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Drug Discrimination and Receptor Binding Studies of *N*-Isopropyl Lysergamide Derivatives

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HUANG, X., D. MARONA-LEWICKA, R. C. PFAFF AND D. E. NICHOLS. *Drug discrimination and receptor binding studies of* N-*isopropyl lysergamide derivatives.* PHARMACOL BIOCHEM BEHAV 47(3) 667-673, 1994.—*N*-Isopropyl (IPLA), *N*-methyl-*N*-isopropyl (MIPLA), *N*-ethyl-*N*-isopropyl (EIPLA), and *N,N*-diisopropyl (DIPLA) lysergamides were evaluated for lysergic acid diethylamide (LSD)-like activity. In rats trained to discriminate 0.08 mg/kg LSD tartrate from saline, each of the subject compounds completely substituted, with an ED_{50} two to three times larger than that of LSD except for DIPLA, which had an ED_{50} about eightfold greater. Similarly, all the compounds displaced [^{125}I](*R*)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ([^{125}I]DOI) from rat cortical homogenates and displaced [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) from rat hippocampal homogenates with K_i values similar to those of LSD, again with the exception of DIPLA, which had about nine- and fourfold lower affinities, respectively. Interestingly, all the compounds had four- to fivefold lower affinities than LSD in displacing [^{3}H]ketanserin from 5-HT_2 binding sites. Molecular modeling studies found that all the compounds had low energy conformations similar to LSD. No correlation between the activity of the compounds and the preferred conformation of the amide substituents was apparent. In summary, *N*-alkyl-*N*-isopropyl analogs of LSD retain LSD-like activity in drug discrimination and 5-HT_{1A} and 5-HT_2 agonist binding assays only until the *N*-alkyl substitution is as large as ethyl; LSD-like activity dramatically drops when the second alkyl substituent is *N*-isopropyl.

Lysergic acid diethylamide (LSD) | *N*-Isopropyl lysergamides | Drug discrimination (DD) | [^{3}H]Ketanserin binding | [^{125}I]R-DOI binding | [^{3}H]8-OH-DPAT binding | Molecular modeling | Hallucinogens | 5-HT_2 receptor

FROM studies relating structure and activity, it is well known that minor molecular modifications of ergot alkaloids produce surprisingly great changes in pharmacological effect (43). Included among these compounds are various amides of lysergic acid. Lysergic acid diethylamide (LSD) is one of the "classic" hallucinogenic agents, but structure-activity studies of lysergamide hallucinogens have largely been neglected since the late 1950s (1,14,20,23). Within the past eight years or so, our laboratory has begun to devote some effort toward filling in some of the obvious gaps that exist in our understanding of this class of hallucinogen.

Because of its structural complexity, there are a number of locations in the lysergamides (Fig. 1) that one could modify for structure-activity studies (11,23,40). Recently, we have reported that the stereochemical properties of the amide substituent of hallucinogenic lysergamides can exert a critical influence on activity. (*R*)- and (*S*)-2-butylamides of lysergic acid completely substituted for LSD in rats trained to discriminate LSD from saline. Both isomers possessed high affinity for [^{125}I](*R*)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ([^{125}I](*R*)-DOI)- and [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT)-labeled binding sites. However, the amide of (*R*)-2-butylamine possessed somewhat higher potency (37).

The focus of this study was to examine further the effects on LSD-like activity of substitution on the amide group of lysergamide. The *N*-isopropylamides were appealing for at least two reasons. First, the *N*-methyl-*N*-isopropyl compound (MIPLA) is an isomer of LSD and would be presumed to have similar lipophilicity and pharmacokinetic parameters. Second,

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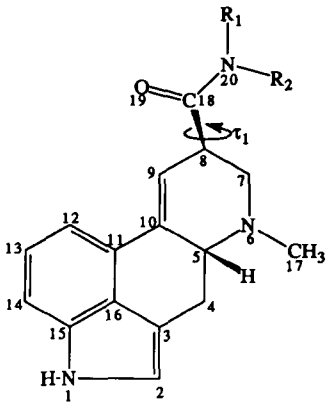

	R_1	R_2
LSD	$-CH_2CH_3$	$-CH_2CH_3$
IPLA	-H	$-CHCH_3$ CH_3
MIPLA	$-CH_3$	$-CHCH_3$ CH_3
EIPLA	$-CH_2CH_3$	$-CHCH_3$ CH_3
DIPLA	$-CHCH_3$ CH_3	$-CHCH_3$ CH_3

FIG. 1. Schematic representation of the structure of LSD derivatives, showing the atom numbering used, the position of τ_1, and the amide substitutions reported in this work.

the isopropyl group is only one carbon larger than an ethyl, but because of its branch provides less conformational mobility than an ethyl. It was anticipated that biological assay and conformational analysis of this small series of *N*-alkyl-*N*-isopropyl compounds might afford additional insight into the reasons for the importance of the amide substituent in lysergamides.

Hallucinogenic activity in humans can be modeled in the drug discrimination (DD) paradigm by studying LSD-like discriminative stimulus (DS) properties in animals (17,33). In a typical procedure, rats are trained to press levers for positive reinforcement and to recognize the interoceptive state associated with the actions of a particular dose of LSD at a particular time after administration. The DS properties of LSD have been studied extensively and seem to be mediated critically by agonist activity at 5-HT_2 receptors (5,16,17,34,42). Therefore, the DD paradigm was employed as a means of evaluating these LSD analogs in rats. In addition, receptor binding studies were employed as an in vitro assessment of the relative 5-HT_2 and 5-HT_{1A} receptor interactions for the four *N*-alkyl-*N*-isopropyl lysergamides. This was accomplished by measuring affinity for the [^{125}I](*R*)-DOI-labeled and the [^{3}H]ketanserin-labeled 5-HT_2 receptor in rat cortical homogenates and the affinity for [^{3}H]8-OH-DPAT-labeled 5-HT_{1A} receptors in rat hippocampal homogenates. Since [^{125}I](*R*)-DOI also binds with high affinity to the 5-HT_{1C} receptor (4,30,38), it is possible that this receptor subtype is also involved in hallucinogenesis. However, the focus in this work is on the 5-HT_2 receptor subtype.

METHOD

Materials

[^{125}I](*R*)-DOI, [^{3}H]ketanserin, and [^{3}H]8-OH-DPAT were purchased from New England Nuclear (Boston) at specific activities of 2200, 61, and 135.5–216 Ci/mmol, respectively. The four *N*-alkyl-*N*-isopropylamides were synthesized in our laboratory following standard methods [e.g., see (37)]. All new compounds met the usual criteria for purity including thin layer chromatography (TLC), nuclear magnetic resonance spectroscopy (NMR), and elemental analysis. (+)-LSD tartrate was obtained from the National Institute on Drug Abuse. Cinanserin was a gift from the SQUIBB Institute for Medical Research. 5-HT was purchased from Sigma (St. Louis).

Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis) weighing 175–200 g were used in all experiments. Animals were either group housed six per cage (radioligand binding experiments) or individually caged in a temperature-controlled room with a 12-h light–dark schedule (DD experiments). With the exception of rats used in the DD experiments, food and water were available ad lib at all times. In DD experiments rats were trained to discriminate LSD tartrate (186 nmol/kg, 0.08 mg/kg) from saline. LSD was administered IP dissolved in saline such that a volume of 1 ml/kg of body weight was used. None of the rats had previously received drugs or behavioral training. Water was freely available in the home cages and a sufficient amount of supplemental feeding (Purina, Lab Blox) was made available after experimental sessions so as to maintain them at approximately 80% of free feeding weight, compared with control rats (housed under the same conditions).

Drug Discrimination

Six standard operant chambers (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, all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, interfaced through a Coulbourn Instruments Dynaport to an IBM PC, controlled reinforcement and data acquisition with a locally written program.

A fixed ratio 50 (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 (35,36). 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. Training sessions were continued until an accuracy of at least 85% (number of correct presses × 100/number of total presses) was attained for 8 of 10 consecutive 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 test sessions. Rats were required to maintain the 85% correct responding criterion on training days to be tested. In addition,

test data (less than 5%) were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. 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 disruption. Treatments were randomized at the beginning of the study.

Radioligand Binding Studies

The procedure of Johnson et al. (25) was employed. Briefly, the frontal cortex or hippocampal brain regions from 20–40 male Sprague–Dawley rats (175–199 g, Harlan) were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in four or eight volumes of 0.32 M sucrose for frontal cortex or hippocampus, respectively. The homogenate was centrifuged at 36 000 × *g* for 10 min, and the resulting pellets were resuspended in the same volume of sucrose. Separate aliquots of tissue were then frozen at −70°C until assay.

For each separate experiment, a tissue aliquot was thawed slowly and diluted 1 to 25 with 50 mM Tris HCl (pH = 7.4). The homogenate was then incubated at 37°C for 10 min and centrifuged twice at 36 500 × *g* for 10 min, with an intermittent wash. The resulting pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na_2EDTA, 0.1% Na ascorbate, and 10 μM pargyline HCl (pH = 7.4). In experiments with [^{125}I](*R*)-DOI and [^{3}H]ketanserin, either 10 mM $MgCl_2$ or 5.7 mM $CaCl_2$ were included, respectively. A second preincubation for 10 min at 37°C was conducted, and the tissues were then cooled in an ice bath.

All experiments were performed with triplicate determinations using the appropriate buffer, to which 200–400 μg of protein was added, giving a final volume of 1 ml. The tubes were allowed to equilibrate for 15 min at 37°C before filtering through Whatman GF/C filters using a cell harvester (Brandel, Gaithersburg, MD), followed by two 5-ml washes using ice-cold Tris buffer. Specific binding was defined as that displaceable with 10 μM cinanserin in both the [^{3}H]ketanserin and [^{125}I](*R*)-DOI binding study and with 10 μM 5-HT in the [^{3}H]8-OH-DPAT binding study. Filters were air-dried and placed into scintillation vials with 10 ml of Ecolite scintillation cocktail and allowed to sit overnight before counting at an efficiency of 37% for tritium, and directly counted in a gamma counter for [^{125}I]-ligand at an efficiency of 79.4%.

[^{125}I](*R*)-DOI saturation experiments were carried out by varying the concentration of unlabeled ligand with a constant amount (0.1 nM) of radioligand, according to the procedure described previously (22,24,25,28). This method is accurate when the unlabeled and labeled ligands have identical physiochemical properties (32). Under the above experimental conditions, [^{125}I](*R*)-DOI was found to bind to a single site (Hill coefficient of 0.91 ± 0.03) with a B_{max} of 44.0 ± 5.67 fmol/mg protein and a K_D of 1.88 ± 0.24 nM. The ability of 8–10 concentrations of test drug to displace 0.1 nM of [^{125}I](*R*)-DOI binding was measured in drug displacement studies. Four to five concentrations of radioligand were used in both [^{3}H]-ketanserin and [^{3}H]8-OH-DPAT saturation experiments. [^{3}H]Ketanserin bound to a single site (Hill coefficient 1.08 ± 0.06) with a B_{max} of 297.3 ± 39.6 fmol/mg protein and a K_D of 1.37 ± 0.20 nM. [^{3}H]8-OH-DPAT bound to a single site (Hill coefficient 1.00 ± 0.01) with a B_{max} of 119 ± 8 fmol/mg protein and a K_D of 2.49 ± 0.23 nM. The ability of 8–10 concentrations of test drug to displace 0.75 nM of [^{3}H]ketanserin or [^{3}H]8-OH-DPAT was determined in drug displacement studies.

Molecular Modeling

Although lysergamides are relatively rigid, they do have conformational flexibility in both the amide substituent and the D-ring. In particular, the D-ring of LSD, and of most related ergolines, has been found to exist in the C(7) "flap-up" conformation in $CDCl_3$ solution (7,21,26) as well as in the crystal structure of LSD iodobenzoate monohydrate (8,9). The D-ring of LSD can undergo a conformational flip into what has been called the "flap-down" conformation, with the C(7) methylene group below the D-ring plane. The C(7)-up and C(7)-down conformations differ in energy by only about 2–4 kcal/mol and have been investigated by Andrews et al. (3). In addition, Pierri et al. (39) have reported that ergotamine exists in the C(7) flap-down conformation in $CDCl_3$ solution. For the current work, however, the D-ring was assumed to be in the conventional flap-up conformation. The amide substituent has a much greater degree of conformational flexibility than the D-ring and is the focus of the conformational studies in this article.

Classical molecular mechanics were performed on LSD and its isopropyl amide derivatives. The molecular flexibility of the compounds is principally described by one dihedral angle, τ_1 (identified in Fig. 1), formed by the O(19)–C(18)–C(8)–C(7) atom set. The dihedral angle was investigated by performing geometry searches, or grid scans, with minimization at each step. The structure as drawn illustrates the compounds with $\tau_1 \approx 180°$. The angles were searched over the range −180° to +180° in 5° increments. To avoid sterically controlled false minima the searches were performed twice, once in the clockwise direction (−180° to +180°) and once in the counterclockwise direction (+180° to −180°), with the smaller energy at each angle being used in the analysis.

All computations were performed on a CAChe Scientific worksystem running CAChe proprietary software, including their implementations of Extended Hückel, ZINDO v3.0, and MOPAC v6.10 (19). CAChe molecular mechanics uses Allinger's MM2 force field (2) as augmented by CAChe Scientific. With MOPAC, the AM1 Hamiltonian (18) was used. The molecular mechanics were investigated on the CAChe system (12) by minimizing the total molecular energy according to the molecular mechanics expression

$$E_{total} = E_{bonding} + E_\theta + E_\phi + E_{improp} + E_{elec} + E_{vdW} + E_{hb}$$

Further, molecular mechanics calculates energies relative to a hypothetical "perfect" geometry, rather than an absolute energy, and uses a temperature of 0 K. The structures cited here are those obtained from molecular mechanics, and the calculated structure of LSD is in good agreement with the published X-ray results (9).

Calculations were performed in vacuo, and no attempt was made to consider the effect of solvation. In addition, the conformations of the lysergamides where the *N*(6)-methyl was equatorial were of lower energy than those in which the methyl was axial.

Statistical Analysis

In the DD study, data were scored in 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. If that drug was one that completely

substituted for the training drug (at least one dose resulted in %SDL = 80% or higher), the method of Litchfield and Wilcoxon (27) was used to determine the ED_{50} and 95% confidence interval (CI). In the receptor binding studies, data were analyzed using the computer programs EBDA and Ligand as described by McPherson (28). The values from three to four separate experiments were combined. Protein determinations were made using the procedure of Bradford (10).

RESULTS

Drug Discrimination

The results of the drug discrimination testing are given in Table 1, and the radioligand displacement data are in Table 2. *N*-Isopropyl (IPLA), MIPLA, and *N*-ethyl-*N*-isopropyl (EIPLA) all substituted in LSD-trained rats with a potency about two- to threefold less than LSD. DD potencies most closely parallel the binding data for [^{125}I]DOI displacement. *N,N*-Diisopropyl (DIPLA) is the only one in the series far less potent (about eightfold) than LSD in the DD paradigm.

Radioligand Binding Studies

At [^{3}H]8-OH-DPAT binding sites, IPLA, MIPLA, and EIPLA all have affinities similar to LSD. However, the affinity of DIPLA for [^{3}H]8-OH-DPAT sites is about four times lower than LSD. At [^{3}H]ketanserin binding sites, all four LSD analogs have about four- to eightfold lower affinities than LSD. At [^{125}I]DOI sites, IPLA and MIPLA have affinities similar to LSD, EIPLA has slightly less affinity, and DIPLA has the lowest affinity of the series, about ninefold less than that of LSD.

Molecular Modeling

The outcome of the geometry searches of τ_1 for the compounds is given in Fig. 2. All of the compounds have similar minimum-energy conformations. The relative energy at unfavored conformations (e.g., $\tau = 180°$) varies considerably, but does not correlate with the potencies of the compounds. It is perhaps likely that only one particular conformation of the amide group allows binding at each of the receptors considered here; however, the present series of compounds possesses enough conformational mobility in the amide region that the preferred conformation can be easily reached by any of the compounds. Given the present data, the variation in potency among these five compounds can best be explained by the presence of a hydrophobic pocket in the receptor which best accommodates about a four-carbon hydrophobic alkyl substitution on the amide nitrogen; the most potent compounds in

TABLE 1

DRUG DISCRIMINATION DATA FROM LSD-TRAINED RATS

Drug	Dose (mg/kg)	*N*	*D*	%SDL	ED_{50} (95% CI) (mg/kg)	ED_{50} (95% CI) (nmol/kg)
1. LSD	0.01	9	0	11		
	0.02	14	0	36		
	0.04	13	1	83	0.021 (0.014-0.032)	48 (32-73)
	0.06	10	3	100		
	0.08	13	0	100		
2. IPLA	0.04	8	2	37		
	0.06	9	1	78	0.046 (0.036-0.058)	110 (86-140)
	0.08	10	1	90		
	0.10	10	2	100		
3. MIPLA	0.02	14	3	9		
	0.04	9	2	56		
	0.06	9	2	89	0.036 (0.024-0.056)	85 (55-130)
	0.08	10	1	90		
	0.10	10	1	100		
4. EIPLA	0.04	11	3	13		
	0.06	11	1	60	0.059 (0.047-0.073)	133 (108-164)
	0.08	11	1	80		
	0.10	11	1	90		
5. DIPLA	0.08	7	0	0		
	0.10	10	3	13	0.161 (0.115-0.226)	351 (250-493)
	0.20	10	1	67		
	0.40	10	2	100		

The ED_{50} values for substitution were calculated according to the method of Litchfield and Wilcoxon (27). For all dose-dependent curves, the slopes are not significantly different (all are parallel). *N* = number of animals tested, *D* = number of animals disrupted (50 presses not completed in 5 min), %SDL = percentage of nondisrupted animals that selected drug level.

TABLE 2

RESULTS OF LSD AND ITS ANALOGS DISPLACING [^{3}H]8-OH-DPAT, [^{3}H]KETANSERIN AND [^{125}I]DOI BINDING

	[^{3}H]8-OH-DPAT		[^{3}H]Ketanserin		[^{125}I]DOI	
	K_i(nM)	n	K_i(nM)	n	K_i(nM)	n
LSD	4.41 ± 0.77	4	4.78 ± 0.53	3	1.43 ± 0.13	4
IPLA	5.23 ± 0.15	3	26.2 ± 0.23	3	1.73 ± 0.26	4
MIPLA	4.60 ± 0.35	3	28.1 ± 4.5	4	1.75 ± 0.27	4
EIPLA	3.67 ± 0.48	3	16.8 ± 2.0	4	3.26 ± 0.19	3
DIPLA	17.9 ± 2.8	3	17.2 ± 2.0	3	12.5 ± 1.8	4

the group (MIPLA and LSD) each have four carbons comprising the amide alkyl chains. Potency declines as the total number of carbons deviates from this ideal.

DISCUSSION

All the *N*-isopropyl analogs of LSD displaced [^{3}H]8-OH-DPAT and [^{125}I](*R*)-DOI binding with affinities similar to LSD except for DIPLA, which had affinity about four- and ninefold lower than LSD for [^{125}I](*R*)-DOI and [^{3}H]8-OH-DPAT binding sites, respectively. All of the four compounds bound to [^{3}H]ketanserin-labeled sites with affinities similar to each other but all had lower affinity than LSD at this site. Curiously, LSD is unique among these five compounds in that it alone has high affinity both at [^{3}H]ketanserin sites and at [^{125}I]DOI-labeled sites; the *N*-isopropyl analogs have four- to eightfold lower affinity at [^{3}H]ketanserin sites than LSD. Perhaps this factor is related to its unique behavioral potency.

The results of the drug discrimination studies show that all the isopropyl analogs tested except for DIPLA fully substituted in LSD-trained rats with ED_{50}s two- to threefold higher than that of LSD. The degree of decrease in potency of DIPLA in the DD paradigm seems to correlate best with the extent of drop in the affinity at the 5-HT_2 agonist binding site. This would be consistent with the cue's being mediated by a 5-HT_2 agonist effect (4,16,17,34,42). However, a role for the 5-HT_{1A} receptor cannot be excluded, particularly if a 5-HT_{1A} effect is synergistic with the 5-HT_2 agonist action (6,33).

The role of the lone pair electrons of the tertiary nitrogen atom in the ergoline D-ring has been discussed extensively with respect to binding forces of the drug-receptor interaction (15,21,29,31,44). For the purposes of the work reported here, it is assumed that the electron pair on N(6) points toward the α (lower) face of the molecule, corresponding to the C(7) flap-up conformation.

On the basis of energy and conformational considerations alone, one would expect in vivo experiments to show little, if any, reduction in the potencies of IPLA, MIPLA, EIPLA, and DIPLA relative to LSD if the compounds adopt a conformation at the receptor similar to their minimum-energy conformation in vacuo. Similarly, if the receptor constrains the compounds to adopt a higher energy conformation, one would expect their potencies to be correlated with the relative ease with which they adopt such a nonpreferred conformation (e.g., decreasing potencies of IPLA > MIPLA > LSD > EIPLA > DIPLA if a configuration of $\tau = -180°$ must be adopted). The fact that neither such prediction is confirmed points to the conclusion that specific steric and hydrophobic constraints in the amide-binding region of the receptor out-

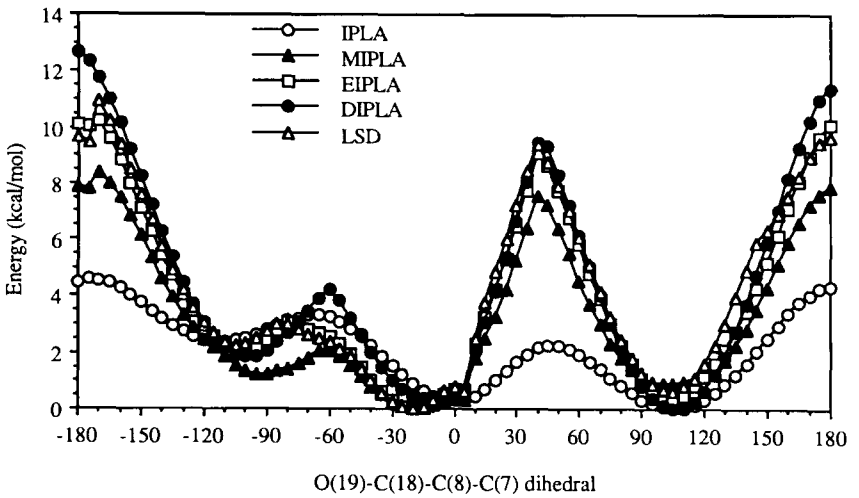

FIG. 2. Potential energy of the subject compounds as a function of τ_1. Each compound's energy has been taken relative to its global minimum (energy of the global minimum is 0 kcal/mol). From top to bottom, τ_1 = 180°, 105°, 45°, −15°, and −60°, respectively.

weigh rotational energy differences about the C(8)-C(carbonyl) bond.

One noteworthy point to consider is the fact that the clinical literature on hallucinogens contains no report of a compound with potency comparable to LSD. Even the closely related *N*-methyl-*N*-ethyl and *N*-ethyl-*N*-allyl lysergamides, which one might expect not to differ significantly in receptor binding and pharmacokinetics from LSD, have merely 30% of the potency of LSD in humans (41). The rat behavioral data in the current study also indicates a unique potency for LSD. As was noted previously, perhaps this finding is related to the particularly high affinity of LSD for the [^{3}H]ketanserin-labeled site. What subjective differences in experience such pharmacological changes may produce must await human trials.

In summary, *N*-isopropyl analogs of LSD, except for DIPLA, retain LSD-like behavioral effects in DD experiments and a pharmacological profile generally similar to that of LSD in [^{3}H]8-OH-DPAT, [^{3}H]ketanserin, and [^{125}I]DOI binding experiments. The DD potency parallels affinity for [^{125}I]DOI-labeled 5-HT$_2$ sites, indicating that all of the compounds are potent 5-HT$_2$ agonists. Though the compounds are likely to have hallucinogenic effects in man, the qualitative differences in these effects produced by the observed variations in receptor affinities will not be known until human studies can be conducted.

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