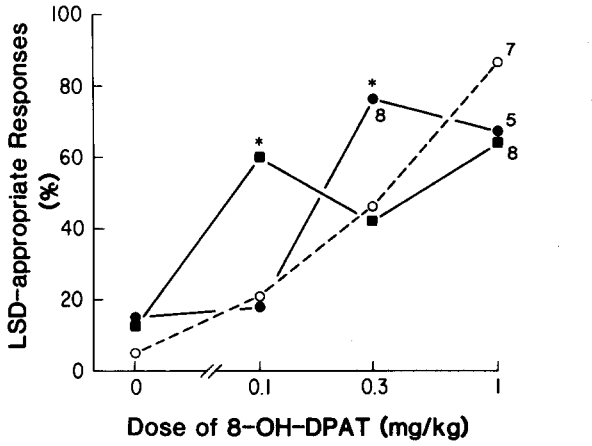

FIG. 4. The effects of 8-OH-DPAT alone (open circles) and in the presence of either pizotyline (closed circles) or pirenperone (closed squares) in rats trained with LSD as a discriminative stimulus. 8-OH-DPAT was injected 15 min before testing. Numbers adjacent to data points indicate the number of animals which completed the session. All other details are as in Fig. 1.

RESULTS

Figures 1 through 6 show the dose-response relationships for LSD, DOM, MDMT, 8-OH-DPAT, TFMPP, and RU-24969, respectively, in rats trained with LSD as a discriminative stimulus. Also seen in the figures are the effects of the same range of doses of each drug following pretreatment with either pizotyline (closed circles) or pirenperone (closed squares). In comparison with the saline value, no significant increase in LSD-appropriate responding occurred following the injection of either pizotyline (15%) or pirenperone (13%).

LSD (Fig. 1) and DOM (Fig. 2) yielded dose-related increases in the percentage of LSD-appropriate responses and each was significantly antagonized by both pizotyline and pirenperone. At a dose of 1.0 mg/kg, DOM substituted completely for LSD. The data shown in Figs. 3 through 6 are more complex.

The extent of substitution of MDMT, 8-OH-DPAT, and TFMPP for LSD was less complete than that of DOM. Maximum values were 79%, 86%, and 60%, respectively. At a dose of 1 mg/kg of RU-24969, 80% of the responses were on the LSD-appropriate lever but only 2 of 10 animals completed the test sessions. In addition, none of this group of drugs was antagonized by pizotyline and pirenperone to the same degree as were DOM and LSD. Indeed, the antagonists significantly potentiated the effects of some doses of MDMT, 8-OH-DPAT, and TFMPP. The potentiation was seen both in terms of the LSD-generalization and with respect to the number of animals completing the sessions. For example, in Fig. 3 the combination of pirenperone with MDMT caused a general trend toward diminished LSD-appropriate responding (antagonism) but a sharp decrease as well in the number of completed sessions (potentiation). The data for 8-OH-DPAT, TFMPP, and RU-24969 (Figs. 4 through 6) provide no evidence of antagonism by either pizotyline or pirenperone. On the contrary, some doses of 8-OH-DPAT and TFMPP substituted more completely for LSD when they were given in combination with an antagonist.

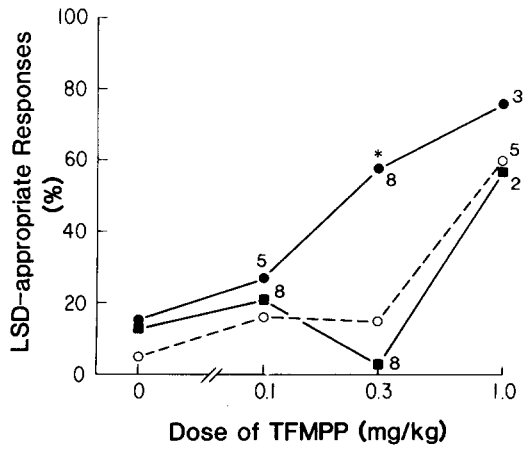

FIG. 5. The effects of TFMPP alone (open circles) and in the presence of either pizotyline (closed circles) or pirenperone (closed squares) in rats trained with LSD as a discriminative stimulus. TFMPP was injected 15 min before testing. Numbers adjacent to data points indicate the number of animals which completed the session. All other details are as in Fig. 1.

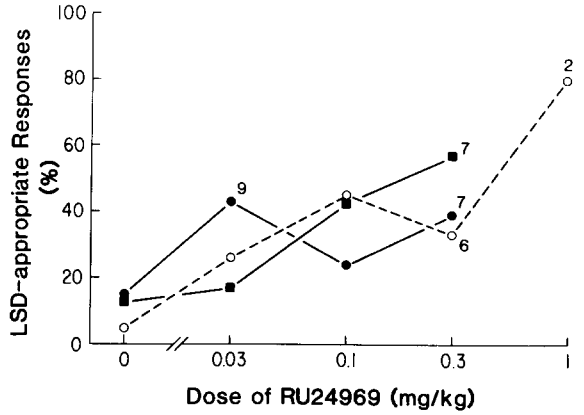

FIG. 6. The effects of RU-24969 alone (open circles) and in the presence of either pizotyline (closed circles) or pirenperone (closed squares) in rats trained with LSD as a discriminative stimulus. RU-24969 was injected 15 min before testing. Numbers adjacent to data points indicate the number of animals which completed the session. All other details are as in Fig. 1.

TABLE 1

DISSOCIATION CONSTANTS FOR THE 5-HT_{1A}, 5-HT_{1B}, AND 5-HT_2 BINDING SITES

	5-HT_1		5-HT_2
	K_H (nM)	K_L (nM)	K_D (nM)
RU-24969	16.8 (B)	960	1,448
	(6–48)	(208–4,432)	(516–4,061)
8-OH-DPAT	19.6 (A)	4,912	6,721
	(4–96)	(1,568–18,388)	(3,868–11,679)
TFMPP	9.97 (B)	1,568	345
	(3–30)	(467–5,260)	(207–575)
MDMT	15.6 (B)	1,239	1,001
	(7–37)	(369–4,155)	(697–1,437)
Pizotyline	13.0 (B)	2,641	1.21
	(6–29)	(758–9,204)	(0.3–6.4)
Pirenperone	37.6 (A)	8,477	0.746
	(17–84)	(242–297,318)	(0.51–1.09)
DOM	IC_{50} >10^{-4}		577
			(442–754)
LSD	6.69 (A,B)	586	9.83
	(4.6–10.6)	(356–963)	(7.5–12.9)

Each value is the geometric mean of 3 to 5 determinations. The 95% confidence interval is given in parentheses. Identification of the high-affinity 5-HT_1 binding site as either 5-HT_{1A} or 5-HT_{1B} is based on the data from Fig. 7. For these experiments, 15 nM (^{3}H)5-HT and 1 nM (^{3}H)ketanserin were used.

The interaction of the above drugs with the various serotonergic receptors was characterized using (^{3}H) ketanserin to measure binding to the 5-HT_2 receptor and (^{3}H)5-HT to measure binding to the 5-HT_1 receptor. Unlike previous studies, identical assay conditions were used for both radioligands. In the presence of 0.1 mM GTP a one-site model best described equilibrium saturation binding of (^{3}H)ketanserin in frontal cortex; the equilibrium dissociation constant (K_D) for (^{3}H)ketanserin was 465 pM(95% confidence limits of 297–727 pM), the density of binding sites (B_{max}) was 225±14.4 fmoles/mg protein and the Hill coefficient was 1.03±0.017 (N=4). Similarly, a one-site model best fit the data from competition experiments of (^{3}H)ketanserin and various serotonergic drugs (Table 1).

Analysis of equilibrium saturation binding of (^{3}H)5-HT in frontal cortical tissue indicated a one-site model also best described the data when 0.1 mM GTP was included in the assay; K_D was 2.72 nM (95% confidence limits of 1.47–5.04 nM), B_{max} was 207±20.2 fmoles/mg protein and Hill coefficient was 1.03±0.04 (N=4). However with the possible ex-

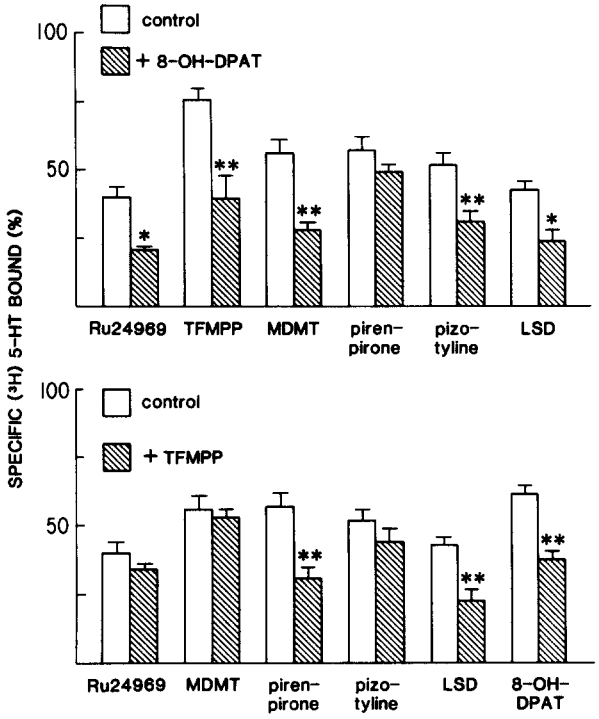

FIG. 7. Selectivity of various serotonergic drugs for the 5-HT_{1A} and the 5-HT_{1B} binding sites. Frontal cortical membranes were incubated as described in the Method section in the presence of 15 nM (^{3}H)5-HT and various serotonergic drugs alone (i.e., control; open bars) or with either 200 nM 8-OH-DPAT (top figure) or 100 nM TFMPP (bottom figure). In these experiments 100 nM RU-24969, 100 nM TFMPP, 100 nM MDMT, 3.3 micromolar pirenperone, 3.3 micromolar pizotyline, 100 nM LSD or 200 nM 8-OH-DPAT were used. Data, which are expressed as percent specific (^{3}H)5-HT bound in the absence of unlabeled drug, are plotted as mean±SEM (N=3–5).

ception of DOM, which had very low affinity for the 5-HT_1 receptor, a two-site model best fit the data from competition experiments of (^{3}H)5-HT and various serotonergic drugs (Table 1). The density of high and low affinity sites was approximately equal for RU-24969, 8-OH-DPAT, TFMPP, and MDMT while the high affinity site comprised 70%, 26%, and 29% of the specific (^{3}H)5-HT binding sites for LSD, pizotyline, and pirenpirone respectively.

Characterization of the high affinity sites as either 5-HT_{1A} or 5-HT_{1B} was made by measuring the ability of the various drugs to inhibit (^{3}H)5-HT binding in the presence of selective 5-HT_{1A} and 5-HT_{1B} drugs. For these experiments a concentration of unlabeled drug that bound primarily to the high affinity site based on the data from competition studies (Table 1) was used. In the presence of 200 nM 8-OH-DPAT, which is highly selective for the 5-HT_{1A} binding site [18, 19, 34], a significant inhibition of (^{3}H)5-HT binding was observed with RU24969, TFMPP, MDMT, pizotyline, and LSD (Fig. 7). Because only binding to the 5-HT_{1B} binding site would be observed in the presence of 200 nM 8-OH-DPAT, the high affinity binding site for these drugs appears to be the 5-HT_{1B} receptor. The data also suggest that the high affinity binding site for pirenperone is the 5-HT_{1A} receptor. These conclusions were tested by repeating the above experiment, but with 100 nM TFMPP so that only binding to the 5-HT_{1A} receptor would be observed. In the presence of TFMPP, significant inhibition of (^{3}H)5-HT binding was only observed with pirenperone, 8-OH-DPAT, and LSD. These data indicate that the high affinity binding site for these drugs is the 5-HT_{1A} receptor. The ability of LSD to cause comparable inhibition of (^{3}H)5-HT binding in the presence of both TFMPP and 8-OH-DPAT indicates LSD binds with comparable high affinity to both 5-HT_1 receptor subtypes.

DISCUSSION

It is generally accepted that the indoles, LSD and MDMT, as well as DOM, a phenethylamine, act at least in part via serotonergic receptors. The pharmacological profiles are less complete for the more recently discovered drugs, 8-OH-DPAT [1,22] RU-24969 [9, 17, 33], and TFMPP [10, 30, 39], but each displays serotonergic activity in one or more test systems. Estimates of the affinities of these drugs and of the serotonergic antagonists, pizotyline and pirenperone, at the various 5-HT receptor subtypes and the role which each subtype plays in behavioral effects remain uncertain. Potentially significant variables include species and brain areas from which the receptors are derived, the radioligand employed, and the specific conditions of the assay [26]. Given these uncertainties, any conclusions regarding the receptor-mediated events which underlie drug-induced stimulus control by serotonergic agents must at this time be somewhat tentative.

With respect to 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors there is general agreement that LSD is nonselective, displaying nanomolar affinities at each (Table 1; [28,41]). However, despite having comparable affinities for these receptors, LSD-induced stimulus control appears dependent only upon the 5-HT_2 site. Primary evidence in support of this conclusion is the complete block of the LSD cue by pirenperone (Fig. 1; [5]), a drug which has high affinity for the 5-HT_2 receptor and significantly lower affinity at the 5-HT_1 site (Table 1; [29]) and by pizotyline whose affinity for the 5-HT_2 receptor is at least 10 times greater than for 5-HT_1 (Table 1; [27,29]). The affinities of pirenperone and pizotyline for the 5-HT_2 site are comparable but they have opposite selectivity at the 5-HT_{1A} and 5-HT_{1B} sites. However, it should be noted as well that pizotyline has nanomolar affinity for histamine$_1$ receptors and for the muscarinic cholinergic receptor [27]. Similarly, pirenperone, while selective with respect to the serotonin receptor types has high affinity for alpha$_1$ and alpha$_2$ adrenoreceptors and for dopamine receptors [27].

The notion that drugs may function as compound stimuli with each element of the stimulus complex reflecting a distinct pharmacological receptor [49] is perhaps relevant to the present data. With respect to LSD, the plausibility of such an hypothesis is demonstrated by an earlier report from our laboratory [50]. In evaluating the stimulus properties of LSD and para-methoxyamphetamine (PMA) it was found that pizotyline did not antagonize stimulus control by PMA when it was trained versus saline. However, in rats trained with LSD, PMA produced a maximum of 75% LSD-appropriate responding and this effect was completely blocked by pizotyline. The conclusion drawn was that PMA-induced stimulus control does not depend upon activation of pizotyline-sensitive serotonergic receptors but that PMA does possess some LSD-like effects which are evident only in animals trained with LSD. Generalizing from this finding, we may assume that any drug with activity at multiple receptors may differ in its apparent stimulus properties depending

upon whether the drug is trained directly or is tested in subjects trained with drugs which may share certain elements of its properties.

For purposes of interpreting the present data, we will assume that LSD functions as a compound stimulus, the most salient elements of which is an action at 5-HT_2 receptors. In addition however, a second element of the LSD stimulus will be assumed to be mediated by occupation of 5-HT_1 receptors. The behavioral consequences of the occupation of 5-HT_1 receptors by LSD will be apparent in drug discrimination experiments only under selected conditions.

The dose-response relationship for LSD (Fig. 1) in rats trained with 0.1 mg/kg LSD versus saline and the effects of pizotyline and pirenperone upon it were as expected from previous studies [5, 38, 48, 54]. Neither of the antagonists had significant agonistic activity. With respect to pizotyline, this result is not incompatible with the findings of Colpaert *et al.* [5] who observed a maximum of 30% LSD-appropriate responding but at a dose of pizotyline of 40 mg/kg; four times greater than that shown in Fig. 1. The present data indicate that at doses equally effective against the LSD cue, pizotyline and pirenperone do not differ in their agonistic properties. In view of this it seems premature to conclude that pirenperone is inherently preferable to pizotyline as an antagonist of the stimulus properties of serotonergic agents. If a choice is to be made between them, one may wish to consider the overall pharmacological activity and receptor affinities of the two agents. It should be noted however that Nielsen *et al.* [38] observed "variable effects" with pizotyline when a higher training dose (0.16 mg/kg) of LSD was used.

The data of Fig. 2 suggest complete generalization of the LSD cue to DOM and complete block of DOM by both pizotyline and pirenperone. These results were not unexpected in view of previous reports that DOM generalizes to LSD [16] and that the direct stimulus properties of the former are antagonized by pizotyline [16] and by pirenperone [46]. Likewise the results are compatible with receptor binding data which indicate a negligible affinity of DOM at 5-HT_1 sites (Table 1, [44]).

Although MDMT is widely regarded as an LSD-like hallucinogenic agent and a number of workers have observed generalization of the LSD cue to MDMT [12, 15, 42, 49], there is reason to believe that MDMT differs somewhat from LSD in its mode of action. Like LSD, it has appreciable affinity for both 5-HT_1 and 5-HT_2 receptors but, with respect to 5-HT_{1A} and 5-HT_{1B} subtypes, MDMT has, unlike LSD, an affinity ratio significantly different from unity. Sills *et al.* [45] found MDMT to be about 50-fold selective for the 5-HT_{1A} site. In contrast, the data of Table 1 indicate a higher affinity for the 5-HT_{1B} subtype.

The data of Fig. 3 are suggestive of less than complete substitution of MDMT for LSD although it must be granted that the degree of LSD-appropriate responding at doses of MDMT of 1 and 3 mg/kg is not significantly different from that following the training dose of LSD. More convincing of a true difference between LSD and MDMT is the relative lack of efficacy of pirenperone and pizotyline in blocking the generalization. Although the trends are certainly suggestive of antagonism by both drugs, only one of six individual points at doses of 0.3 to 3 mg/kg of MDMT reached statistical significance. Furthermore, the reduction in the number of subjects whose response rates were decreased below criterion value by the combination of pirenperone and MDMT contrasts clearly with the interaction between either LSD (Fig. 1) or DOM (Fig. 2).

The greater degree of antagonism of MDMT by pizotyline at an MDMT dose of 0.3 as compared with higher MDMT doses is in general agreement with the results of Young *et al.* [53] who trained rats with either 1.5 mg/kg or 3.0 mg/kg MDMT and were able to block completely only the lower training dose with pizotyline. In contrast, Glennon *et al.* [16] observed complete antagonism of the generalization of DOM to MDMT, a finding explicable on the basis that DOM is more selective with respect to 5-HT_2 receptors than is LSD. Thus generalization of DOM to MDMT might involve only 5-HT_2 receptors while generalization of LSD to MDMT would include 5-HT_1 receptors as well.

In contrast with LSD, the affinity of 8-OH-DPAT is several hundred-fold greater at the 5-HT_1 receptor than at the 5-HT_2 receptor (Table 1; [6, 18, 19]). With respect to the 5-HT_1 receptor subtypes, 8-OH-DPAT appears to be the most selective of those drugs so far tested. In preparations derived from rat frontal cortex, 8-OH-DPAT has an affinity at 5-HT_{1A} which is at least 200 times greater than that at 5-HT_{1B} (Table 1; [18, 19, 23, 34]). Given the clear differences between 8-OH-DPAT and LSD in terms of their binding properties, the degree to which the LSD stimulus generalized to 8-OH-DPAT was unexpected (Fig. 4). Furthermore, in rats trained with a 0.2 mg/kg dose of 8-OH-DPAT versus saline, the maximum degree of genralization to DOM and to MDMT was 23% and 38%, respectively [11]. The present observation that neither pirenperone nor pizotyline antagonized 8-OH-DPAT in LSD-trained animals is in agreement with Glennon's finding that neither ketanserin nor spiperone blocks 8-OH-DPAT-induced stimulus control [11]. Once again, the simplest explanation of these data is that LSD produces effects on 5-HT_1 receptors which are apparent in drug discrimination studies only when drugs active at 5-HT_1 receptors are tested.

The data of Fig. 5 indicate that TFMPP is able to mimic LSD to a limited extent. The maximum degree of generalization was 60% at a dose of 1.0 mg/kg. However, only five of ten rats completed the test. Partial generalization between LSD and TFMPP appears to be symmetrical in that animals trained with TFMPP gave approximately 50% TFMPP-appropriate responses when tested with LSD [7,30]. In line with the suggestion that DOM produces a somewhat less complex discriminative stimulus than does LSD, Glennon *et al.* [16] observed a maximum generalization of DOM to TFMPP of only 28%.

The apparent potentiation of the LSD-like stimulus properties of TFMPP by pizotyline was completely unexpected. Earlier studies in which TFMPP was directly trained found no antagonism with ketanserin [14], tetrahydrotrazodone, an effective antagonist of the DOM cue [13], pizotyline, or pirenperone, and only a modest degree of antagonism by metergoline and spiperone [7].

In Fig. 6 it is seen that LSD generalizes to RU-24969 to only a limited extent. This is as would be expected from the earlier observation that DOM does not generalize to RU-24969 [16]. However, two groups have reported that in rats trained with TFMPP, there is generalization to RU-24969 [7,14]. This is in keeping with the data of Table 1 which indicate that the two drugs have comparable affinities for the 5-HT_{1B} site. We are unaware of attempts to train RU-24969

directly or to antagonize its discriminative effects. The data of Fig. 6 provide no evidence of blockade by either pirenperone or by pizotyline.

Although TFMPP and RU-24969 are often reputed to be agonists at 5-HT_{1B} receptors, there is considerable uncertainty regarding their selectivity. Based on earlier data [24,32], Glennon [12] recently estimated that TFMPP has a 3- to 18-fold greater affinity at 5-HT_1 sites as compared with 5-HT_2 receptors. This is in agreement with the data of Table 1 which indicates 35-fold selectivity. With respect to 5-HT_{1A} and 5-HT_{1B} subtypes, the data of Table 1 suggest that TFMPP has at least 100-fold higher affinity at the latter site. Others have concluded that TFMPP has a 4-fold [18,19] to 67-fold [45] greater affinity for 5-HT_{1B}. In contrast with LSD, the affinity of RU-24969 is at least several hundred times greater at 5-HT_1 than at 5-HT_2 receptors [6, 18, 19, 23]. With respect to 5-HT_{1A} and 5-HT_{1B} subtypes, the present data (Table 1) indicate that RU-24969 is selective for the 5-HT_{1B} site. This is in agreement with earlier estimates of selectivity ranging from about 2-fold [18, 19, 23] to 67-fold [45]. In addition, Heuring *et al.* [20] have recently presented evidence that RU-24969 has significant interactions at non-5-HT_{1A} sites. These sites have not as yet been pharmacologically characterized.

The present data confirm and extend many previous observations regarding the generalization of LSD to other serotonergically-active drugs. In addition, they provide evidence of an unexpected interaction with the serotonergic antagonists, pizotyline and pirenperone.

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