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Complex stimulus properties of LSD: a drug discrimination study with α_2-adrenoceptor agonists and antagonists

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Abstract The influence of several α_2-adrenergic agents on the discriminative stimulus (DS) properties of lysergic acid diethylamide (LSD) was studied in rats trained to discriminate 0.08 mg/kg (186 nmol/kg) of LSD from saline in a two-lever operant paradigm. Only yohimbine fully mimicked LSD with an ED_{50} of 2.05 mg/kg (5.24 μmol/kg). Yohimbine's 5-HT_{1A} agonist properties may be responsible for this substitution. Other α_2-adrenoceptor antagonists, idazoxan with an agonist/antagonist profile at 5-HT_{1A} receptors and RS 26026–197, a highly selective α_2-adrenoceptor antagonist, failed to produce substitution. Clonidine, an α_2-adrenoceptor agonist, did not substitute for LSD but the response rate was dose-dependently reduced. None of the α_2-adrenergic agents used for pretreatment before LSD inhibited the response to the LSD training dose. Coadministration of clonidine with LSD produced a leftward shift of the dose-response relationship of LSD without a significant change in the slope of the dose-response line. Simultaneous administration of α_2-adrenergic agents with LSD shifted the dose-response curve to the left only when the adrenergic agent also possessed at least moderate affinity for the 5-HT_{1A} receptor. In addition, radioligand competition experiments were performed that showed LSD to have relatively high affinity (K_i=37 nM) for [^{3}H]clonidine-labeled sites in rat cortex with lower affinity for [^{3}H]yohimbine labeled sites. While previous studies have suggested that the nature of the LSD cue may be essentially expressed by 5-HT_2 receptor activation, the present data show that this cue can be modulated by effects of LSD at 5-HT_{1A} and at other monoamine neurotransmitter receptors.

Key words Drug discrimination · Discriminative stimulus · LSD; α_2-Adrenoceptor · 5-$HT_{2A/2C}$ receptor · 5-HT_{1A} receptor · Clonidine · Yohimbine · Idazoxan · RS 26026–197

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Introduction

Historically, the behavioral effects of lysergic acid diethylamide (LSD) have been attributed to its actions on central monoamine neurotransmitter receptors, particularly those for the indoleamine serotonin (5-hydroxytryptamine, 5-HT), but also to a lesser extent on those for the catecholamines, dopamine (DA) and norepinephrine (NE). Radioligand competition studies demonstrate that LSD is a non-selective agent that binds to several different populations of 5-HT receptors and other neurotransmitter binding sites (Creese et al. 1975; Hoyer 1988; Pierce and Peroutka 1989, 1990; Hoyer and Schoeffter 1991; Huang et al. 1994). It is perhaps not surprising, therefore, that the use of many diverse physiological, pharmacological, and behavioral procedures, have failed to elucidate completely the mechanisms underlying the complex spectrum of LSD's biobehavioral actions.

Substantial evidence suggests, however, that the discriminative stimulus effect of LSD is mediated by postsynaptic 5-HT_{2A} receptors (White et al. 1980; Glennon et al. 1984; Cunningham and Appel 1987; Titeler et al. 1988; Arnt 1989). Nevertheless, results from drug discrimination studies are sometimes difficult to interpret. For example, in rats trained to discriminate LSD from saline complete generalization was reported with quipazine, a non-hallucinogenic, non-selective serotonin agonist (Kuhn et al. 1977; White et al. 1977; Colpaert et al. 1979; Friedman et al. 1984; Appel and Cunningham 1986; Cunningham and Appel 1987). Although this substitution has been characterized as mediated by a 5-HT_{2A} agonist effect, quipazine has relatively high affinity at 5-HT_3 recognition sites (Ireland and Tyers 1987; Hoyer 1988; Van Wijngaarden et al. 1990). Substitution in LSD-trained rats is also reported for lisuride, a dopaminergic ergot derivative (Holohean et al. 1982; Mokler et al. 1983), yohimbine, an α_2-adrenoceptor antagonist (Colpaert 1984) and methysergide and mianserin, non-selective 5-HT agents with mixed agonist and antagonist properties (Colpaert et al. 1979, 1982). Furthermore, spiperone does not block the LSD cue, despite the fact that it is an extremely potent

5-HT_{2A} receptor antagonist (Cunningham and Appel 1987). Thus, there is reason to believe that the LSD stimulus may also reflect its nonselective nature.

Previous studies have demonstrated that adrenergic receptors do not mediate the LSD cue, since it is neither blocked nor mimicked either by α-adrenergic (phentolamine) or β-adrenergic (propranolol) antagonists (Kuhn et al. 1978). Nevertheless, biochemical and electrophysiological data indicate that LSD interacts with noradrenergic systems, and α_2-adrenergic agents are able to modulate serotonergic function. For example, in contrast to the depressant effect of LSD on raphe and nigral cells, LSD causes an increase in the cellular activity of some NE neurons in the locus coeruleus (Rogawski and Ahhajanian 1979). Svensson et al. (1975) have shown that very small intravenous doses of the α-adrenergic agonist clonidine are able to inhibit most 5-HT neurons in the dorsal raphe. Recent studies have demonstrated that α_2-adrenoceptors can modulate 5-HT release in the rat hippocampus (Mongeau et al. 1993), and activation of serotonin 5-HT_{2A} receptors decreases norepinephrine release in the same brain area (Done and Sharp 1992). Colpaert (1984) has demonstrated cross substitution between yohimbine and LSD and full substitution of clonidine in LSD-trained rats, accompanied by reduction of the response rate. However, an earlier report (Winter 1978) failed to observe either complete generalization of yohimbine to LSD or antagonism of the yohimbine cue by pizotyline. Additionally, Winter (1988) has shown cross substitution between yohimbine and the 5-HT_{1A} receptor agonists, 8-OH-DPAT and ipsapirone, and more recently has demonstrated that a significant component of yohimbine-induced stimulus control is mediated by the 5-HT_{1A} receptor (Winter and Rabin 1992, 1993).

The present study was designed to determine the extent to which the α_2-adrenoceptor antagonists, yohimbine, idazoxan, and RS 26026–197, and the α_2-adrenoceptor agonist, clonidine, could produce substitution in rats trained to discriminate LSD from saline, or could influence the stimulus properties of LSD. The α_2-adrenoceptor antagonists were chosen from a series of compounds with gradually increasing affinity for the α_2-adrenoceptor and decreasing affinity at the 5-HT_{1A} receptor. Yohimbine has similar affinity at the α_2-adrenoceptor and 5-HT_{1A} receptor; idazoxan binds with higher affinity to the α_2-adrenoceptor than to the 5-HT_{1A} receptor (Kawai et al. 1994) but may also bind to "imidazoline-recognition sites" (Michel et al. 1990). RS 26026–197 is proposed to be a very selective α_2-adrenoceptor antagonist (Clark et al. 1991).

Materials and methods

Subjects

Twenty male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, Ind.) weighing 200–220 g at the beginning of the drug discrimination study were used as subjects trained to discriminate LSD tartrate from saline. None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 80% of free-feeding weight (350–410 g). Lights were on from 0700 to 1900 hours. The laboratory and animal facility temperature was 22–24°C and the relative humidity was 40–50%. Experiments were performed between 0830 and 1700 hours each day, Monday–Friday.

For receptor binding studies male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, Ind.) weighing 250±50 g were used. The animals were kept in groups of five rats per cage, at the same conditions described above, but with free access to food and water.

Apparatus

Six standard operant chambers (model E10-10RF, Coulbourn Instruments, Lehigh Valley, Pa.) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a food hopper (combination dipper pellet trough, model E14-06, module size 1/2), all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, connected through a Med Associates interface to a 486-based microcomputer, controlled reinforcement and data acquisition with a locally written program.

Discrimination training and testing

A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. The drug discrimination procedure details have been described elsewhere (Oberlender and Nichols 1988, 1990; Marona-Lewicka and Nichols 1994). After habituation to the experimental conditions (1 week after isolation in the individual home cages and at the beginning of the food deprivation) each rat's initial shaping was started. During the first two or three sessions rats were trained only to associate a characteristic noise (click) after lever pressing with a delivered food pellet (without drug injections). Initially, rats were shaped to lever press on an FR1 schedule so that one food pellet was dispensed for each press. Half of the rats were trained on drug-L (left), saline-R (right) and the other half on drug-R, saline-L to avoid positional preference. Training sessions lasted 15 min and were conducted at the same time each day. Levers were cleaned between animals with 10% ethanol solution to avoid olfactory cues (Extance and Goudie 1981). Only one appropriate lever was present during the first ten sessions of initial learning (after beginning to administer saline or LSD, IP 30 min before sessions). Afterwards both levers were present during all following phases of training, but reinforcements were delivered only after responses on the appropriate lever. Presses on the incorrect lever had no programmed consequences. As response rates stabilized (during the next 15 sessions), the schedule of reinforcement was gradually increased to an FR50. Once at the FR50, training continued until an accuracy of at least 85% (number of correct presses×100/number of total presses) was attained for eight of ten consecutive sessions (approximately 40–60 sessions). Once criterion performance was attained, test sessions were interspersed between training sessions, either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session; the test procedure was repeated once again after the animal had regained the training accuracy criterion. Test drugs were administered IP 30 min prior to the sessions; test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min the session was ended and scored as a dis-

ruption but the number of presses and latency time (time measured from the start of the session to the first lever press) were recorded. Treatments were randomized at the beginning of the study.

Radioligand competition

For these studies the animals were killed by decapitation. The brains were rapidly removed from the skull, and the cerebral cortex was quickly dissected over ice. The tissues were homogenized (Brinkman Polytron, setting 5, 2×14 s) in 25 vol 50 mM TRIS-HCl buffer, pH 7.4 (at 24°C). For the clonidine binding assay 5 mM EDTA was added to the TRIS-HCl buffer. The homogenate was centrifuged twice for 15 min at 40 000 *g* and 4°C. For the binding assay (according to Brown et al. 1990, with minor modifications) the final pellets were reconstituted in TRIS-HCl buffer (for [^{3}H]clonidine binding, or TRIS-HCl plus EDTA for [^{3}H]yohimbine binding) to obtain a final protein concentration of approximately 0.6–0.7 mg/ml. Specific binding was estimated with 10 µM phentolamine. Incubations were carried out at 25°C using 1 nM [^{3}H]clonidine or 1 nM [^{3}H]yohimbine. Displacement at each concentration of *d*-LSD was determined in triplicate.

Drugs

The training drug was (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg, 186 nmol/kg, NIDA). Other drugs used during the drug discrimination study include: clonidine hydrochloride, yohimbine hydrochloride, idazoxan hydrochloride (2-[2-(1, 4-benzodioxanyl)]-2-imidazoline) (all purchased from Sigma, St Louis, Mo.) and RS 26026–197 (a generous gift from Syntex, Palo Alto, Calif.). All drugs were dissolved in 0.9% saline and were injected IP in a volume of 1 ml/kg, 30 min before the session (time chosen from the time-response cue for the training dose of LSD, unpublished observation) or 60 min before the training drug (90 min before the session) in pretreatment tests. Drugs used for radioligand competition studies were: [^{3}H]clonidine (specific activity 57.8 Ci/µmol, New England Nuclear), [^{3}H]yohimbine (specific activity 88 Ci/µmol, Amersham, Chicago, Ill.), and phentolamine hydrochloride (Sigma, St Louis, Mo.).

Data analysis

Data from the drug discrimination study were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (%SDL) for each dose of test compound was determined. The degree of substitution was determined by the maximum %SDL for all doses of the test drug. "No substitution" is defined as 59% SDL or less, and "partial" substitution is 60–79% SDL. If the drug was one that completely substituted for the training drug (at least one dose resulted in a %SDL=80% or higher) the method of Litchfield and Wilcoxon (1949) was used to determine the ED_{50} (log-probit analysis of the dose producing 50% drug-lever responding) and 95% confidence interval (95% CI). This method also allowed for tests of parallelism between dose-response curves. If the percentage of rats disrupted (%D) was 50% or higher, the ED_{50} value was not determined, even if the %SDL of nondisrupted animals was higher than 80%.

Results of the radioligand competition studies were analyzed using a four-parameter non-linear least squares regression analysis program to estimate the IC_{50} (concentration of competing drug to displace 50% of specifically bound radioligand). Values are reported as the mean ±SEM for three separate determinations.

Results

Figure 1A–D shows the dose-response relationships for clonidine (A), yohimbine (B), idazoxan (C), and RS 26026–197 (D) in rats trained with 186 nmol/kg (0.08 mg/kg) LSD as a discriminative stimulus. Clonidine, an α_2-adrenoceptor agonist did not substitute for LSD (Fig. 1A) at any tested dose. Maximum drug-lever responding (57%) was obtained at a dose of 0.08 mg/kg (0.3 µmol/kg) of clonidine. In addition, clonidine strongly decreased response rates; all ten rats were disrupted at the highest tested dose (0.16 mg/kg, 0.6 µmol/kg). The ED_{50} value for this *suppressant* effect of clonidine was 0.034 mg/kg (0.13 µmol/kg).

The α_2-adrenoceptor antagonist yohimbine mimicked LSD (Fig. 1B) with an ED_{50} of 2.05 mg/kg (5.24 µmol/kg) and CI 1.73–2.42 mg/kg (4.43–6.20 µmol/kg). However, at the highest dose tested, yohimbine depressed response rates, and produced 60% disruption. Neither idazoxan (Fig. 1C) nor RS 26026–197 (Fig. 1D) substituted for LSD, although both these α_2-adrenoceptor antagonists produced a high percentage of drug lever selection at the lower tested dosages. Idazoxan and RS 26026–197 did not reduce response rates in rats trained to discriminate LSD from saline (data not shown).

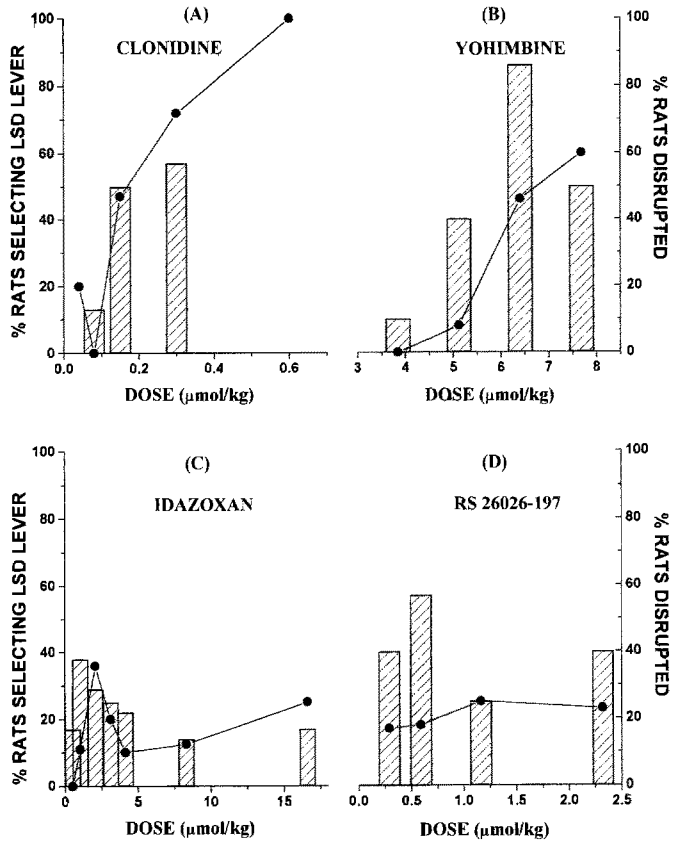

Fig. 1 Effects of clonidine (**A**), yohimbine (**B**), idazoxan (**C**), and RS 26026–197 (**D**) in rats trained with LSD (0.08 mg/kg, 186 nmol/kg) as a discriminative stimulus. The abscissa indicates dosages of appropriate test compounds (µmol/kg). The *left ordinate* indicates percentage of rats selecting the LSD-lever (*hatched bars*), the *right ordinate* shows percentage of rats that did not complete 50 presses on one lever within 5 min of the test session (*line* and *filled circle*). All points are percentage data from 10–17 subjects

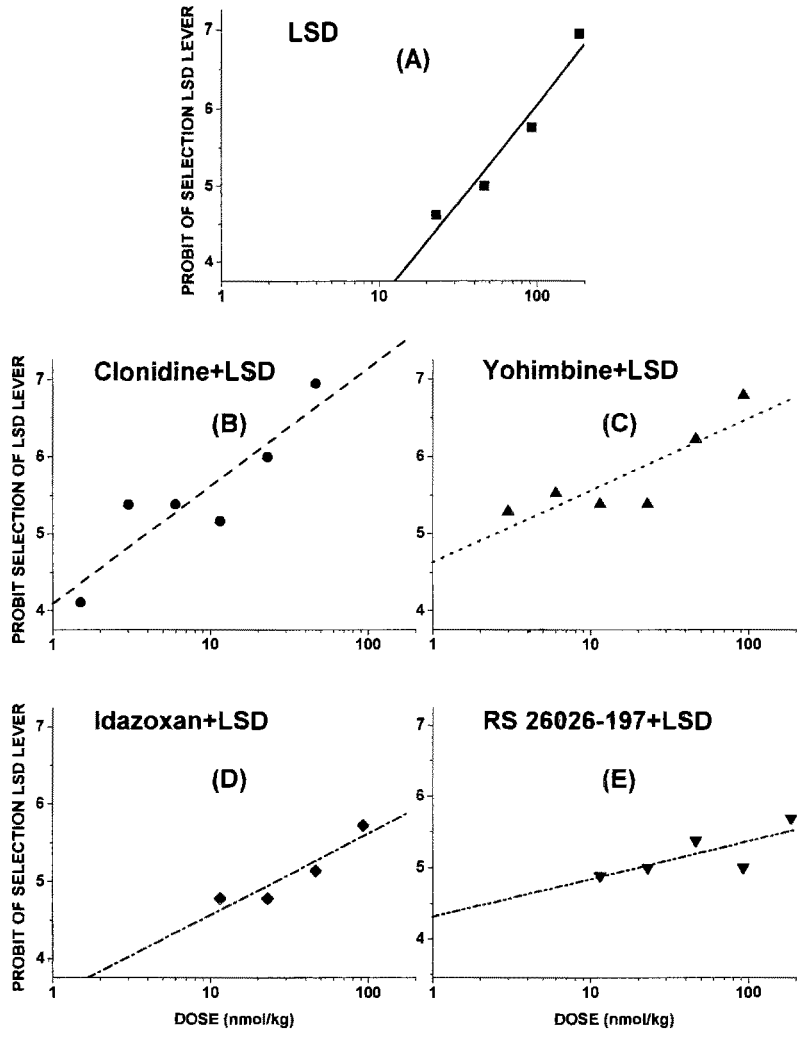

Fig. 2 Regression lines fitting dose-response data of α_2-adrenergic agents coadministered with different doses of LSD from substitution tests in rats trained to discriminate 0.08 mg/kg (186 nmol/kg) of LSD from saline. The *abscissa* indicates LSD dose (nmol/kg). The *left ordinate* indicates probit from percentage of rats selecting the LSD-lever. The ED_{50} values and 95% confidence intervals (*CI*) were calculated according to the method of Litchfield and Wilcoxon (1949) and were as follows: 38 (26–59) nmol/kg [0.016 (0.011–0.024) mg/kg] for LSD, 4 (2–9) nmol/kg [0.002 (0.001–0.004) mg/kg] for LSD+clonidine, 2.5 (1–8) nmol/kg [0.001 (0.001–0.004) mg/kg] for LSD+yohimbine, 26 (12–57) nmol/kg [0.011 (0.005–0.025) mg/kg] for LSD+idazoxan, and 20 (4–102) nmol/kg [0.009 (0.001–0.044) mg/kg] for LSD+RS 26026–197. The Litchfield and Wilcoxon method also allowed for tests of parallelism. The slopes of the LSD and LSD +clonidine curves did not differ ($P<0.05$) whereas the slopes for each antagonist+LSD did differ from that of the LSD alone curve. The slopes of α_2-adrenoceptor antagonists+LSD curves did not differ from each other ($P<0.05$ for each combination)

In order to evaluate the possible interactions between LSD and adrenergic agents the effect of combinations of an α_2-adrenoceptor agonist or antagonist with LSD was studied in rats trained to discriminate LSD from saline. In the first series of experiments rats were injected with different doses of clonidine, yohimbine, idazoxan, or RS 26026–197 60 min before LSD. None of the α_2-adrenergic agents used for pretreatment in these tests inhibited the response to the training dose of LSD (data not shown).

In the second series of experiments to evaluate the interaction between LSD and adrenergic agents, simultaneous injections (two successive injections) were given of different doses of LSD with clonidine (0.04 mg/kg, 0.15 μmol/kg), yohimbine (2.0 mg/kg, 5.12 μmol/kg), idazoxan (0.5 mg/kg, 2.08 μmol/kg) or RS 26026–197 (0.25 mg/kg, 0.58 μmol/kg). Figure 2 illustrates the dose-response relationship for LSD alone (Fig. 2A) and for LSD plus α_2-adrenoceptor agents. The log-probit plots show orderly, linear dose-response relationships. Coadministration of clonidine with LSD (Fig. 2B) produced a ten-fold leftward shift of the dose-response relationship of LSD without a significant change in the slope of the dose-response line. During these tests there was no increase in the numbers of disruptions. Indeed, the percentage of disruption was lower than for clonidine alone. This could be interpreted to mean that LSD blocked (or reversed) the rate suppressant effect of clonidine. Simultaneous administration of yohimbine and LSD (Fig. 2C) also evoked a significant 15-fold leftward shift of the dose-response curve of LSD, but accompanied by a significant decrease in the slope. Coadministration of idazoxan with LSD (Fig. 2D) produced a slight but non-significant shift of the dose-response curve to the left. The highly selective α_2-adrenoceptor antagonist RS 26026–197 given with LSD (Fig. 2E) did not change the ED_{50} value for LSD, but the slope was significantly different than for LSD alone. The dose-response curves for the combinations of LSD with α_2-adrenoceptor antagonists were parallel one to each other (Fig. 2C–E) but not to the dose-response curve for LSD (Fig. 2A) alone.

Radioligand competition experiments showed that LSD has relatively high affinity for α_2-adrenoceptor binding sites labeled by [^{3}H]clonidine and [^{3}H]yohimbine, with K_i values of 37±3 and 127±6 nM, and Hill coefficients of 1.14±0.14 and 0.83±0.12, respectively. LSD

clearly has high affinity for [^{3}H]clonidine-labeled sites, but whether it is an agonist or antagonist is presently unknown.

Discussion

These experiments provide behavioral evidence that the discriminable state elicited by LSD can be mimicked by yohimbine, an α_2-adrenoceptor antagonist with 5-HT_{1A} agonistic effects, and that stimulation of α_2-adrenoceptors by clonidine is able to potentiate the stimulus properties of LSD in the two lever drug discrimination paradigm. Other α_2-adrenoceptor antagonists, idazoxan and RS 26026–197 did not mimic LSD at any dose tested in rats trained to discriminate 0.08 mg/kg (186 nmol/kg) LSD from saline.

Any drug with activity at multiple receptors may differ in its apparent stimulus properties depending upon whether the drug is used for training or is tested in subjects trained with drugs that may share certain of its pharmacological elements. For purposes of interpreting the substitution of yohimbine in LSD trained rats, we assume that LSD functions as a compound stimulus, the most salient feature of which is an action at 5-HT_{2A} receptors. However, the binding-inhibition data of Leysen et al. (1982) suggest that the affinity of yohimbine is only 1/1000 of the affinity of ketanserin for 5-HT_2 receptors in rat brain membranes labeled with [^{3}H]ketanserin. However, a second element of the LSD stimulus may be mediated by occupation of 5-HT_1 receptors. Data from drug discrimination studies in pigeons suggest that LSD may express some 5-HT_{1A} receptor-mediated effects (Walker et al., 1991; Yamamoto et al., 1991). The selective 5-HT_{1A} receptor agonist, 8-OH-DPAT is able to mimic LSD in rats trained to discriminate 0.16 mg/kg (372 nmol/kg) LSD from saline (Winter and Rabin 1988; Meert et al. 1990) but LSD can only partially mimic 8-OH-DPAT in rats trained to discriminate the latter agent (Cunningham and Appel 1987). The simplest explanation of these facts is that LSD produces effects on 5-HT_1 receptors that are apparent in drug discrimination studies only when drugs active at the 5-HT_1 receptor are tested.

There is considerable evidence that yohimbine has high affinity at 5-HT_1 binding sites (Fozard et al. 1987; Broadhurst et al. 1988; Hoyer 1988; McCall et al. 1991; Schoeffter and Hoyer 1991; Winter and Rabin 1992). Moreover, Winter and Rabin (1988, 1989, 1992, 1993) demonstrated that cross substitution occurs between yohimbine and the 5-HT_{1A} receptor agonists 8-OH-DPAT and ipsapirone, and suggested that yohimbine-induced stimulus control is mediated to a significant degree by actions at the 5-HT_{1A} receptor. Thus, yohimbine substitution in LSD-trained rats may result from its 5-HT_{1A} agonistic properties while its α_2-adrenoceptor antagonism might play only a supporting role for this effect of yohimbine.

Idazoxan and RS 26026–197 did not mimic LSD at any doses tested. Although it has been reported that idazoxan competes at 5-HT_{1A} binding sites with a K_i of 63 nM, it also inhibits forskolin-stimulated adenylate cyclase activity (Kawai et al. 1994) and in drug discrimination substitution tests only partially generalized to 8-OH-DPAT or flesinoxan (Ybema et al. 1994). Idazoxan also can block some effects induced by 5-HT_{1A} agonists, e.g. the hyperglycemic and hypoinsulinemic effects of 8-OH-DPAT (Jhanwar-Uniyal et al. 1994), and completely blocked the effect of 8-OH-DPAT on the 5-HIAA oxidation peak recorded in the suprachiasmatic nucleus of anaesthetized rats (Marsden and Martin 1986). It appears therefore, that idazoxan is a weak partial agonist or antagonist at 5-HT_{1A} receptors. Thus, the agonist efficacy of idazoxan at 5-HT_{1A} receptors may be too weak for it to mimic LSD in this drug discrimination study. RS 26026–197, which is a highly selective antagonist at α_2-adrenoceptors, did not substitute for LSD.

The data from dose-response effects for substitution tests of idazoxan and RS 26026–197 might be interpreted to suggest that inhibition of α_2-adrenoceptors could, albeit weakly, mimic the stimulus properties of LSD. Both compounds gave the highest percentage of substitution for LSD at very low doses: 38% at 1.25 μmol/kg (0.3 mg/kg), and 59% at 0.6 μmol/kg (0.25 mg/kg) for idazoxan and RS 26026–197, respectively. Blockade of somatodendritic and terminal α_2-adrenoceptors facilitates noradrenergic transmission, causing activation of raphe neurons via α_1-adrenoceptors, thus increasing hippocampal 5-HT release (Clement et al. 1992). An inhibitory control is probably mediated via α_2-adrenoceptors located on 5-HT terminals in the hippocampus that are tonically activated by endogenous norepinephrine (Mongeau et al. 1993). Direct blockade of inhibitory α_2-heteroreceptors located on 5-HT terminals is held responsible for the increase in extracellular 5-HT and indirect activation of 5-HT postsynaptic 5-HT_1 receptors. It thus seems possible that the indirect activation of postsynaptic 5-HT_{1A} receptors may occur in response to low doses of α_2-adrenoceptor antagonists.

Clonidine did not substitute for LSD but the response rate was dose-dependently reduced, which may be due to general behavioral suppression induced by clonidine. Nevertheless, coadministration of clonidine with LSD gave a parallel leftward shift of the LSD dose-response curve. In general, when dose-response curves of two treatments are parallel, it suggests that the drugs may be acting via a common site and/or mechanism of action (Levine 1990). Thus, the present results could be interpreted to mean that coadministration of clonidine is able to potentiate the LSD stimulus properties without changing the nature of the cue. A mechanism responsible for this effect is hard to explain on the basis of action by clonidine only in a particular brain structure or by action at a single receptor subpopulation. Moreover, as we have shown here, LSD itself has significant affinity at α_2-adrenoceptor binding sites, especially at those labeled by [^{3}H]clonidine.

Several literature reports suggest a functional relationship between effects produced by hallucinogens and

activation of α_2-adrenoceptors. Geyer and Lee (1984) reported that clonidine, like LSD, suppressed 5-HT neuronal firing in the dorsal raphe nucleus but its action was mediated through noradrenergic neurons. Clonidine induces GH (growth hormone) secretion by a mechanism that has similarities to the effects of direct 5-$HT_{2A/2C}$ receptor agonists. Aulakh and coworkers (1992) demonstrated that clonidine-induced GH secretion is mediated by stimulation of α_2-adrenergic autoreceptors, which in turn enhances 5-HT activity via stimulation of postsynaptic 5-HT_{2C} receptors to promote GHRF (growth hormone-releasing factor). In that study, only the lowest dose of clonidine produced maximum increases in GH level, whereas the higher doses did not produce further increases. The effect of clonidine on GH secretion was completely blocked not only by α_2-adrenoceptor antagonists, but also by metergoline and mesulergine pretreatment while mianserin and ritanserin partially blocked the effect.

This effect of low dose clonidine is consistent with electrophysiologic evidence reported by Mongeau and coworkers (1994). Low doses of clonidine decrease norepinephrine output by activating α_2-adrenergic autoreceptors on norepinephrine neurons, reducing the tonic inhibitory action of endogenous norepinephrine on α_2-adrenergic heteroreceptors on 5-HT terminals, resulting in increased 5-HT release. The inhibitory effect of high doses of clonidine on 5-HT neurotransmission was not abolished by 6-OHDA treatment, suggesting that high dose clonidine directly activates α_2-adrenergic heteroreceptors to decrease 5-HT release. A biphasic effect on 5-HT neurotransmission is also consistent with the notion that α_2-adrenergic heteroreceptors and α_2-adrenergic autoreceptors are not identical, as suggested in the report by Raiteri et al. (1983) and by Maura et al. (1985), who showed that the potency of clonidine is 10 times higher at α_2-adrenergic autoreceptors that at α_2-adrenergic heteroreceptors.

Abrupt termination of clonidine dependence by yohimbine administration produces a withdrawal syndrome (e.g., jumping, jerks, wet dog shakes, and diarrhea) that also has certain similarities to activation of 5-HT_2 receptors (Thoolen et al. 1983). All of this evidence might lead one to predict that the discriminative stimulus properties of LSD would be potentiated by clonidine.

Potentiation of LSD effects by coadministration with yohimbine or idazoxan appears to change the site and/or mechanism of action. Coadministration of idazoxan with LSD shifted the dose-response curve slightly but nonsignificantly to the left and this curve was parallel to that obtained after simultaneous administration of yohimbine and LSD, but was significantly different from that produced by LSD alone. Coadministration of RS 26026–197 did not change the ED_{50} value for LSD but significantly changed the slope of the dose-response curve. Thus, potentiation of LSD by α_2-adrenergic agents used in this study exists only for compounds that have at least moderate affinity at 5-HT_{1A} receptors.

Glennon (1990) has suggested that LSD is a high-affinity, low-efficacy, nonselective 5-HT agonist; in the absence of another agonist it may function as an agonist, whereas in the presence of a high-efficacy agonist, it will function as an antagonist. There are several reports in the literature suggesting that a functional relationship may exist between 5-HT_2 and HT_{1A} receptors (Backus et al. 1990; Berendsen and Broekkamp 1990; Darmani et al. 1990; Yocca et al. 1990).

The potent presynaptic inhibition by LSD of serotonergic neurotransmission may also contribute to the central action of this drug. The [^{3}H]serotonin release from slices of rat hippocampus elicited by electrical stimulation was inhibited by LSD in a concentration-dependent manner (Langer and Moret 1982). Changes in serotonergic neurotransmission produced by the indirect action of α_2-adrenoceptor antagonists may be responsible for the different slopes of the dose-response curves resulting from coadministration of LSD and α_2-adrenoceptor antagonists. Although parallel to each other, these curves are significantly different from the dose-response relationship for LSD alone.

In conclusion, the effects of LSD are multifaceted and divergent, implicating a variety of different but probably interrelated neuronal mechanisms to account for the various components of the LSD experience. Although the essential nature of the LSD cue appears to be expressed through 5-HT_2 receptor activation, this cue may be modulated by effects of LSD at other monoamine receptors. These ancillary monoamine interactions could be important in conferring the extraordinarily high potency possessed by LSD.

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