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Stereospecific Receptor Sites for *d*-Lysergic Acid Diethylamide in Rat Brain: Effects of Neurotransmitters, Amine Antagonists, and Other Psychotropic Drugs

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SUMMARY

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A method *in vitro* designed to determine specifically the stereospecificity of tissue receptor sites was used to study the characteristics of *d*-lysergic acid diethylamide (LSD) binding by rat brain particulates and the effects of selectively chosen drugs. There is a high- and a low-affinity binding, and both are stereospecific. The high-affinity binding is saturable (half-saturation at 4 nM) and shows definite regional and subcellular differences. The highest binding relative to protein content occurs in the striatum regionally and in microsomal fractions subcellularly. The subcellular distribution data also suggest that the *d*-LSD acceptor substance need not be confined to the neuronal soma or terminal membrane. The effects of related hallucinogens, neurotransmitters, serotonin (5-HT) and dopamine antagonists, and other drugs were studied to help determine whether central 5-HT receptors are identical with a site of LSD binding. Of the drugs tested, methiothepin was most effective in blocking the high-affinity, stereospecific binding of *d*-LSD. The pattern of drug effects suggests that the high-affinity, stereospecific binding site on brain membranes may not be identical with a 5-HT or dopamine receptor, but that LSD and nonpsychotomimetic congeners can bind to such receptors while simultaneously binding to one or more other points on the membrane in the immediate vicinity of the receptor. This is consistent with the view that LSD can act either agonistically or antagonistically at central 5-HT, and possibly dopamine, receptors *in vivo*.

INTRODUCTION

A growing body of data indicates that the potent biological effects of *d*-lysergic acid diethylamide are mediated by its interactions with the brain amines serotonin, dopamine, and norepinephrine (2-11). LSD[3] readily enters the brain and penetrates cell membranes. It has extremely potent agonist action on serotonergic neurons (12). Theories about LSD emphasize interactions particularly at 5-HT receptors, with either agonist or antagonist ef-

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[3] The abbreviations used are: LSD, lysergic acid diethylamide; 5-HT, serotonin (5-hydroxytryptamine).

fects or both (13). However, the precise nature of the acceptor site for LSD on neuronal membranes has been elusive.

With radioautography of freeze-dried sections, Diab *et al.* (14) observed "firm" binding of [^{3}H]LSD *in vivo* to the periphery of the neurons and their processes within brain regions known to contain 5-HT; these observations are consistent with binding to 5-HT receptors. Farrow and Van Vunakis (15), using equilibrium dialysis, observed the high-affinity binding (as well as medium- and low-affinity binding) of [^{3}H]LSD to rat cerebral cortex synaptosomes and synaptic membranes *in vitro*.

Our preliminary studies of the effect of LSD on 5-HT uptake in brain slices and subcellular particulates indicated that LSD binds to neuronal and subneuronal membranes (16). We studied the binding of [^{3}H]LSD of high specific activity by brain fractions and subfractions to determine whether it was stereospecific and, through drug interactions, to determine some characteristics of this site and its possible identity with a 5-HT receptor.

As this report was in preparation, other studies bearing on the stereospecific binding of LSD appeared. Bennett and Aghajanian (17, 18) investigated high-affinity binding of [^{3}H]LSD in rat brain homogenates which appeared to be stereospecific and was diminished by 5-HT; they suggested that the receptor for this binding may mediate the physiological action of LSD in inhibiting the firing of serotonergic neurons. Bennett and Snyder reported a high-affinity, stereospecific binding site for LSD in rat and monkey brain membranes which they concluded was the postsynaptic 5-HT receptor (19, 20); they also proposed that LSD and 5-HT bind to two distinct conformations of the same postsynaptic 5-HT receptor (20).

The experiments reported here show that a high-affinity, stereospecific LSD binding site on neuronal membranes exists and suggest that the binding of LSD to this site involves a 3-point attachment to the membrane by the lone-pair electrons of the 3 nitrogen atoms in the molecule. Therefore this site may be larger than a 5-HT or dopamine receptor.

MATERIALS AND METHODS

Materials. *d*-[^{3}H]Lysergic acid diethylamide was obtained from New England Nuclear Corporation (14.2 or 17.01 Ci/mmole) and from Amersham/Searle (21 Ci/mmole). Soluene-350 was obtained from Packard Instrument Company. Scintillation counting fluid was prepared by dissolving 4 g of Omnifluor (New England Nuclear) in 1 liter of scintillation-grade toluene. Drugs were supplied by the following companies: Janssen Pharmaceutical Research Laboratories, pimozide; Endo Laboratories, molindone; Eli Lilly and Company, Lilly 110-140; Hoffmann-La Roche, methiothepin; Merck and Company, cyproheptadine; McNeil Laboratories, haloperidol; Regis Chemical Company, mescaline; Sandoz Pharmaceuticals, methysergide, thioridazine, clozapine, and clothiapine; Smith Kline & French, phenoxybenzamine and chlorpromazine; and E. R. Squibb and Sons, cinanserin. Hallucinogenic drugs were supplied by the Center for Studies of Narcotic and Drug Abuse, National Institute of Mental Health. All other reagents and chemicals were supplied locally. All solutions were prepared from deionized water. Drug concentrations were calculated on a molar basis, using the molecular weight of the free base (not the salt) in each case.

Subcellular fractionation. Male Sprague-Dawley rats (200–250 g) were killed by decapitation and their brains were immediately removed. The forebrains were separated by a single transection anterior to the corpora quadrigemina (dorsally) and posterior to the mammillary bodies (ventrally), homogenized (12 strokes, 1 min) in 10 volumes of ice-cold 0.32 M sucrose in Tri-R glass homogenizers with Teflon pestles (clearance, 0.009–0.011 inch), and centrifuged at 1000 × *g* for 10 min in a Sorvall RC 2- B centrifuge. The supernatant, nuclei-free homogenate was carefully separated from the pellet (P_1) and reserved for binding and protein assays. Unless otherwise indicated, we used this P_1 supernatant (P_1S) from two rat forebrains for all binding assays. In experiments involving further subfractionation, P_1 was washed once with 5 ml of 0.32 M sucrose, the wash was added to P_1S, and

the mixture was centrifuged at 12,000 × *g* for 20 min in the Sorvall RC 2-B to give the crude mitochondrial pellet (P_2). This P_2 pellet was subfractionated into myelin (P_2A), synaptosomes (P_2B), and purified mitochondria (P_2C) according to Gray and Whittaker (21). The P_2 supernatant (P_2S) was centrifuged at 100,000 × *g* for 60 min in an International B-60 ultracentrifuge to obtain the cellular microsomal fraction (P_3). Another microsomal fraction was obtained when the P_2 pellet was subfractionated into a different set of components, following osmotic lysis by suspension in ice-cold deionized water according to Whittaker *et al.* (22). This latter procedure was modified slightly by centrifuging the five-step gradient at 100,000 × *g* for 30 min (instead of 53,500 × *g* for 120 min). This modification produced the same well-defined bands obtained by Whittaker *et al.* in a much shorter time.[4] Examination of electron micrographs[5] of these bands verified that their contents corresponded to the contents of the bands as described by Whittaker *et al.* (22) as follows: P_2D, synaptic vesicles; P_2E, microsomes and some synaptic vesicles; P_2F and P_2G, synaptosome ghosts and membrane fragments; P_2H, damaged or undisrupted synaptosomes; and P_2I, small mitochondria. All density gradient centrifugations were carried out in the SB-110 rotor of an International B-60 ultracentrifuge.

Binding assay. We used the method of Goldstein *et al.* (23) for determination of stereospecific binding. All initial and final incubations were carried out in 15-ml Corex tubes at 37° with shaking in a total volume of 2 ml of Krebs-Ringer-phosphate medium (pH 6.8–7.0) containing 1 μM pargyline, 10 mM glucose, 1 mM ascorbic acid, and 0.2 mM EDTA. Separate experiments showed that maximum binding in this medium occurs at pH 6.8–7.0. Aliquots (0.2 ml) of tissue suspension were initially incubated for 10 min without drug or with either unlabeled *d*-LSD or unlabeled *l*-LSD, each at 100-fold excess (relative to the [^{3}H]LSD concentration). This 100-fold excess was chosen on the basis of the procedure of Goldstein *et al.* (23); we checked whether more or less of an excess improved the degree of stereospecific binding and found that a 100-fold excess was optimal. After initial 10-min incubation, [^{3}H]*d*-LSD was added; for routine binding assays the concentration was varied between 2 and 4 nM, depending on the specific activity of the particular batch of [^{3}H]LSD. The tubes were incubated for 30 min, since maximum binding under these conditions was not achieved until then (Fig. 1). Following this incubation, the tubes were placed in an ice-water mixture until centrifugation at 6000–7000 × *g* for 20 min in a Sorvall RC 2-B refrigerated centrifuge. The supernatants were removed by aspiration, the pellets and walls of the tubes were flushed once with 2 ml of ice-cold 0.9% NaCl solution, and the pellets were dissolved by incubation with 1 ml of Soluene-350 at 50° for 30 min. The resulting solutions were transferred to glass counting vials, 10 ml of toluene counting fluid were added, and radioactivity was measured in a Isocap/300 scintillation counter (Searle Radiographics, Inc.). Ef-

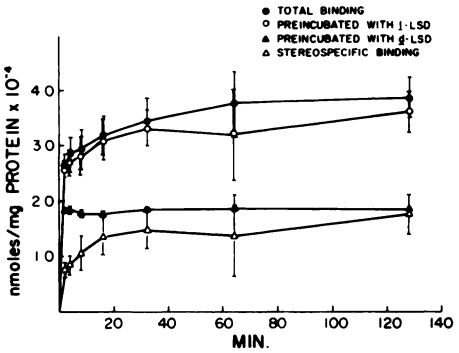

FIG. 1. *Variation of total, nonspecific, and stereospecific high-affinity binding of [^{3}H]d-LSD by rat forebrain (P_1S) suspensions with time of incubation*

Each point is the mean of quadruplicate binding assays carried out as described in MATERIALS AND METHODS. The actual amount of radioactivity for the total binding curve ranged from 3500 cpm/mg protein (8300 dpm/mg) at 2 min to 5100 cpm/mg (12,200 dpm/mg) at 128 min; for stereospecific binding, the range was 1000 cpm/mg (2400 dpm/mg) to 2300 cpm/mg (5500 dpm/mg). The specific activity of the [^{3}H]*d*-LSD was 14.2 Ci/mmol.

[4] A. E. Halaris, unpublished observations.

[5] The electron micrographs were prepared for us by Dr. Nicholas Lenn, Department of Neurology, University of Chicago.

ficiency of counting was determined from experimentally determined quenching curves for samples with identical Soluene-toluene mixtures. Radioactivity from blank tubes (no tissue) handled in the same way as sample tubes was always less than 500 cpm.

In this binding assay, preliminary incubation in Krebs-Ringer-phosphate medium alone gives total binding of [^{3}H]*d*-LSD, i.e., specific plus nonspecific binding (top curve in Fig. 1). Initial incubation with a 100-fold excess of *l*-LSD reduces nonspecific binding slightly (second curve from top, Fig. 1), while initial incubation with a 100-fold excess of *d*-LSD reduces binding substantially by blocking stereospecific binding (third curve, Fig. 1). Thus, by this assay, stereospecific binding is defined as the difference between binding after incubation with *l*-LSD and binding after incubation with *d*-LSD (bottom curve in Fig. 1). This difference was determined each time to make sure that stereospecific binding was being measured, particularly when testing the effects of drugs. The drug to be tested was always included in the initial as well as the final incubation mixture, either alone (for total binding) or along with *l*-LSD or *d*-LSD (for assay of stereospecific binding).

The protein content of 0.2-ml aliquots of the tissue suspensions was determined by the method of Lowry *et al.* (24); 0.2 ml of the P_1S forebrain suspension contained between 1.5 and 2 mg of protein. Figure 2 shows that binding was linear up until about 2 mg.

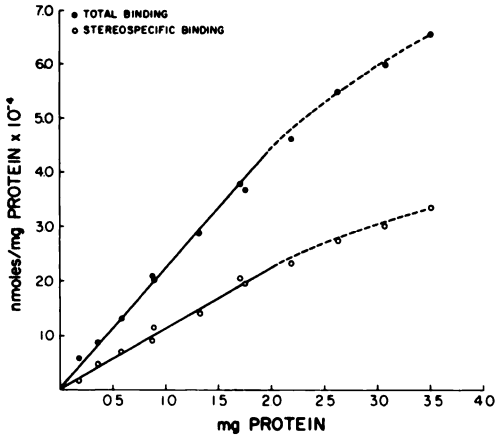

FIG. 2. *Variation of total and stereospecific high-affinity binding of [^{3}H]d-LSD with protein content of P_1S suspensions of rat forebrain*

Each point is the mean of quadruplicate assays carried out as described in MATERIALS AND METHODS. Incubation time was 30 min.

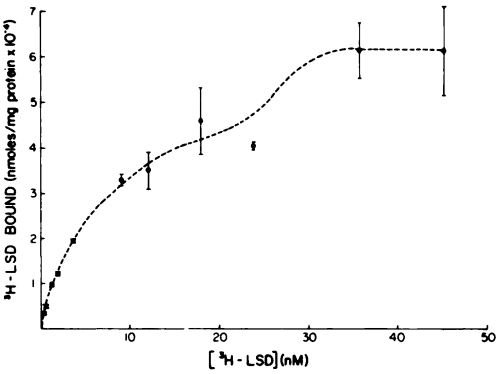

FIG. 3. *Variation of stereospecific binding of [^{3}H]d-LSD with concentration of [^{3}H]d-LSD in incubation medium*

The data from a total of seven experiments are represented; in each single experiment, a different P_1S suspension was used and the high-affinity, stereospecific binding values for at least six different concentrations were determined in quadruplicate assays as described in MATERIALS AND METHODS. Each point is the mean ± standard error for two to six trials with each concentration.

RESULTS

Variation of binding with [^{3}H]LSD concentration. The changes in stereospecific binding with increasing concentrations of [^{3}H]LSD in the assay medium are shown in Fig. 3. The shape of the curve suggests the existence of more than one binding system, with differing affinities. A reciprocal plot of the data in Fig. 3 yields two straight lines, from which half-saturation values can be graphically estimated at 4 nM for the high-affinity system and at 25 nM for the low-affinity system. The existence of more than one group or type of binding site is also suggested by the shape of the Scatchard plot (Fig. 4) of the same data. The experimental, curved Scatchard plot can be resolved into two binding lines as shown in Fig. 4. The graphical technique of Rosenthal (25), as modified by Pennock (26), was used for this purpose. It

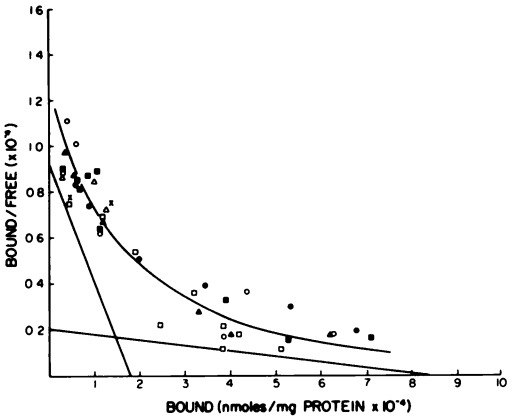

FIG. 4. *Scatchard plot of stereospecific binding data from Fig. 3*

The points shown are the means of quadruplicate binding assays on a single rat forebrain P_1S suspension; seven such suspensions (from 14 forebrains) were used, as indicated by the different symbols. The two straight binding lines were resolved from the curved, experimental Scatchard plot by the graphical technique of Rosenthal (25), as modified by Pennock (26).

is the high-affinity binding site which is studied in the remainder of this paper.

Distribution of high-affinity, stereospecific binding in rat tissues. Although there was considerable LSD binding in tissues other than brain, there was little or no stereospecific binding, with the exception of the kidney, where a small amount was observed (Table 1). The distribution of stereospecific binding in rat brain regions is also shown in Table 1. Most of the whole brain stereospecific binding was accounted for by the forebrain. Relative to protein content, the highest amount of stereospecific binding occurred in the striatum. The cortical areas selected (amygdaloid, temporal) and the septum also showed substantial binding. There was less in the midbrain raphe region. It is noteworthy that the ratio of stereospecific to total binding was highest in those regions which showed the highest stereospecific binding (see Table 1), because this indicates an enrichment of stereospecific binding sites in these regions. While there was a rough correlation between the rank orders of stereospecific binding and of the ratio of stereospecific to total binding, it would be possible to miss areas rich in stereospecific binding without specifically testing for them.

A preliminary study of the distribution of the high-affinity, stereospecific binding in eight regions of monkey brain was carried out, using one animal. Again the cortical areas and the caudate nucleus showed the highest binding per milligram of protein, and in this case the rank order of stereospecific binding was exactly the same as that of the ratio to total binding.

TABLE 1

Stereospecific binding of d-LSD by rat tissues relative to protein content and total binding

Binding was determined in P_1S suspensions as described under MATERIALS AND METHODS. Values are means and standard errors of quadruplicate assays. The values for whole brain, forebrain, and hindbrain are typical ones obtained by the routine binding assay, performed on the same day on which the brain regions were assayed. For the nonbrain tissues and brain regions, 20–25 rats were used in order to obtain 400–500 mg of tissue for all regions, and quadruplicate binding assays were performed on P_1S suspensions from this amount of tissue. The ratio of stereospecific to total (specific and nonspecific) binding were determined at the same time the stereospecific binding measurements were performed.

Tissue	Stereospecific binding	Ratio of stereospecific to total binding
	nmoles/mg protein $\times 10^{-4}$	
Whole brain	1.190 ± 0.111	0.369
Forebrain	1.348 ± 0.005	0.364
Hindbrain	0.388 ± 0.094	0.199
Amygdaloid cortex	1.578 ± 0.046	0.427
Temporal cortex	1.230 ± 0.099	0.397
Striatum	2.972 ± 0.220	0.544
Septum	1.238 ± 0.124	0.356
Thalamus	0.768 ± 0.186	0.277
Hypothalamus	0.959 ± 0.121	0.321
Raphe	0.860 ± 0.051	0.367
Medulla	0.606 ± 0.179	0.263
Cerebellum	0.381 ± 0.230	0.236
Spinal cord	0.328 ± 0.093	0.141
Liver	0.057 ± 0.098	0.026
Lung	0.022 ± 0.056	0.025
Spleen	0.066 ± 0.038	0.080
Kidney	0.272 ± 0.066	0.186
Muscle	0.002 ± 0.022	0.009

Subcellular distribution of high-affinity, stereospecific binding in rat brain. While P_1 (nuclei) and P_3 (microsomes) showed substantial binding, the highest proportion (40%) of stereospecific binding in a whole rat brain homogenate was found in the P_2 (crude mitochondrial) fraction. Relative to protein content, however, P_3 bound substantially more LSD than P_2 or P_1 (Table 2). Subfractionation of P_2 (without osmotic lysis) revealed that both P_2A (myelin) and P_2B (synaptosomes) bind LSD stereospecifically and in approximately equivalent amounts. There was little binding in the purified mitochondrial (P_2C) fraction. When the crude mitochondrial fraction (P_2) was subjected directly to mild osmotic lysis, the synaptic vesicles (P_2D) showed little binding, while the microsomes (P_2E) had the highest binding. The synaptosome ghosts (P_2F and P_2G) showed less binding. Consistent with binding in P_2B, and in P_2F and P_2G, the damaged or partially disrupted synaptosomes (P_2H) showed a substantial degree of binding. Table 2 shows the specific activities (in terms of protein content) and the ratios to total binding for these various subfractions. The unlysed (P_3) and lysed (P_2E) microsomal fractions had the highest specific activities, as well as the highest proportion of total binding.

Effects of selected neurotransmitters and drugs on high-affinity, stereospecific binding. Table 3 shows the IC_{50} values of a series of LSD congeners, transmitters, hallucinogens, 5-HT and dopamine antagonists, and other drugs of interest, listed in order of decreasing potency. Of all the agents tested, methiothepin, a putative central 5-HT antagonist, was the most potent blocker, its affinity for the LSD binding site being almost as high as that of *d*-LSD itself. Other peripherally characterized 5-HT antagonists fell into two groups: effective blockers like *d*-LSD in structure (2-bromo-*d*-LSD, methysergide) and those unlike *d*-LSD in structure (cyproheptadine, cinanserin), which were less potent in blocking than most of the tranquilizers tested. Dopamine antagonism does not predict affinity for the LSD binding site, since the most specific antagonist, pimozide (27), was less potent than haloperidol and the other tranquilizers. Thus neuroleptics as a group (except for methiothe-

TABLE 2

Stereospecific binding of d-LSD by rat brain subcellular fractions relative to protein content and total binding

Two whole rat brains were homogenized in 10 volumes of 0.32 M sucrose and fractionated subcellularly as described under MATERIALS AND METHODS. For the experiments involving subfractions P_2D–P_2I, four whole brains were used. Aliquots (0.2 ml) of the homogenate and supernatants (P_1S and P_2S) were assayed directly for binding as described in the text. Particulates were resuspended in 0.32 M sucrose (P_1 and P_2 in 10 ml; P_2C, P_2I, and P_3 in 6 ml), and 0.2-ml aliquots of these suspensions were assayed for binding. In the density gradient experiments, the supernatant phases overlying each band were discarded and the entire band was collected; 0.2-ml aliquots of each band were assayed directly unless the volume was too small, in which case the material was diluted to the smallest appropriate volume with either 0.32 M sucrose or deionized water. In the binding assays, in order to ensure the formation of pellets, the centrifugation speed at the end of the incubation was increased to 10,000 × *g* for P_2, P_2A–P_2C, and P_2F–P_2I, and to 180,000 × *g* for P_2D, P_2E, P_2S, and P_3. These latter high-speed centrifugations were carried out in 4-ml polyallomer tubes in the International B-60 ultracentrifuge. Values are the means and standard errors of the numbers of experiments shown in parentheses; each experiment involved quadruplicate binding assays.

Fraction	Stereospecific binding	Ratio of stereospecific to total binding[a]
	nmoles/mg protein × 10^{-4}	
Homogenate	1.273 ± 0.067 (12)	0.404
P_1	1.340 ± 0.124 (5)	0.356
P_1S	1.492 ± 0.089 (5)	0.456
P_2	2.145 ± 0.109 (13)	0.450
P_2A	1.774 ± 0.062 (8)	0.401
P_2B	1.703 ± 0.212 (8)	0.428
P_2C	0.481 ± 0.115 (7)	0.142
P_2D	0.314 ± 0.109 (4)	0.229
P_2E	3.998 ± 0.229 (4)	0.547
P_2F	1.828 ± 0.546 (3)	0.285
P_2G	2.669 ± 0.644 (3)	0.408
P_2H	2.676 ± 1.828 (3)	0.308
P_2I	1.614 ± 1.817 (3)	0.142
P_2S	1.377 ± 0.093 (6)	0.533
P_3	3.908 ± 0.521 (6)	0.622

[a] Defined in the legend to Table 1.

TABLE 3

Inhibition of high-affinity, stereospecific binding of d-LSD in rat forebrain P_1S suspensions

P_1S suspensions of rat forebrain, prepared as described under MATERIALS AND METHODS, were incubated in the presence of five to eight different concentrations of each drug (except for 5-HT, which was tested at 10 different concentrations) in two or three experiments. Nonspecific binding that occurred in the presence of a 100-fold excess of unlabeled *d*-LSD was subtracted from all binding values, and the percentage inhibition of this stereospecific binding by each drug was calculated. The inhibition values were plotted against each drug concentration both on log probit paper and on ordinary graph paper (values between 20% and 80% inhibition only). The concentration of each drug that inhibited stereospecific binding 50% (IC_{50}) was read from the two plots, and the values were averaged to give the results shown.

Drug	IC_{50}
	*n*M
d-LSD	4.7
Methiothepin	5.3
2-Bromo-*d*-LSD	5.8
Methysergide	10
d-Lysergic acid monoethylamide	40
d-Lysergic acid dimethylamide	51
Chlorpromazine	75
Clozapine	75
Thioridazine	94
Clothiapine	100
Cyproheptadine	200
Haloperidol	310
Cinanserin	720
Psilocin	890
Bufotenine	930
5-Methoxy-*N*,*N*-dimethyltryptamine	970
Molindone	980
Pimozide	1,500
Serotonin	1,900
N,*N*-Dimethyltryptamine	2,200
Lilly 110-140	3,000
Phenoxybenzamine	4,300
Dopamine	42,500
l-LSD	120,000
Norepinephrine	305,000
Mescaline	440,000

pin) were intermediate in potency between LSD-like drugs and the non-LSD-like 5-HT antagonists.

The most potent of the neurotransmitters in inhibiting the binding was 5-HT, but its affinity was 400 times less than that of *d*-LSD; dopamine had about 10,000 times, and norepinephrine about 60,000 times, less affinity than *d*-LSD. While closeness or analogy to the molecular configuration of *d*-LSD is an important factor in inhibitory potency, psychotomimetic potency clearly is not. Thus 2-bromo-*d*-LSD and *l*-LSD are both nonpsychotomimetic, but the former is an effective binding blocker and the latter is not. While psychotomimetic congeners of *d*-LSD are also effective blockers, the simple indole psychotomimetics are much less potent than the LSD congeners; they are, however, more potent than 5-HT, with the interesting exception of *N*,*N*-dimethyltryptamine. Mescaline, which shows cross-tolerance with LSD, was even less potent than *l*-LSD.

At concentrations 100 times that of [^{3}H]LSD, the following drugs had no effect on the high-affinity, stereospecific binding: tryptophan, tryptamine, 5-hydroxytryptophan, *N*-acetylserotonin, melatonin, yohimbine, corticosterone, γ-aminobutyric acid, deoxypyridoxine, cyclic 3',5'-AMP, *d*-amphetamine, *l*-amphetamine, fenfluramine, propranolol, disulfiram, phencyclidine, Δ^9-tetrahydrocannabinol, chlordiazepoxide, diphenylhydantoin, and morphine.

Prior treatment with d-LSD in vivo. Administration of unlabeled *d*-LSD *in vivo* (520 μg/kg intraparitoneally) significantly diminished the stereospecific binding of [^{3}H]*d*-LSD assayed *in vitro*. The degree of diminution depended on the time of death and followed the time course of the clearance of LSD *in vivo* (2); maximum diminution (47%) occurred 15 min after injection, a time when the concentration of *d*-LSD in the brain is maximal (2).

DISCUSSION

The high-affinity binding of *d*-LSD (K_D = 9 nM) to synaptic membrane fractions was first found with equilibrium dialysis (15), and was inexplicably confined to the cerebral cortex. Stereospecificity was inferred, but not directly demonstrated for binding or for the inhibitory drug effects (15).

Bennett and Aghajanian (18) reported a half-saturation value of 4 nM, identical with our observed value. They assumed a priori that the binding site for *d*-LSD is

the 5-HT receptor, as indicated by their method; specific binding of *d*-LSD was both defined and determined as the difference between binding in the presence of 0.1 or 1.0 mM 5-HT and binding in the absence of 5-HT (18). Our method was designed precisely to detect stereospecific binding of *d*-LSD itself to brain membranes. While the pattern of inhibitory drug effects is generally similar in all studies, it remains possible that these different methods assay somewhat different sites, especially in view of the 400-fold difference in affinity of LSD and 5-HT for the binding site. Using essentially the same stereospecific assay (23) reported here, Bennett and Snyder (19) reported high-affinity, stereospecific binding of LSD (K_D = 10 nM).

Our experiments (Figs. 3 and 4) consistently indicated the possibility of dual binding sites, one characterized by high- and the other by lower-affinity binding, and both requiring stereospecific binding. While we investigated only the high-affinity site, even the nonspecific binding could be pharmacologically significant.

Our findings on the regional distribution of the high-affinity site agree with previous studies (18–20). We found in rat brain that areas of higher stereospecific binding had higher ratios of stereospecific to total binding. This enrichment of binding was highest in the striatum. While this is consistent with reports that LSD (9, 10, 28–30) and methiothepin (31, 32) can interact with dopamine receptors, dopamine and its antagonist, pimozide, have negligible affinity for the site studied here (Table 3).

Although subcellular studies cannot definitively localize sites of receptor interactions, certain conclusions are suggested. First, the LSD acceptor site is probably not confined to the neuronal soma or nerve terminal membrane, since we observed as much binding in the myelin (P_2A) as in the synaptosomal (P_2B) subfraction. Binding in P_2A probably is not due to contaminating synaptosomes, since (a) our electron micrographs did not indicate this, (b) the ($Na^+ + K^+$)-ATPase activity of P_2A is low relative to that of P_2B (33), and (c) the high-affinity synaptosomal uptake of [^{3}H]5-HT in P_2A is less than 10% of that in P_2B.[6] There is other evidence of a possible LSD binding site in myelin (34–36).

Second, enrichment of stereospecific binding in microsomal fractions argues somewhat against a firm conclusion that the binding site is identical with a postsynaptic transmitter receptor, for these are thought to be associated with P_2 or P_2B fractions and not with P_3 (37, 38). The enrichment and the finding of the highest degree of high-affinity, stereospecific LSD binding in microsomal fractions are not artifacts due to membrane fragmentation, because (a) binding was the same, relative to protein content, whether the microsomal fractions were lysed (P_2E) or not (P_3), (b) the ratio of stereospecific to total binding was highest in these two fractions irrespective of lysis, and (c) administration of [^{3}H]*d*-LSD *in vivo* or its addition *in vitro* resulted in the highest recovery of [^{3}H]*d*-LSD in the microsomal (P_3) fraction.[4] Nor are synaptic vesicles contributory, since that subfraction (P_2D) was quite low in binding, and LSD given *in vivo* is not found in such fractions.[4] Dense-core granules in the P_2E subfraction (39) are not accountable, because these appear in P_2E as a result of synaptosome lysis, and high binding of a specific activity (per milligram of protein) equivalent to that of P_2E was observed in the unlysed P_3.

Raphe lesions indicate there are postsynaptic acceptor sites (18–20). Yet, in view of the variety and character of neuronal elements capable of high-affinity, stereospecific binding, these *d*-LSD acceptor sites cannot presently be viewed as exclusively postsynaptic.

Nature of high-affinity, stereospecific binding site for d-LSD. For the LSD acceptor, relationship to the over-all configuration of the *d*-LSD molecule is the crucial factor, as exemplified by the blocking potency of 2-bromo-*d*-LSD. A characteristic is the orientation of the N-6 lone pair of electrons downward from the fused ring plane (40, 41). Methiothepin had the high-

[6] R. A. Lovell and D. X. Freedman, unpublished observations.

est blocking potency. Framework molecular models (Prentice-Hall) showed the hypothetical possibility that the lone-pair electrons on the 3 nitrogen atoms in *d*-LSD, and on the 2 sulfur atoms and N-4 of the piperazine ring in methiothepin, could be oriented downward from the over-all plane of each molecule to make a tripod of electron donor pairs. The dimensions so formed are approximately similar in the two molecules (Fig. 5). For methiothepin, some flexibility (due to free rotation about the single bond, which holds the piperazine ring to the 2-carbon bridge) permits slight variability in the dimensions of the triangle. The relative rigidity of the *d*-LSD molecule would enable it to retain its active conformation and account for both its characteristic potency and its persistence of action on 5-HT-mediated systems (42–50). We could thus postulate a site in brain membranes which binds *d*-LSD, its congeners, and methiothepin by a 3-point attachment involving electron donor-acceptor interactions with charge points on the membrane surface.

This site is probably larger than that needed to block 5-HT. It is conceivable that as *d*-LSD approaches the vicinity of 5-HT receptors—depending on its orientation—it could either agonistically stimulate the 5-HT receptors or bind to adjacent charge points, so that a portion of the molecule would cover the 5-HT sites and thus block them antagonistically, in accord with the extensive evidence of both properties of the drug on 5-HT systems.

There are some difficulties in conceiving of the 5-HT and LSD acceptor sites as equivalent. Although there are commonalities between the steric and electronic configurations of 5-HT and *d*-LSD (50), it is not possible experimentally or theoretically to establish an unambiguous conformation of 5-HT. It is thus inherently more difficult to characterize the 5-HT receptor than the LSD acceptor. Accordingly, the structure of the 5-HT receptor site has

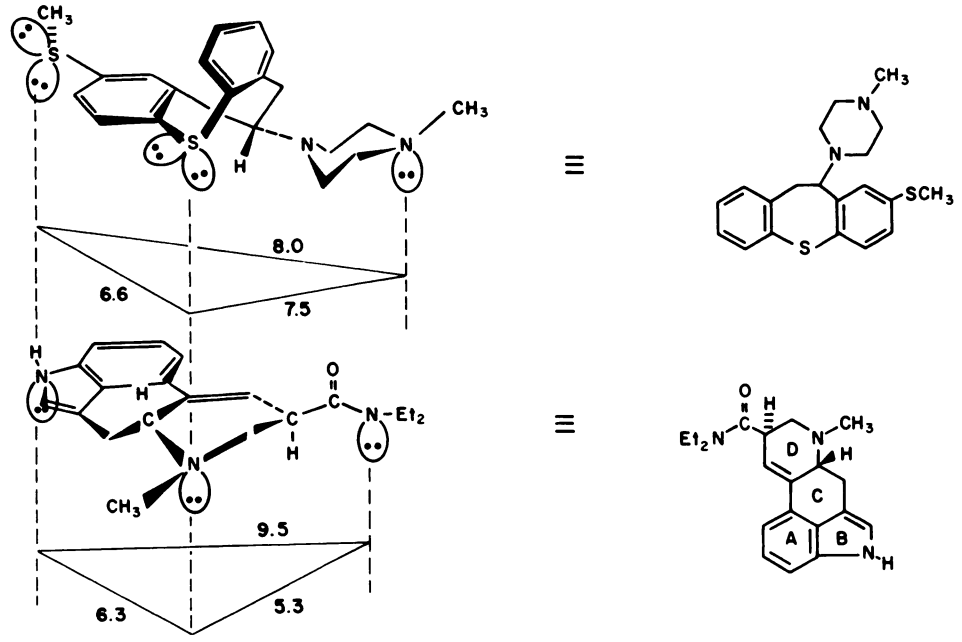

FIG. 5. *Drawings of steric and electronic configurations of d-normal-LSD and methiothepin molecules made from scale models*

The drawings are intended to illustrate the approximate similarity in the dimensions of the triangle formed by the lone-pair electrons on the 3 nitrogen atoms in d-normal-LSD and the triangle formed by the lone-pair electrons on the 2 sulfur atoms and the outermost nitrogen atom of the piperazine ring in methiothepin. Numerical values shown are in Angstrom units (1 inch = 1 A).

been viewed as a major determinant of the 5-HT conformer that binds, and the hope has been that LSD might probe the 5-HT receptor (51). But our data show that LSD is not a precise probe. What we can more confidently describe is a stereospecific acceptor site for *d*-LSD, which is probably larger than that needed to bind 5-HT. This, and the small percentage of available 5-HT molecules with the right conformation to bind, may account for the large excesses of 5-HT required to block the LSD binding (17, 18). In any event, the properties of both the LSD molecule and acceptor site might be considered in accounting for the pharmacological effects of this drug.

The 5-HT binding site on nerve endings studied by Marchbanks (52, 53) is probably independent of the *d*-LSD binding site studied here, because of a lack of stereospecificity (53). Attempts to separate from brain nerve endings proteolipid or protein components which bind 5-HT and/or LSD (54–56) have not yet provided definitive evidence of the identity of LSD and 5-HT binding sites.

The evidence appears to us insufficient to equate this high-affinity, stereospecific binding of LSD definitively to its range of central actions *in vivo*. *d*-LSD administered *in vivo* does diminish the stereospecific binding assayed *in vitro*, and we would calculate (2) that after doses that inhibit the midbrain raphe (18) *in vivo*, LSD concentrations are in the high-affinity range of stereospecific binding. Yet even though both 2-bromo-*d*-LSD (57) and methiothepin (58) can block some behavioral effects of LSD, and 2-bromo-*d*-LSD may block or attenuate the effects of LSD in man (59), Aghajanian (12) has shown that 2-bromo-*d*-LSD does not block LSD effects on the raphe neurons.

As we review our data, it appears that neither psychotomimetic potency nor antagonism of 5-HT or dopamine correlates directly with this LSD stereospecific binding site. A molecule does not have to act like LSD or always antagonize its effects to block the binding. Perhaps high-affinity, stereospecific binding could be a necessary first step in a complex sequence of biochemical and neural events, and 2-bromo-*d*-LSD or methiothepin might have the right configuration to bind, but because of a particular structural feature or lack of it subsequent events are somehow prevented. A membrane conformational change affecting access of 5-HT to receptors (60) may occur early in the sequence. Elucidation of the precise steps and the sequences following stereospecific binding of LSD is clearly required, and the multiple morphological loci of the LSD acceptor sites must be kept in mind.

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