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LSD and structural analogs: pharmacological evaluation at D_1 dopamine receptors

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Abstract The hallucinogenic effects of lysergic acid diethylamide (LSD) have been attributed primarily to actions at serotonin receptors. A number of studies conducted in the 1970s indicated that LSD also has activity at dopamine (DA) receptors. These latter studies are difficult to interpret, however, because they were completed before the recognition of two pharmacologically distinct DA receptor subtypes, D_1 and D_2. The availability of subtype-selective ligands (e.g., the D_1 antagonist SCH23390) and clonal cell lines expressing a homogeneous receptor population now permits an assessment of the contributions of DA receptor subtypes to the DA-mediated effects of LSD. The present study investigated the binding and functional properties of LSD and several lysergamide analogs at dopamine D_1 and D_2 receptors. Several of these compounds have been reported previously to bind with high affinity to serotonin $5HT_2$ (i.e., ^{3}H-ketanserin) sites in the rat frontal cortex ($K_{0.5}$ 5–30 nM). All tested compounds also competed for both D_1-like (^{3}H-SCH 23390) and D_2-like (^{3}H-spiperone plus unlabeled ketanserin) DA receptors in rat striatum, with profiles indicative of agonists (n_H<1.0). The affinity of LSD and analogs for D_2 like receptors was similar to their affinity for $5HT_2$ sites. The affinity for D_1 like receptors was slightly lower (2- to 3-fold), although LSD and several analogs bound to D_1 receptors with affinity similar to the prototypical D_1 partial agonist SKF38393 ($K_{0.5}$ ca. 25 nM). A second series of experiments tested the binding and functional properties of LSD and selected analogs in C-6 glioma cells expressing the rhesus macaque D_{1A} receptor. LSD and the analogs tested bound to C-6 mD_{1A} cells with affinity and kinetics similar to those obtained in rat straitum. Additionally, LSD and selected analogs were able to increase cAMP accumulation, albeit only as partial agonists. Similar to the actions of SKF38393, they could stimulate, as well as block, DA-stimulated cAMP synthesis. These results represent the first clear demonstration of the interaction of LSD with DA D_1 receptors, and provide a basis for evaluating the contribution of D_1 receptors to the biobehavioral actions of LSD.

Key words *LSD* · Lysergic acid diethylamide · Hallucinogen · Dopamine · D_1 receptor · D_2 receptor · cAMP synthesis · Adenylate cyclase · Ergolines · Lysergamides

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Introduction

Historically, the hallucinogenic effects of lysergic acid diethylamide (LSD) have been attributed to its actions at serotonin receptors (e.g., Freedman and Boggan 1982; White 1986; Aghajanian 1994). In considering the pharmacology of the hallucinogens as a class, the most salient feature of their mechanism of action appears to be an interaction with receptors of the serotonin $5\text{-}HT_{2A/2C}$ subtype (Glennon et al. 1984). This conclusion was developed based on, at least in part,

the finding that hallucinogens of the phenethylamine class had low affinity for any other type of brain receptor. LSD, however, possesses affinity for a variety of central monoamine receptors that includes not only serotonin, but also adrenergic and dopamine receptors. Indeed, LSD has remarkable submilligram potency in man, yet its affinity for 5-$HT_{2A/2C}$ receptor subtypes is comparable to that of phenethylamine compounds that are an order of magnitude less potent than LSD as hallucinogens.

A number of studies conducted in the 1970s indicated that LSD interacts with dopamine (DA) receptors. For example, radioreceptor studies (Burt et al. 1976; Creese et al. 1976) demonstrated that LSD binds with high affinity to sites labeled with either dopamine agonists (^{3}H-dopamine) or antagonists (^{3}H-haloperidol). Several other biochemical studies suggested that LSD could produce functional effects at DA receptors, although these findings were not consistent (see reviews by Freedman and Boggan 1982; White 1986). The reported actions of LSD included changes in DA synthesis, metabolism, and release (Da Prada et al. 1975; Persson et al. 1977, 1978; Pieri et al. 1978), DA cell firing (Christoph et al. 1977; Walters et al. 1979), and cAMP synthesis (Von Hungen et al. 1974; Da Prada et al. 1975; Spano et al. 1975; Ahn and Makman 1979). Additionally, LSD has been reported to produce behavioral effects similar to those caused by dopamine agonists. Two such effects are the induction of stereotypies in normosensitive rats (Dixon 1968; Fog 1969) and contralateral circling in rats with unilateral dopamine denervation (Pieri et al. 1974; Trulson et al. 1977). Further, in rats trained to discriminate LSD from saline, the dopaminergic ergot lisuride gives complete substitution (Holohean et al. 1982; Mokler et al. 1983); LSD itself produced partial substitution in rats trained to discriminate apomorphine from saline (Appel et al. 1982; Holohean et al. 1982).

A number of remarkable developments in dopamine receptor pharmacology have occurred since these initial reports of the dopaminergic activity of LSD. The most important is the recognition of two pharmacologically distinct families of dopamine receptors, D_1 and D_2, and a better understanding of their functional roles in the central nervous system (Garau et al. 1978; Kebabian and Calne 1979). More recently, molecular cloning studies have revealed multiple molecular forms of dopamine receptors, although it is still useful to classify them pharmacologically as either D_1-like or D_2-like. D_1-like (D_{1A} and D_{1B} or D_5) dopamine receptors selectively recognize the phenyltetrahydrobenzazepines, and are coupled positively to adenylate cyclase. D_2-like (D_2, D_3, and D_4) receptors recognize benzamides and butyrophenones, but are either uncoupled or negatively coupled to adenylate cyclase (see Civelli et al. 1991; Sibley and Monsma 1992; Gingrich and Caron 1993 for reviews of molecular nomenclature).

Although there is significant evidence that LSD can produce effects linked to dopamine receptor occupation, this action has generally been ignored in considering the mechanism of action of LSD, in favor of an exclusive role for 5HT receptors in hallucinogenesis (e.g., Aghajanian 1994). This conclusion is surprising (and perhaps premature) when one considers that the interaction of LSD with D_1 receptors has never been tested directly. Such a conclusion is understandable, however, when one considers that LSD often is grouped with the pharmacologically less complex phenethylamine hallucinogens, with which a serotonergic mechanism seems to be the only reasonable explanation for their biological activity. Thus, while a serotonergic mechanism may be an essential feature of hallucinogen action, and may define some key neurochemical interaction, LSD is nevertheless both more complex pharmacologically and a more potent hallucinogen than the phenethylamines. It seems quite likely that some of these differences might derive from the additional monoamine interactions of LSD.

When viewed retrospectively, many of the early binding and functional studies conducted with LSD provide evidence for the actions of LSD at D_2-like receptors. LDS appears to be a partial agonist at these sites, as evidenced by its lower functional potency compared to prototypical D_2 agonists, as well as its ability to anatagonize the actions of D_2 agonists (e.g., Walters et al. 1979; White 1986). There is no report, however, of the ability of LSD to compete for D_1 labeled binding sites, and no functional studies have employed the D_1 selective antagonist SCH23390. The single suggestive piece of evidence for the effects of LSD on D_1 receptors is the ability of this compound to stimulate adenylate cyclase (von Hungen et al. 1974; Da Prada et al. 1975; Spano et al. 1975; Ahn and Makman 1979). It is especially difficult to evaluate these reports, however, because the tissue preparations employed typically contained both D_1 and D_2 receptors, which are known to have opposing effects on adenylate cyclase (Stoof and Kebabian 1981). The presence of multiple subtypes of 5HT receptors coupled to adenylate cyclase (see Boess and Martin 1994) further complicates interpretation of these studies.

The availability of radiolabeled D_1 selective compounds and clonal cell lines expressing a homogeneous D_1 receptor population now permits an unambiguous evaluation of the actions of LSD at dopamine D_1 receptors. Toward this aim, two series of experiments were conducted. The first examined the binding affinity of LSD for dopamine D_1 and D_2 receptors in native tissues from rat brain. Additionally, a variety of structural analogs of LSD were tested to examine specific aspects of structure-activity relationships of lysergic acid amides. Those analogs chosen for testing have been reported to be of similar or greater potency compared to LSD when tested in rodents trained to discriminate LSD from saline (Hoffman and Nichols 1985;

Oberlender et al. 1992; Huang et al. 1994). A second series of experiments was designed to examine the D_1 dopamine receptor functional activity of LSD and selected structural analogs in a molecular expression system, C-6 glioma cells expressing recombinant rhesus macaque D_{1A} receptor (Machida et al. 1992). Unlike native tissues, neither D_2 nor $5HT_2$ receptors are expressed in this cell line. Thus, this system allows functional assessments of LSD actions at D_1 receptors without the confounding effects due to actions via other receptor types.

The results from the present study demonstrate, for the first time, that LSD and several other lysergamide analogs are high affinity D_1 like dopamine receptor ligands. In addition, this work defines more precisely the nature of the interaction of LSD with D_2-like receptors. Interestingly, the D_1 binding affinities of LSD and some of the other lysergamides tested were similar to that of the prototypical D_1 ligand SKF38393. LSD and its structural analogs were able to stimulate cAMP accumulation significantly, although none of the test compounds was as efficacious as DA. This partial agonist profile was similar to that of SKF83893; LSD and the analogs we tested were able to increase cAMP synthesis when tested alone, yet also antagonize the cAMP accumulation caused by dopamine.

Materials and methods

Materials

[^{3}H]SCH23390 (7-chloro-8-dihydroxy-3-[^{3}H]methyl-1-phenyl-2,3,4,5-tetrahydro-1h-3-benzazepine) was synthesized as described by Wyrick and Mailman (1985). [^{3}H]-spiperone was purchased from Amersham (Arlington Heights, Ill.) [^{3}H]-Ketanserin was obtained from New England Nuclear (Boston, Mass.). *R*(+)-SCH23390 and (±)-SKF38393 were purchased from Research Biochemicals (Natick, Mass.). LSD (*d*-lysergic acid diethylamide tartrate) was obtained from NIDA. Two *N*(6)-alkyl-nor LSD derivatives were prepared, as described previously (Hoffman and Nichols 1985); these were *N*(6)-ethyl norLSD (EthLAD: 9,10-didehydro-*N*,*N*-diethyl-6-ethylergoline-8β-carboxamide) and *N*(6)-alkyl-norLSD (AllylLAD: 9,10-didehydro-*N*,*N*-diethyl-6-allyllergoline-8β-carboxamide). The isomeric 2-butyl lysergamide mixture was prepared (LASBA: *N*(*R*,*S*-methylpropyl)-9,10-didehydro-6-methylergoline-8β-carboxamide), and the two diastereomers (*R*-LASBA and *S*-LASBA) were resolved, as reported by Oberlender et al. (1992). The lysergamide of *N*-methyl-*N*-isopropylamine (LAMIDE: *N*-methyl-*N*(2-propyl)-9,10-didehydro-6-methylergoline-8β-carboxamide) was synthesized by standard methods (e.g., see Oberlender et al. 1992). (±)-DHX (*trans*-10,11-dihydroxy5,6,6a,7,8,12b-hexahydrobenzo[a]phenathridine) was synthesized as previously described (Brewster et al. 1990). The enanitomers of DHX were resolved and the active enantiomer identified as (+)-(6*aR*, 12*bS*)-DHX (Knoerzer et al. 1994). Cinanserin was a kind gift from Squibb Institute for Medical Research. Dopamine, cAMP, and isobutyl methylxanthine (IBMX) were obtained from Sigma (St. Louis, Mo.). cAMP primary antibody was obtained from Dr. Gary Brooker (George Washington University, Washington D.C.), and secondary antibody (rabbit anti-goat IgG) covalently attached to magnetic beads was purchased from Advanced Magnetics (Cambridge, Mass.).

Subjects

Adult male Sprague Dawley rats (200–250 g) were obtained from Charles River Breeding Laboratories (Raleigh, N.C.). or Harlan Laboratories (Indianapolis, Ind.). Rats were killed by decapitation, and the whole brains removed and chilled briefly in ice-cold saline. For dopamine receptor assays, the brains were sliced with the aid of a dissecting block (Heffner et al. 1980), and central striata were then dissected from two coronal sections containing the majority of this region. Tissue was frozen immediately on dry ice and stored at −70° C until the day of the assay. The tissue for the serotonin receptor binding studies was prepared according to the method of Middlemiss and Fozard (1983). Briefly, the frontal cortex region was dissected rapidly on ice, and the tissue homogenized in 7 vol 0.32 M sucrose in a Brinkman Polytron (setting 6 for 20 s). The homogenate was centrifuged (36 500 *g* for 10 min), and the pellet rehomogenized in the same volume of buffer. Aliquots were removed and stored at −70° C for up to 3 weeks. The animal protocols used in the present experiments were consistent with current NIH principles of animal care and were approved by the University of North Carolina Institutional Animal Care and Use Committee, and by the Purdue Animal Care and Use Committee.

Cell cultures

C-6 glioma cells expressing the rhesus macaque D_{1A}receptor, (C-6-mD_{1A}, Machida et al. 1992) were grown in DMEM-H medium containing 4500 mg/l glucose, L-glutamine, 5% fetal bovine serum and 600 ng/ml G418 or 2 g/ml puromycin. Cells were maintained in a humidified incubator at 37° C with 5% CO_2.

Membrane preparation

Cells were grown in 75 cm^2 flasks until confluent. The cells were rinsed and lysed with 10 ml ice-cold hypo-osmotic buffer (HOB) (5 mM Hepes, 2.5 mM $MgCl_2$, 1 mM EDTA; pH 7.4) for 10 min at 4° C. Cells were then scraped from the flasks using a sterile cell scraper from Baxter (McGaw Part, Ill.). Flasks received a final rinse with 5 ml HOB. The final volume of the cell suspension recovered from each flask was ca. 14 ml. Scraped membranes from several flasks were then combined. The combined cell suspension was homogenized (10 strokes), 14 ml at a time, using a 15-ml Wheaton Teflon-glass homogenizer. The cell homogenates were combined and spun at 43 000 *g* (Sorval RC-5B/SS-34, Du Pont, Wilmington, Del.) at 4° C for 20 min. The supernatant was removed, and the pellet was resuspended (10 strokes) in 1 ml ice-cold HOB for each original flask of cells homogenized. This homogenate was then spun again at 43 000 *g* at 4° C for 20 min. The supernatant was removed, and the final pellet was resuspended (10 strokes) in ice-cold storage buffer (50 mM Hepes, 6 mM $MgCl_2$, 1 mM EDTA, pH 7.4) to yield a final concentration of ca. 2.0 mg protein/ml. Aliquots of the final homogenate were stored in microcentrifuge tubes at −80° C. Prior to their use for radioligand binding or adenylate cyclase assays, protein levels for each membrane preparation were quantified using the BCA protein assay reagent (Pierce, Rockford, Ill.) adapted for use with a microplate reader (Molecular Devices; Menlo Park, Calif.).

Dopamine receptor binding assays-rat homogenates

Frozen rat striata were homogenized by several manual stokes in a Wheaton Teflon-glass homogenizer in 8 ml ice-cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$ (pH 7.4). Tissue was centrifuged at 27 000 *g* for 10 min, the supernatant was discarded, and the pellet was homogenized (5 strokes) and resuspended in ice-cold

buffer and centrifuged again. The final pellet was suspended at a concentration of 2.0 mg wet weight/ml. The amount of tissue added to each assay tube was 1.0 mg, in a final assay volume of 1.0 ml. D_1 receptors were labeled with ^3H SCH23390 (0.30 nM); D_2 receptors were labeled with ^3H spiperone (0.07 M); unlabeled ketanserin (50 nM) was added to mask binding to $5HT_2$ sites. Total binding was defined as radioligand bound in the absence of any competing drug. Nonspecific binding was estimated by adding unlabeled SCH23390 (1 μM) or unlabeled chloropromazine (1 μM) for D_1 and D_2 receptor binding assays, respectively. As an internal standard, a competition curve with six concentrations of unlabeled SCH23390 (D_1 binding) or chlorpromazine (D_2 binding) was included in each assay. Triplicate determinations were made for each drug concentration. Assay tubes were incubated at 37° C for 15 min, and binding was terminated by filtering with ice-cold buffer on a Skatron 12 well cell harvester (Skatron, Sterling, Va.) using glass fiber filter mats (Skatron no. 7034). Filters were allowed to dry and 1.0 ml Optiphase HI-SAF II scintillation fluid were added. Radioactivity was determined on an LKB Wallac 1219 RackBeta liquid scintillation counter (Wallac, Gaithersburg, Md.). Tissue protein levels were estimated using the BCA protein assay reagent (Pierce, Rockford, Ill.).

Dopamine receptor binding assays-C-6-mD_{1A} cells

Receptor binding assays were done in C-6-mD_{1A} cells as described for rat striatum with the following modifications. Frozen membranes were thawed and resuspended in assay buffer (50 mM Hepes with 4 mM $MgCl_2$ and 0.001% bovine serum albumin; pH 7.4). The amount of tissue added to each tube was ca. 50 μg in a final assay volume of 500 μl. Duplicate determinations were performed at each drug concentration.

Serotonin receptor binding assays–rat homogenates

A frozen tissue aliquot was thawed in ice and diluted 1:20 with 50 mM TRIS-HCl (pH 7.4). The suspension was centrifuged at 36 500 *g* for 10 min. The pellet was resuspended in the same volume of buffer, incubated at 37° C for 10 min to allow degradation of residual monoamines by monoamine oxidase, then centrifuged at 36 500 *g* a second time. The final pellet was resuspended in 50 mM TRIS-HCl containing 0.1% ascorbic acid, 5.7 mM $CaCl_2$, and 10 μM pargyline (final pH 7.4) and preincubated for 10 min at 37.5° C. Assays were performed in triplicate with the buffer described above, to which 200–400 μg protein was added. The ability of the test compounds to compete with 0.75 nM ^3H-ketaserin was studied, with unlabeled cinanserin (10 μM) used to define nonspecific binding. Tubes containing tissue and drug were incubated at 37.5° C for 15 min, then filtered through Whatman GF/C filters in a Brandel cell harvester, modified for receptor binding studies, followed by two 3-s washes with ice-cold buffer. Filters were counted by liquid scintillation spectrometry.

Data analysis for radioreceptor assays

Binding data from each assay were analyzed separately. Data were normalized by expressing the average dpm at each competitor concentration as a percentage of total binding. These data were then subjected to nonlinear regression analysis using the algorithm for sigmoid curves in the curve-fitting program InPlot (Graphpad, San Francisco, Calif.) or EBDA and LIGAND software, as adapted for the IBM-PC by McPherson (1985), to generate $K_{0.5}$ values and a Hill coefficient (n_H) for each curve. Analysis of the residuals indicated an excellent fit; *r* values were above 0.99 for all curves in the present experiments.

Adenylate cyclase assay

Frozen membranes were thawed and added to assay tubes (10 μg protein/tube) containing a prepared reaction mixture [100 mM Hepes, (pH 7.4), 100 mM NaCl, 4 mM $MgCl_2$, 2 mM EDTA, 500 μM isobutyl methylxanthine (IBMX), 0.01% ascorbic acid, 10 μM pargyline, 2 mM ATP, 5 μM GTP, 20 mM phosphocreatine, 5 units creatine phosphokinase (CPK), 1 μM propranolol] and selected drugs. The final reaction volume was 100 μl. Basal cAMP activity was determined by incubation of tissue in the reaction mixture with no drug added. Tubes were assayed in duplicate and, after a 15-min incubation at 30° C, the reaction was stopped by the addition of 500 μl 0.1 N HCl. Tubes were vortexed briefly, and then spun in a BHG HermLe Z 230 M microcentrifuge for 5 min at 15000 *g* to precipitate particulates.

Radioimmunoassay (RIA) of cAMP

The concentraton of cAMP in each sample was determined with an RIA of acetylated cAMP, modified from that previously described (Harper and Brooker 1975). Iodination of cAMP was performed using a method reported by Patel and Linden (1988). Assay buffer was 50 mM sodium acetate buffer with 0.1% sodium azide (pH 4.75). Standard curves of cAMP were prepared in buffer at concentrations of 2–500 fmol/assay tube. To improve assay sensitivity, all samples and standards were acetylated with 10 μl of a 2:1 solution of triethylamine:acetic anhydride. Samples were assayed in duplicate. Each assay tube (total volume 300 μl) contained 25 μl of each sample, 75 μl buffer, 100 μl primary antibody (sheep, anti-cAMP, 1:100 000 dilution with 1% BSA in buffer) and 100 μl [^{125}I]-cAMP (50 000 dpm/100 μl of buffer). Tubes were vortexed and stored at 4° C overnight (approximately 18 h). Antibody-bound radioactivity was separated by the addition of 25 μl BioMag rabbit, anti-goat IgG (Advanced Magnetics, Cambridge, Mass.), followed by vortexing and incubation at 4 °C for 1 h. To these samples, 1 ml 12% polyenthylene glycol/50 mM sodium acetate buffer (pH 6.75) was added and tubes were centrifuged at 1700 *g* for 10 min. Supernatants were aspirated and radioactivity in the pellet was determined using an LKB Wallac gamma counter (Gaithersburg, Md.).

Data analysis for cAMP accumulation studies

Data for each sample were expressed initially as pmol/mg per min cAMP. Baseline values of cAMP were subtracted from the total amount of cAMP produced in each drug condition. Data for each drug were expressed relative to the stimulation produced by 100 μM DA.

Results

Radioreceptor binding

Native rat tissue

LSD and its *N*-ethyl analog (EthLAD) bound with high affinity ($K_{0.5} < 30$ nM) to D_1 labeled sites in rat striatum, whereas the *N*-allyl compound (AllylLAD) displayed somewhat lower affinity (see Table 1 and left panel of Fig. 2). The affinities of the LAMIDE and LASBA analogs were similar to that of LSD. In most cases, the D_1 receptor affinity of the lysergamides

Table 1 Summary of affinities of LSD and analogs at dopamine and serotonin receptors in rodent brain. Radioligand binding studies for dopamine receptors were conducted in rat striatal homogenates, using 0.3 nM ^{3}H-SCH23390 (D_1 sites) or 0.07 nM ^{3}H-spiperone in the presence of 50 nM unlabeled ketanserin (D_2 sites). Competition curves were analyzed by nonlinear regression to determine estimates for the $K_{0.5}$ and Hill slope (n_H). $5HT_2$ affinity was assessed by competition with 0.75 nM ^{3}H-ketanserin in rat frontal cortex. Data represent the mean and standard error from at least three independent assays for each test compound

Drug	D_1 Binding:rat $K_{0.5}$ (nM)	n_H	D_2 Binding: rat $K_{0.5}$ (nM)	n_H	$5HT_2$ Binding: rat $K_{0.5}$ (nM)
LSD	27.1 ± 4.8	0.81 ± 0.03	6.43 ± 0.57	0.71 ± 0.04	4.3 ± 0.2
R-LASBA	22.6 ± 2.9	0.82 ± 0.06	5.57 ± 0.98	0.70 ± 0.03	5.0 ± 0.2
S-LASBA	30.3 ± 9.2	0.81 ± 0.08	11.2 ± 3.8	0.73 ± 0.06	18.8 ± 1.2
LAMIDE	25.9 ± 8.0	0.80 ± 0.04	7.11 ± 1.8	0.79 ± 0.07	28.1 ± 4.5[a]
EthLAD	22.1 ± 6.8	0.81 ± 0.02	4.4 ± 0.69	0.74 ± 0.02	5.2 ± 0.4[b]
AllylLAD	189 ± 6.5	0.67 ± 0.04	12.3 ± 3.1	0.68 ± 0.01	8.1 ± 0.6[b]
SCH23390	0.39 ± 0.04	1.01 ± 0.05	N.D.	–	N.D.
CPZ	N.D.	–	0.85 ± 0.13	0.96 ± 0.17	N.D.

[a]Data from Huang et al. (1994)
[b]Data from Nichols et al. (1991)

was similar to that of the prototypical D_1 agonist SKF38393. The test compounds also competed with high affinity for D_2 receptors in the rat striatum (Table 1); with one exception (AllylLAD), the D_2 affinities were approximately 4-fold greater than those for D_1 receptors. There was limited stereoselectivity at either D_1 or D_2 receptors for the *R*- and *S*-LASBA compounds. All of the lysergamides tested displayed shallow Hill slopes ($n_H < 1.0$) at both D_1 and D_2 receptors. Table 1 also provides information on the affinity of these lysergamide compounds for ^{3}H-ketanserin labeled ($5HT_2$) sites in the rat frontal cortex; LSD, *R*-LASBA and EthLAD displayed the high affinity ($K_{0.5}$ ca. 5 nM) for these sites.

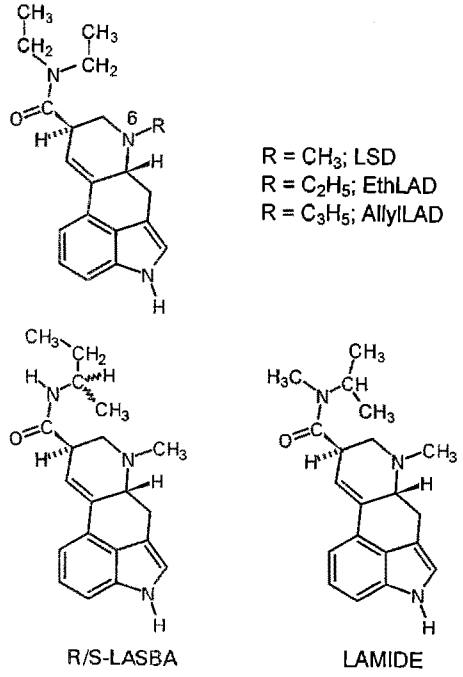

Fig. 1 Schematic representation of the structures of the lysergamide derivatives used in the present experiments

C-6 glioma cells

Prior to examining the functional properties of LSD and analogs in C-6-mD_{1A} cells, we examined the binding potencies of selected compounds i n this cell system. In addition to LSD, the two *N*(6)-substituted analogs were selected for testing because they displayed the highest (EthLAD) and lowest (AllylLAD) affinity for D_1 receptors in the rat striatum. As shown in Fig. 2 (right panel) and Table 2, the rank order of binding potency of LSD and *N*(6)-substituted analogs in C-6mD_1 cells was identical to the rat striatal data. Moreover, the absolute affinities ($K_{0.5}$ values) of these compounds at the human D_1 receptor expressed in C-6-mD_{1A} cells were similar to that seen in rat striatal membranes.

Table 2 Summary of binding affinities and functional activity of LSD and analogs at dopamine D_1 receptors in C-6-mD_{1A} cells. Radioligand binding studies for dopamine D_1 receptors (labeled with 0.3 nM ^{3}H-SCH23390) were conducted in membranes prepared from C-6-mD_{1A} cells. Competition curves were analyzed by nonlinear regression (using a sigmoid algorithm) to determine $K_{0.5}$ values and Hill coefficients (n_H). As a functional endpoint, the ability of each compound (10 μM) to stimulate cAMP accumulation was measured in C-6-mD_{1A} cell membranes in the presence or absence of 10 μM dopamine. Data for binding and functional measurements represent the mean values from 2–4 independent experiments

Drug	D_1binding: C-6 mD_1cells $K_{0.5}$(nM)	n_H	cAMP accumulation (% 100 μM dopamine Drug alone (10 μM)	Drug +10 μM DA
DA	106[a]	0.57	96 ± 3	N.D.
LSD	30.3	0.70	38 ± 5	48 ± 8
R-LASBA	N.D.	–	40 ± 4	54 ± 14
S-LASBA	N.D.	–	39 ± 3	35 ± 8
LAMIDE	N.D.	–	46 ± 7	47 ± 1
EthLAD	23.7	0.62	57 ± 7	68 ± 8
AllylLAD	183.0	0.59	42 ± 11	63 ± 8
DHX (+)	4.5[a]	0.57	95 ± 5[a]	N.D.
SKF38393 (+)	17.1[a]	0.58	38 ± 5	54 ± 4
SCH23390	0.54	0.99	N.A.	N.D.

[a] Data from Watts et al. (1993)

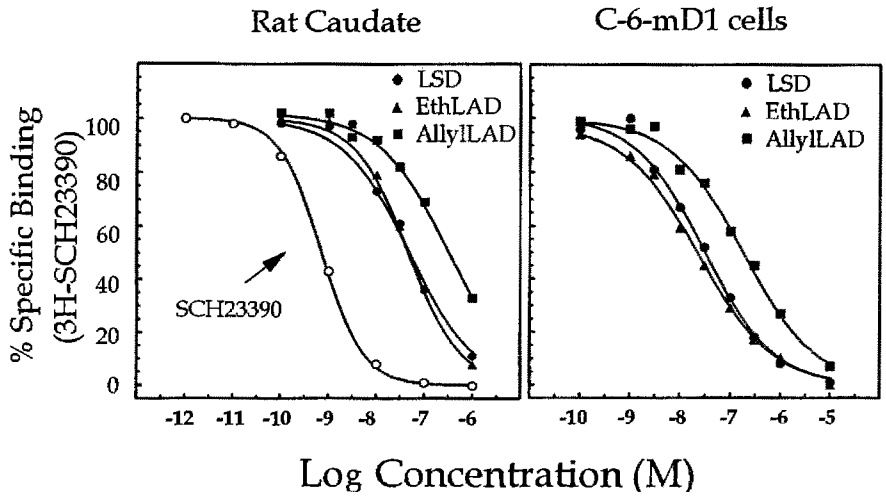

Fig. 2 Lysergamides bind with similar affinity to D_1 receptors in rat striatum and C-6-mD_{1A} cells. D_1 receptors were labelled with 0.3 nM ^{3}H-SCH23390. Competition curves were analyzed by nonlinear regression. Two or three independent assays were conducted for each test compound

Adenylate cyclase activity

C-6-mD_{1A} cells

We examined the ability of LSD to stimulate cAMP accumulation in C-6-cells expressing the rhesus macaque D_1 dopamine receptor (see Fig. 3 and Table 2). All compounds were tested at 10 μM; based on the D_1 binding affinities, this concentration was expected to occupy the large majority of D_1 receptors. LSD and the analogs tested displayed partial agonist activity, stimulating cAMP accumulation to a lesser extent than either DA or the full agonist dihydrexidine (DHX; Lovenberg et al. 1989; Brewster et al. 1990; Mottola et al. 1992). Interestingly, the extent of cAMP activation by LSD was similar to that of the prototypical D_1 ligand SKF38393. As can be seen in Fig. 3, the lysergamide compounds (and the benzazepine D_1 agonist SKF38393) were able to antagonize the effects of DA at D_1 receptors. The effects of LSD were completely blocked by the D_1 antagonist SCH23390 (data not shown).

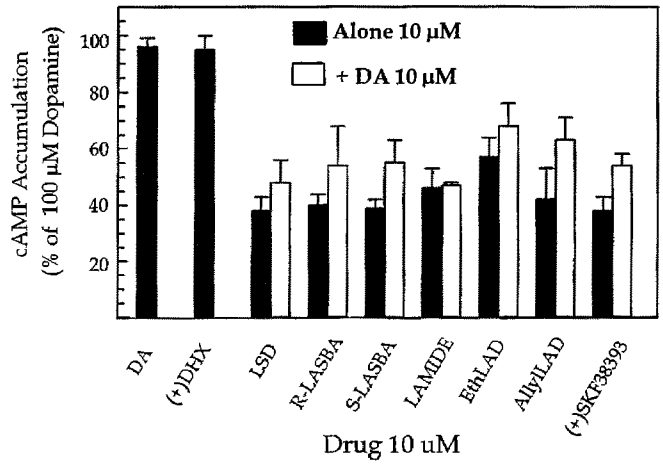

Fig. 3 LSD and related lysergamides are partial agonists at D_1 receptors. The ability of lysergamide analogs to stimulate and block dopamine-mediated adenylate cyclase activity was measured in C-6-mD_{1A} cell membranes. The high efficacy agonist (+)-DHX and the partial agonist (+)-SKF38393 were included for comparison purposes. Data represent means ±SEM from four experiments

Discussion

Our results indicate that LSD and certain other lysergamide analogs are potent dopaminergic ligands that possess agonist activity at D_1 dopamine receptors. These data represent the first clear demonstration of the interaction of hallucinogenic lysergamides with DA D_1 receptors. Although the affinities of LSD and its analogs are somewhat lower for D_1 than for either D_2 or $5HT_2$ sites, it is likely that a significant population of D_1 receptor sites are occupied at drug doses that produce psychoactive effects. This suggests the need for a re-examination of the potential contributions of dopamine D_1 receptor-mediated actions to the diverse biobehavioral effects of LSD.

The present results are consistent with several previous reports suggesting that LSD is a partial agonist at dopamine receptors linked to adenylate cyclase in native tissues (Von Hungen et al. 1974; Da Prada 1975; Spano et al. 1975; Ahn and Makman 1979). It is important to note, however, that D_1 selective antagonists were not available at the time these earlier studies were conducted; thus, it was not possible to attribute the actions of LSD directly to D_1 receptors. Some of these earlier studies reported that both haloperidol and chlorpromazine blocked the stimulatory actions of LSD on adenylate cyclase (Von Hungen et al. 1974; Ahn and Makman 1979). Although both of these drugs are primarily regarded as dopamine antagonists (with some selectivity for D_2 receptors), they also are antagonists for other receptors (e.g., α-adrenergic and histaminergic) at the concentrations at which they were employed. The present data indicate conclusively that the stimulation of cAMP synthesis in these systems was due primarily to activation of D_1 receptors.

The finding that the D_1 receptor affinities and efficacies of the lysergamides are similar to those of the prototypical D_1 ligand SKF38393 underscores the potential functional implications of our data. For example, it is well known that SKF38393 can act synergistically to augment both the behavioral and electrophysiological actions of dopamine D_2 selective ligands (e.g., Braun and Chase 1986; Hu and Wang 1988). With respect to the actions of LSD-like compounds, it is conceivable that the D_1 actions could potentiate the effects of that same compound (or of endogenous DA) acting at D_2 receptors. The partial agonist actions of LSD and other lysergamides in C-6-mD_{1A} glioma cells also have important implications for predicting the extent of activation at D_1 receptors in mammalian brain. Specifically, the functional effects of these compounds are expected to vary widely, depending upon the characteristics of the target tissue (see Kenakin 1987). For example, the partial D_1 agonist SKF38393 produces a maximal response indistinguishable from that of either DA or the high efficacy agonist dihydrexidine (DHX) in a system with a large number of spare receptors (Watts et al. 1993). Inasmuch as there is suggestive evidence that the number of spare receptors may vary significantly across species, brain region, and developmental period (e.g., see Pifl et al. 1991; May and Sugawa 1993), the dopaminergic effects of LSD would be expected to vary concomitantly.

It is notable that much of the work to clarify the mechanisms underlying hallucinogenic potency has been conducted in rodents. This species may not be suitable for examining the possible influence of D_1 receptors, however, because there are striking differences in both the chemoanatomy and the functional role for D_1 receptors in primate versus rodent brain (see Berger et al. 1991; Berger 1992; Goldman-Rakic et al. 1992). In rodent brain, dopaminergic innervation to cortex is restricted to prefrontal, entorhinal, and anterior cingulate cortical areas. In contrast, dopaminergic innervation occurs throughout the cortical mantle in primates; D_1 receptors are located in the molecular layer in all cortical areas, and also display a bilaminar pattern in certain regions. This expanded distribution of dopaminergic cortical input (and D_1 receptors) parallels the enhanced importance of dopamine neurotransmission in a number of complex cognitive tasks in primates, including memory-related operation (Sagwaguchi and Goldman-Rakic 1991). Important species differences in dopaminergic systems may underlie the inconsistencies reported in the extent of stimulation of adenylate cyclase by LSD (presumably mediated by dopamine receptors) in different brain regions between rat versus primate (cf. Spano et al. 1975; Ahn and Makman 1979).

The need for a re-examination of a possible dopaminergic involvement is psychotomimetic effects in primates is strengthened further when one considers the current focus on DA-5HT interactions in the etiology and treatment of psychosis (Meltzer and Nash 1991; Joyce 1993; Leysen et al. 1993). It appears that many of the atypical antipsychotics have significant affinity for $5HT_2$ receptors (in addition to dopamine D_2 receptors), and this may confer special benefits with respect to reduced propensity for extrapyramidal symptoms and a reduction in negative symptoms. More recently, the use of a D_1 antagonist has been suggested for the treatment of psychosis (McQuade et al. 1992), although it is not clear to what extent the antispsychotic effects of D_1 receptor antagonists might be modified by concurrent manipulation of serotonergic systems. Because LSD has high affinity at a variety of both serotonin and dopamine receptors, it seems likely that these interactions play important roles in defining the overall action of the drug.

Finally, the data from the present study provide some interesting observations for discerning structure-activity relationships. The D_1 receptor affinities (and functional activity) of the lysergamide compounds tested were similar in most cases. In contrast, there was more variability in $5HT_2$ receptor affinity (for the ^{3}H-ketanserin site) among these compounds. This observation indicates a lack of sensitivity of the D_1 receptor, but not the $5HT_2$ receptor, to the structural modifications explored in the present study. For example, it appears that the dopamine D_1 receptor is not sensitive to the stereochemistry of the *N*-butylamides of LSD (*R*- and *S*-LASBA). In contrast, the $5HT_2$ receptor site appears to be quite sensitive to this specific stereochemical influence (Oberlender et al. 1992). The lack of strong covariation in D_1 and $5HT_2$ receptor affinities among compounds suggests that, within this series of compounds, it may be possible independently to manipulate the actions of the lysergamides at these two biological targets. This ability should prove useful for elucidating the possible contribution of D_1 receptor actions to the serotonin-mediated effects of the lysergamides. It should also be noted that this structural series may be of some use in molecular modeling studies to understand the binding and activation of D_1 receptors.

In summary, we believe that the present data raise the possibility that D_1 receptor activation may contribute to aspects of the neurochemical, behavioral and perceptual sequelae elicited by LSD-like compounds. As noted in the Introduction, this hypothesis clearly does not preclude a primary role for serotonin receptors (e.g., $5HT_{2A}$) in hallucinogenesis. It is well established that drugs within at least two major classes of hallucinogens display a high affinity for serotonin $5HT_2$ receptors (e.g., Titeler et al. 1988), although there is still controversy concerning whether other serotonin receptor subtypes (e.g., $5HT_{1A}$ and $5HT_{2C}$) modulate aspects of hallucinogenesis (McKenna et al. 1990; Sanders-Bush and Breeding 1991). It is ironic that the effects of LSD at dopamine receptors were recognized more than 20 years ago, yet this information has not

been utilized in the intervening years. A specific role for D_1 sites did not appear to have been considered, however. It seems likely that a complete explanation of the myriad components of the LSD experience may require a consideration of the interaction of a number of different neuronal systems, including actions at dopaminergic and serotonergic systems. Thus, while serotonergic systems may play an essential role in defining the action of hallucinogenic drugs as a general class, the unique effects and high potency of LSD, in particular, may be attributed to actions in a variety of neuronal systems.

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