Biochemical Pharmacology, Vol. 22, pp. 1103–1113. Pergamon Press, 1973. Printed in Great Britain.

CHARACTERISTICS OF D-LYSERGIC ACID DIETHYLAMIDE BINDING TO SUBCELLULAR FRACTIONS DERIVED FROM RAT BRAIN*

JOHN T. FARROW and HELEN VAN VUNAKIS

Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154, U.S.A.

(*Received* 27 *January* 1972; *accepted* 27 *October* 1972)

Abstract—Synaptosomes of rat cerebral cortical gray matter contain low concentrations of high affinity ($K_{D_1} = 9 \times 10^{-9}$ M) and medium affinity ($K_{D_2} = 1{\cdot}2 \times 10^{-6}$ M) binding sites for LSD (D-lysergic acid diethylamide). Cerebral cortical synaptic membranes contain 2·2-times as many high affinity binding sites per milligram of protein as synaptosomes, but only about 0·18-times as many medium affinity sites per milligram of protein. These binding sites were not detected in synaptosomes from other regions of the brain, or in myelin or mitochondria from either the cortex or the rest of the brain. All these fractions, however, do exhibit low affinity binding ($K_{D_3} > 10^{-4}$ M). Binding was determined by equilibrium dialysis employing ^{3}H-LSD of high specific activity. The high affinity binding component is stable for at least 90 min at 37°. Incubation with proteolytic enzymes reduces the binding. Of the LSD bound to cortex both *in vivo* and *in vitro*, 20–25 per cent is irreversibly bound. There is a pH dependency of the binding which suggests that at 5×10^{-9} M and at 2×10^{-5} M, LSD binds preferentially in the protonated form to a fraction of the binding sites. LSD binding to synaptosomes is inhibited by ten hallucinogenic compounds of widely varying structures, but not by their nonhallucinogenic congeners. All of these hallucinogenic compounds may produce their effects by binding to the same sites which bind LSD. Chlorpromazine also inhibits LSD binding. Prostaglandin E_1 and cyclic AMP substantially increase LSD binding.

WE HAVE examined LSD (D-lysergic acid diethylamide) binding to subcellular particles from rat brain, on the assumption that the drug must bind to LSD receptors in brain in order to produce its extraordinary effects. Some characteristics of this binding have been reported previously[1] as have studies on the antibody receptor site to D-lysergamide.[2] We report here the subcellular and gross anatomical localization of LSD binding in brain, its stability, pH dependence, reversibility *in vivo* and *in vitro* and the effect on binding of various hallucinogenic and nonhallucinogenic compounds.

MATERIALS AND METHODS

Materials. The sources of some of the compounds used in this study have been reported.[1] D-Lysergic acid amide (Sandoz batch No. 12901), L-isolysergic acid amide (Sandoz batch No. 13001), L-LSD, cannabidiol, cannabicyclol and 1-*trans*-Δ^9-tetrahydrocannabinol were all provided by the U.S. Food and Drug Administration and the National Institute of Mental Health. Atropine sulfate was obtained from Lilly, Inc. and reserpine from the Regis Chemical Company. Chlorpromazine was kindly

* This work was supported in part by a research grant from the National Institute of Mental Health (No. MH-19009). J.T.F. is a post-doctoral fellow (Training Grant NS 05241 from the National Institute of Neurological Diseases and Stroke). H.V.V. is a PHS Research Career Awardee (Award No. 5K6-AI-2372 from the National Institute of Allergy and Infectious Diseases).

donated by Smith, Kline & French Laboratories and prostaglandin E_1 by Upjohn Company. The preparation of psilocin from psilocybin has been previously described.[2] Enzymes were obtained from Worthington Biochemical Corp., except for neuraminidase, which was from Microbiological Associates.

Preparation of subcellular fractions. Synaptosomes derived from cerebral cortical gray matter and myelin, synaptosomes and mitochondria derived from whole brain exclusive of cerebral cortical gray matter, were prepared from 190 to 250 g Sprague–Dawley rats by a slight modification[3] of the procedure of Gray and Whittaker.[4] The crude mitochondrial fraction in 0·32 M sucrose was layered on top of a discontinuous sucrose density gradient (1·2 and 0·8 M sucrose) and centrifuged at 200,000 *g* for 75 min in a Spinco SW-50 rotor to yield myelin, synaptosomes and mitochondria. Centrifugation at 100,000 *g* for 75 min gave fractions exhibiting LSD binding consistent with data in Fig. 1 and previous experiments.[1] The fractions were either used immediately or were diluted with water to 0·32 M sucrose and centrifuged at 200,000 *g* for 45 min to give pellets which were then resuspended in buffer. These two alternatives gave equivalent LSD binding. The morphology of the fractions was substantiated by electron microscopy.

Synaptic membranes (fraction G) were prepared by suspending the crude mitochondrial pellet (derived from cerebral cortical gray matter) in water (2 ml/g fresh wt of tissue), layering the suspension on a discontinuous sucrose density gradient (0·4, 0·8 and 1·0 M sucrose), and centrifuging the gradient at 200,000 *g* for 75 min.[3,5]

Determination of binding. We studied the binding of LSD to subcellular fractions by equilibrium dialysis. Dialysis bags (8 mm) containing 0·40 ml of the tissue sample were suspended in a 50-ml bath of modified Krebs–Ringer solution, pH 7·25. The bath contained varying concentrations of unlabeled LSD and generally labeled ^{3}H-LSD (1·9 Ci/m-mole, New England Nuclear Corp.). The appropriate concentration of another drug was added with the LSD to the bath for the inhibition experiments.[6–8] To reduce oxidation of ^{3}H-LSD, the bath was flushed with N_2 and contained 0·01 % ascorbic acid. We dialyzed the samples overnight in the dark at 4°, with shaking, and then measured in triplicate, by liquid scintillation techniques, ^{3}the radioactivity in 0·1 ml each of bag and bath. $\text{Dis/min}_{in} - \text{dis/min}_{out}$, the difference between the radioactivity in the dialysis bag and the radioactivity in the bath, represents the amount of ^{3}H-LSD taken up by the tissue. $(\text{Dis/min}_{in} - \text{dis/min}_{out}) \times [\text{LSD}]_{total}/[^3\text{H-LSD}]$ represents total LSD taken up.

pH studies. To determine the pH dependency of the LSD binding, we measured binding in the usual way, except that the pH of the bath was altered by the addition of HCl or NaOH. The ionic strength varied less than 5 per cent from pH 6 to 8. To exclude the possibility that the observed variation in LSD binding with pH could be due to pH-dependent tritium loss from ^{3}H-LSD, we adjusted dialysis solution containing 5×10^{-9} M ^{3}H-LSD to pH 8·0 for 2–16 hr, than readjusted it to pH 6·5 just prior to addition of a dialysis bag containing synaptosomes. One control flask was kept at pH 6·5 and another at pH 8·0 for the same period of time prior to addition of dialysis bags. The LSD binding was then measured as usual in all three flasks without further pH adjustments.

Inhibition studies. In all cases the LSD concentration was $5{\cdot}4 \times 10^{-9}$ M. Both LSD and inhibitor were present in the bath at the beginning of the dialysis.

Effect of enzymes and temperature on binding. The synaptic membrane fraction was

incubated with or without enzyme (125 μg/10 mg protein) in the buffer mentioned above at 37°. The fraction was then placed in dialysis bags and LSD binding was measured at 4° as usual. The pH was 7·25 for the temperature studies and for RNase, DNase, neuraminadase, trypsin and chymotrypsin digestions. Peptic digestion of the synaptic membranes was carried out at pH 3, and LSD binding measured as usual at pH 7·25.

Reversibility in vitro. Synaptosomes from cerebral cortical gray matter were dialyzed in the usual manner against $5{\cdot}0 \times 10^{-9}$ M ^{3}H-LSD for 24 hr. The bags were then transferred to 1000 ml of buffer, or $5{\cdot}0 \times 10^{-7}$, $2{\cdot}0 \times 10^{-5}$ or 10^{-4} M unlabeled LSD in buffer and dialyzed for an additional 24 hr. The amount bound after the second dialysis represents irreversible binding under these conditions. Control samples were dialyzed for 48 hr against $5{\cdot}0 \times 10^{-9}$ M ^{3}H-LSD. The amount bound represents total binding at $5{\cdot}0 \times 10^{-9}$ M LSD. Irreversible binding is expressed as a percentage of the control.

Reversibility in vivo. Unanesthetized rats were injected intravenously with 0·10 μg of ^{3}H-LSD in 0·20 ml of 0·9% saline, and killed 15 or 90 min later by decapitation. Cerebral cortical gray matter and the rest of the brain were homogenized separately. Radioactivity was determined before and after dialysis of 0·4-ml samples of the homogenized tissue against 1000 ml of buffer. The amount bound after dialysis represents irreversible binding under these conditions.

Stability of ^{3}H-LSD in vitro *and* in vivo. The synaptosomal fraction (1·0 ml) was dialyzed in a miniature dialysis cell overnight at 4° against 1·0 ml of 5×10^{-7} M ^{3}H-LSD in Ringer solution. The synaptosomes were then extracted twice with equal volumes of benzene–dichloroethane (3 : 1). The extracts were combined and the volume was reduced under vacuum. Three μg of unlabeled LSD in 1 μl of ethanol was added to the reduced extract, which was then spotted on an Eastman Alumina TLC Chromagram and developed in benzene–chloroform–ethanol (30 : 10 : 1). Unlabeled LSD was detected by u.v. absorption. The chromatogram was cut into strips and tritium was measured.

To determine stability of ^{3}H-LSD *in vivo*, rats were injected as previously described and killed 90 min later. Cerebral cortical gray matter and the rest of the brain were homogenized separately. Both fractions were extracted and the extracts chromatographed, as described above.

Other methods. The purity of the ^{3}H-LSD was periodically checked by thin-layer chromatography on Eastman Alumina Chromagrams. The solvent system was benzene–chloroform–ethanol (30 : 10 : 1). The protein content of dialyzed samples was determined by the method of Lowry *et al.*[9] Nitrogen was measured by the method of Lang.[10]

RESULTS AND DISCUSSION

Equilibrium dialysis of myelin and mitochondrial fractions derived either from cerebral cortical gray matter[1] or from whole brain exclusive of cerebral cortical gray matter showed binding characterized by constant $(\text{dis/min}_{in} - \text{dis/min}_{out})$ per dis/min_{out} between 10^{-10} M and 5×10^{-5} M LSD (Fig. 1). This binding could result from the known lipid solubility of LSD or from a large concentration of low affinity LSD binding sites (dissociation constant $= K_{D_2} > 10^{-4}$ M). The synaptosomal fraction and the synaptic membrane fraction, both from cerebral cortical gray matter, also

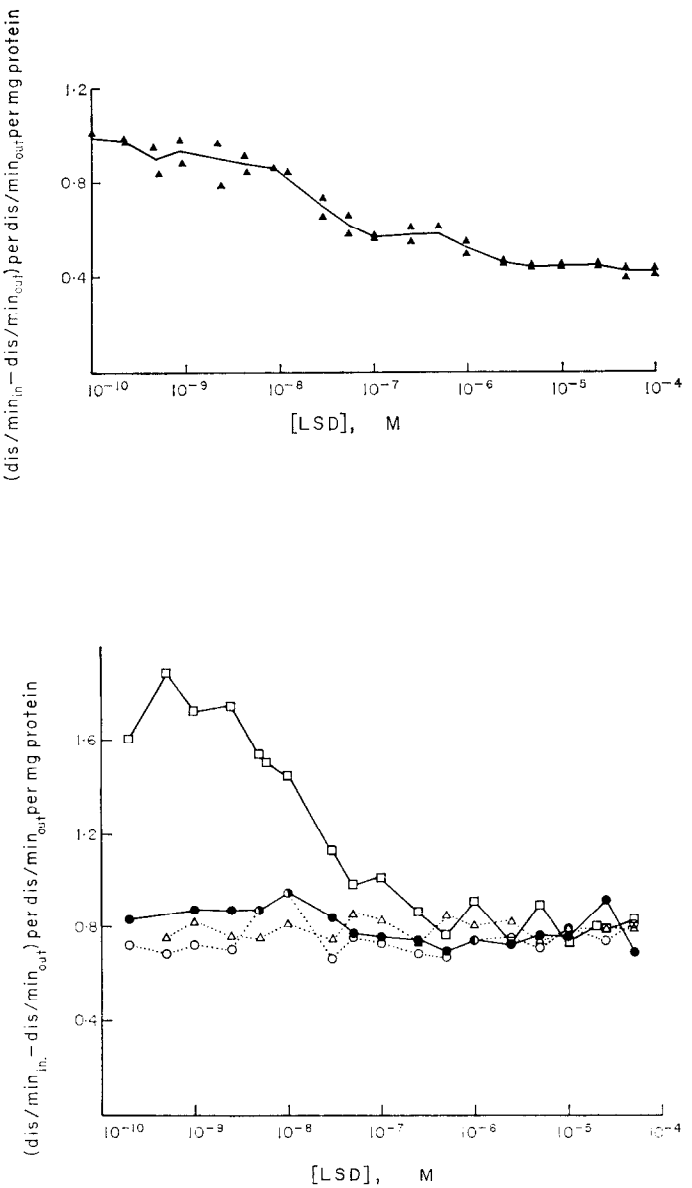

FIG. 1. Binding of LSD to synaptosomes (▲) and synaptic membranes (□) from cerebral cortical gray matter, and myelin (○), synaptosomes (●), and mitochondria (△) from whole brain exclusive of cerebral cortical gray matter. For multiple binding sites:

$$\frac{\text{dis/min}_{in} - \text{dis/min}_{out}}{\text{dis/min}_{out}} = \frac{[B_1]}{K_{D_1} + [\text{LSD}]} + \frac{[B_2]}{K_{D_2} + [\text{LSD}]} + \ldots + \frac{[B_n]}{K_{D_n} + [\text{LSD}]}$$

where dis/min$_{in}$ = dis/min/0·10 ml of dialysis bag contents, dis/min$_{out}$ = dis/min/0·10 ml of bath, B = LSD binding site, and K_D = dissociation constant. If (dis/min$_{in}$ − dis/min$_{out}$) per dis/min$_{out}$ per milligram of protein is greater at a low LSD concentration than at higher LSD concentrations, high affinity binding is present. The data for synaptosomes are individual experimental values. The other curves are pooled data (2–5 experiments/point).

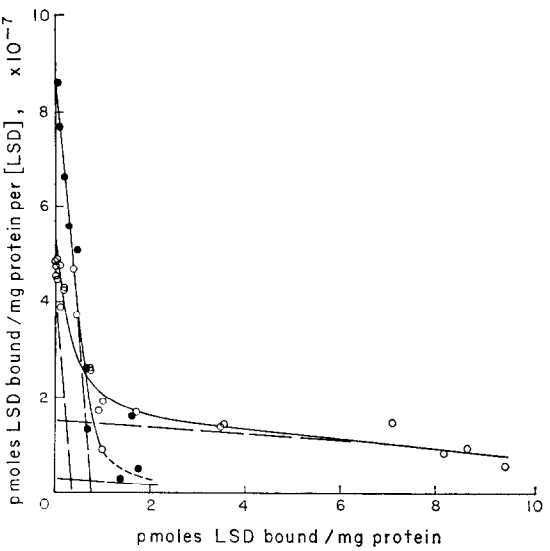

FIG. 2. LSD binding to synaptosomes (○) and synaptic membranes (●) derived from cerebral cortical gray matter is expressed as a Scatchard plot[11] in order to determine the number, affinity and concentration of high affinity binding sites. In both cases, the nonspecific binding ($K_D > 10^{-4}$ M) has been subtracted from the total LSD bound. For synaptosomes, the curve is a theoretical curve for binding by two classes of binding sites having dissociation constants of $9{\cdot}0 \times 10^{-9}$ M and $1{\cdot}2 \times 10^{-6}$ M, and present at 0·35 pmoles/mg protein (6·4 pmoles/g fresh wt of tissue, 2·9 pmoles/mg nitrogen) and 18·4 pmoles/mg protein (340 pmoles/g fresh wt of tissue, 160 pmoles/mg nitrogen) respectively. The straight dashed lines indicate the two classes of synaptosomal binding sites. The points for synaptosomes are unpooled experimental data. The amount of medium affinity binding (and protein) in each dialysis bag containing synaptic membranes is much lower than in the case of synaptosomes. $dis/min_{in} - dis/min_{out}$ is correspondingly smaller, and errors are inevitably larger, especially at higher LSD concentrations. Therefore, all experimental data at each LSD concentration were pooled (2–5 experiments/point) to obtain the plot for synaptic membranes. The incomplete (dashed) portion of the curve indicates the difficulty of obtaining data at relatively high LSD concentrations. To calculate the concentration of medium affinity binding sites, we assumed that the binding constant was the same as that in synaptosomes, $K_{D_2} = 1{\cdot}2 \times 10^{-6}$ M. The high affinity binding sites ($K_{D_1} = 9{\cdot}0 \times 10^{-9}$ M) are present in synaptic membranes at 0·77 pmoles/mg protein, and the medium affinity sites ($K_{D_2} = 1{\cdot}2 \times 10^{-6}$ M, assumed) at approx. 3·3 pmoles/mg protein.

possessed this type of binding, but in addition showed binding at low LSD concentrations that was not present in myelin or mitochondria (Fig. 1). This binding was nearly absent in synaptosomes derived from whole brain exclusive of cerebral cortex (Fig. 1).

Two classes of LSD binding sites, one of high affinity ($K_{D_1} = 9{\cdot}0 \times 10^{-9}$ M) and one of medium affinity ($K_{D_2} = 1{\cdot}2 \times 10^{-6}$ M) account for the additional binding in cerebral cortical synaptosomes (Fig. 2). Cerebral cortical synaptic membranes contain the high affinity sites ($K_{D_1} = 9{\cdot}0 \times 10^{-9}$) and perhaps the medium affinity sites as well. The concentration of the high affinity sites is exceedingly low in synaptosomes, 6·4 pmoles/g fresh weight of tissue from which the fraction was derived.[1] On a protein basis, these sites are 2·2-times more concentrated in the synaptic membrane fraction than in synaptosomes, 0·77 pmoles/mg of protein as compared with 0·35 pmole/mg of protein. By contrast, the medium affinity sites are only about 0·18-times as concentrated in the synaptic membrane fraction as in synaptosomes, about 3·3 pmoles/mg of protein as compared with 18·4 pmoles/mg of protein (Fig. 2). The low affinity

binding sites ($K_{D_3} > 10^{-4}$ M) are present in synaptosomes at > 10 nmoles/mg of protein (Fig. 1).

We extracted synaptosomes after overnight dialysis against ^{3}H-LSD and chromatographed the extract (see Methods) to be certain that LSD remains unchanged during dialysis. Of the total radioactivity in the synaptosomal fraction 24 per cent was not extractable, perhaps corresponding to the 25 per cent irreversible binding discussed below. Of the extracted radioactivity, 93 per cent coincided with true LSD on chromatography.

To determine whether the high affinity binding is localized within regions of the cerebral cortex, we arbitrarily divided the cortex into several areas and measured LSD binding to synaptosomes from each area (Fig. 3). Within experimental error, differences among the areas were not found indicating that either the binding sites are uniformly distributed or our dissection was too gross to reveal detailed regional differences.

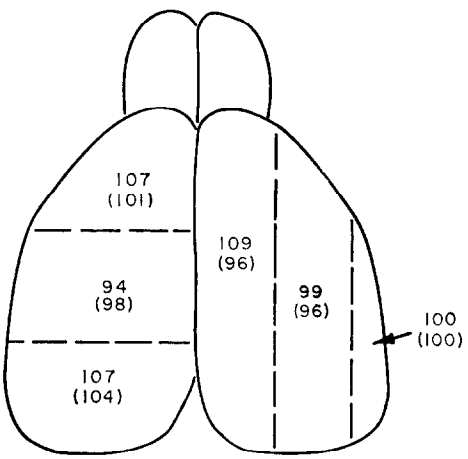

FIG. 3. Cerebral cortical gray matter was divided as indicated in this dorsal view of the cerebrum and LSD binding to the six resulting areas was determined at 2×10^{-5} M LSD (parentheses) and $5{\cdot}4 \times 10^{-9}$ M LSD (no parentheses). ($dis/min_{in} - dis/min_{out}$) per dis/min_{out} per milligram of protein is expressed as a percentage of the value for the area at the far right of the diagram. This area included the pyriform lobe. Olfactory bulbs were included in the areas adjacent to them.

Both the low affinity and the high affinity binding components in synaptic membranes are stable for at least 90 min at 37° (Table 1). After incubation at pH 3, 37° for 30 min, binding of LSD is increased, perhaps through exposure of sites that are occluded at pH 7·25. Incubation at 37° for 30 min with RNase, DNase or neuraminidase does not appreciably decrease the binding, but incubation with trypsin, chymotrypsin or pepsin reduces the binding by 30–35 per cent suggesting that protein may be present in the LSD binding sites. The enzymes alone do not appreciably bind LSD at these concentrations.

We examined reversibility of LSD binding by dialyzing cerebral cortical synaptosomes first against $5{\cdot}0 \times 10^{-9}$ M ^{3}H-LSD and then against a large volume of buffer, or a high concentration of unlabeled LSD in buffer. Of the original binding, 25 ± 4 per cent (S.E., $n = 8$) was irreversible under these conditions. We also injected rats

TABLE 1. STABILITY OF LSD BINDING TO SYNAPTIC MEMBRANES

Conditions	LSD concn (M)	Binding as per cent of untreated control
37° × 0 min	$5{\cdot}0 \times 10^{-9}$	100
37° × 0 min	$1{\cdot}0 \times 10^{-5}$	100
37° × 30 min	$5{\cdot}0 \times 10^{-9}$	99·5
37° × 30 min	$1{\cdot}0 \times 10^{-5}$	90·5
37° × 90 min	$5{\cdot}0 \times 10^{-9}$	100·5
37° × 90 min	$1{\cdot}0 \times 10^{-5}$	102·5
RNase, 37° × 30 min	$5{\cdot}0 \times 10^{-9}$	98
DNase, 37° × 30 min	$5{\cdot}0 \times 10^{-9}$	95
Neuraminidase 37° × 30 min	$5{\cdot}0 \times 10^{-9}$	93
Trypsin + chymotrypsin 37° × 30 min	$5{\cdot}0 \times 10^{-9}$	70
Pepsin (pH 3) 37° × 30 min	$5{\cdot}0 \times 10^{-9}$	65
pH 3, 37° × 30 min	$5{\cdot}0 \times 10^{-9}$	135

with ^{3}H-LSD, killed them 15 or 90 min later, and measured irreversibly bound label (Table 2). The concentration of total radioactivity was somewhat higher in cerebral cortex than in the rest of the brain at both times, whereas the per cent of the label that was irreversibly bound after 15 or 90 min was substantially higher in cortex and was about the same as that observed *in vitro*. We extracted both fractions and chromatographed the extracts (see Materials and Methods). Twenty per cent of the radioactivity in the cerebral cortical fraction and 22 per cent of the radioactivity in the rest of the brain were not extractable under these conditions. Ninety-four per cent of the radioactivity extracted from cerebral cortex and 90 per cent of that extracted from the rest of the brain coincided with an LSD standard on chromatography.

TABLE 2. REVERSIBILITY OF LSD BINDING *in vivo**

Tissue	Time of death after injection (min)	Total LSD (pmoles/ mg protein)	Irreversible binding (%)
Cerebral cortical gray matter	15	0·42	24
Cerebral cortical gray matter	90	0·27	20
Whole brain except for cerebral cortical gray matter	15	0·31	9·5
Whole brain except for cerebral cortical gray matter	90	0·22	8·5

* Rats (125–150 g) were injected intravenously with 0·10 μg of ^{3}H-LSD and killed 15 or 90 min later and brain tissue was homogenized (see Materials and Methods). Tritium was measured before and after dialysis against buffer to determine total radioactivity and radioactivity irreversibly bound under these conditions. The ratio of irreversibly bound tritium to total tritium is the per cent of irreversible binding. Data are mean values from two rats (15 min) or three rats (90 min).

A fraction of the LSD binding to synaptosomes at both $5{\cdot}0 \times 10^{-9}$ M and $2{\cdot}0 \times 10^{-5}$ M LSD varied with pH in a manner that coincides with theoretical proton dissociation curves having pK values of 7·65 and 7·70 (Fig. 4). The nitrogen at position 7 of LSD has a dissociable proton, pK = 7·72,[12] which could account for the observed

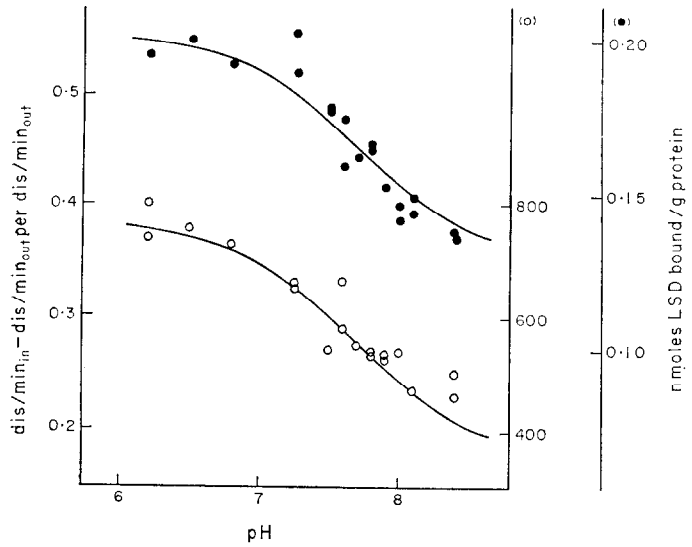

FIG. 4. Binding of 5×10^{-9} M LSD (top) and 2×10^{-5} M LSD (bottom) to synaptosomes at various pH values. Points are experimental data, and the curves are theoretical curves for proton dissociation, pK = 7·70 (top), pK = 7·65 (bottom). LSD has a pK value of 7·72.[12]

variation in binding. To exclude the possibility that the observed change in LSD binding with pH could be due to pH-dependent tritium loss from ^{3}H-LSD, the pH was adjusted to 8·0 for 2–16 hr, then to 6·5 before binding was measured (see Materials and Methods). Binding under these conditions differed from the binding at pH 6·5 (Fig. 4) by only 4 per cent of the difference between binding at pH 6·5 and pH 8·0, indicating that tritium was not lost from ^{3}H-LSD at pH 8·0. LSD ($2{\cdot}0 \times 10^{-5}$ M) probably binds preferentially in the protonated form to the class of low affinity binding sites that is pH sensitive. It is not possible to assign the pH dependent binding observed at $5{\cdot}0 \times 10^{-9}$ M LSD to one or another class of binding sites on the basis of the data.

Despite their widely varying structures, all the hallucinogenic compounds examined to date have inhibited $5{\cdot}4 \times 10^{-9}$ M LSD binding to synaptosomes (Table 3). Psilocybin is probably not hallucinogenic but is rapidly dephosphorylated in the body[13] to the potent hallucinogen psilocin. Psilocybin did not inhibit LSD binding, but psilocin did. Mescaline, psilocin and LSD produce similar symptoms and show cross-tolerance despite considerable differences in their structures. They may therefore produce their effects at a common set of postsynaptic receptors.[14–16] The ratio of their hallucinogenic potencies in man (1 : 32 : 4500–9275; mescaline–psilocin–LSD) is in good agreement with the ratio of their potency of inhibition of LSD binding (0·32 : 32 : 6400),[1] which implies that all three act at the LSD binding sites.

Lysergic acid amide is a much less potent hallucinogen than LSD. At a concentration 20 times greater than the LSD concentration, it inhibits 28 per cent of the LSD binding. The nonhallucinogenic stereoisomers, L-LSD and L-isolysergic acid amide, also at 10^{-7} M, exhibit negligible inhibition, which implies that LSD binding is stereospecific.

Chlorpromazine decreases the hallucinogenic effects of LSD and also substantially

inhibits LSD binding. Reserpine has the opposite behavioral effect and does not inhibit. Atropine is hallucinogenic, but only at very high doses,[17,18] and is only inhibitory at a high concentration, 10^{-5} M.

The major active ingredient of marijuana, 1-*trans*-Δ^9-tetrahydrocannabinol, also inhibits LSD binding, whereas its inactive congener, cannabicyclol, does not. Cannabidiol is inactive but inhibits. 1-*trans*-Δ^9-Tetrahydrocannabinol is not commonly thought of as a hallucinogenic drug, but in large doses produces a clinical syndrome having many aspects in common with LSD.[19]

N,*N*-dimethyltryptamine and *N*,*N*-dimethyl-5-methoxytryptamine are much more effective inhibitors than their nonmethylated congeners in competing for the receptor sites on the antibodies to D-lysergamide.[2] As inhibitors of $5{\cdot}4 \times 10^{-9}$ M LSD binding to synaptosomes, however, these compounds are equally effective. In pooled results, the difference in per cent inhibition between dimethylated and nonmethylated tryptamine and 5-methoxytryptamine is only $0{\cdot}8 \pm 2{\cdot}3$ per cent (S.E., $n = 16$).

Prostaglandin E_1 and cyclic AMP both substantially increase LSD binding. The enhancement of binding by prostaglandin E_1 is quite variable but significant, 41 ± 32 per cent (S.E., $n = 6$). The cause of this variability is unknown at present.

We cannot know which of the three binding modalities are involved in the inhibition experiments on the basis of these data. By regarding inhibition by 10^{-4} M unlabeled LSD as "100 per cent inhibition", we are reporting inhibition data as if only the high and medium affinity sites are involved. This assumption seems probable considering the extremely low LSD doses required to produce physiological effects. LSD receptor isolation would confirm this assumption.

Other workers have studied some aspects of LSD binding to brain. Marchbanks[20] reported 50 per cent inhibition by LSD at 4×10^{-7} M of serotonin binding sites in rat brain synaptosomes characterized by $K_D = 5 \times 10^{-7}$ M. LSD itself bound with $K_D = 1{\cdot}25 \times 10^{-7}$ M. The serotonin binding sites were localized mainly in rhinencephalon, subcortical and midbrain regions. The binding sites were destroyed by freezing, neuraminidase, acid pH and exposure to room temperature for short periods, and could be extracted with retention of binding activity using *n*-butanol. By contrast, the high affinity LSD binding we have observed ($K_{D_1} = 9 \times 10^{-9}$ M) is localized in cerebral cortex, retains activity after freezing, neuraminidase (Table 1), exposure to acid pH (Fig. 4) or 37° for 90 min (Table 1) and is not extractable using *n*-butanol. Therefore, these binding sites are probably not the same as those reported by Marchbanks.[20]

In a preliminary report, Mehl and Demus[21] measured ^{3}H-LSD binding at a single concentration (10^{-6} M) to subfractions of a rat brain crude mitochondrial fraction after osmotic shock. 2-Bromo LSD (10^{-4} M) did not significantly reduce LSD binding, except to the myelin fraction. Pronase destroyed LSD binding. Binding was maximal at pH 6·4 and half-maximal at pH 4·8 and 8·0. Calculations based on our data indicate that 99 per cent of the binding observed by Mehl and Demus[21] at 10^{-6} M LSD was to low and medium affinity LSD binding sites.

Using another technique to localize the binding sites of LSD, Diab *et al.*[22] have observed the binding of ^{3}H-LSD to neurons in autoradiographs of rat brain cortex. Our understanding of the physiology of the LSD receptor would be greatly enhanced if it could be isolated, its macromolecular structure determined, and its interaction with LSD and other drugs described in molecular terms.

TABLE 3. INHIBITION OF LSD BINDING TO SYNAPTOSOMES BY VARIOUS COMPOUNDS*

	Concn (M)	Inhibition (%)
Transmitters and metabolites		
Serotonin	10^{-6}	70
5-Hydroxyindoleacetic acid	10^{-6}	−7
Tryptamine	10^{-6}	40
Melatonin	10^{-6}	14
L-Epinephrine	10^{-6}	5
L-Norepinephrine	10^{-6}	23
DL-Dopa	10^{-6}	−6
γ-Aminobutyric acid	10^{-6}	2
Hallucinogens		
LSD	10^{-4}	"100"
LSD	10^{-7}	68
Mescaline	10^{-4}	50
Mescaline	10^{-6}	1
Psilocybin	10^{-6}	5
Psilocin	10^{-6}	56
5-Methoxytryptamine	10^{-6}	55
5-Methoxy-*N,N*-dimethyltryptamine	10^{-6}	46
N,N-dimethyltryptamine (DMT)	10^{-6}	27
2,5-Dimethoxy-4-methylamphetamine (DOM)	10^{-6}	25
Bufotenine	10^{-6}	52
Harmaline	10^{-6}	28
Atropine	10^{-5}	23
Atropine	10^{-6}	0
Lysergic acid amide	10^{-7}	28
Lysergic acid derivatives L-LSD	10^{-7}	4
L-Isolysergic acid amide	10^{-7}	9
Cannabinol derivatives		
1-*trans*-Δ^9-Tetrahydrocannabinol	10^{-6}	30
Cannabicyclol	10^{-6}	2
Cannabidiol	10^{-6}	26
Others		
L-Tryptophan	10^{-6}	3
L-Phenylalanine	10^{-6}	0
L-Tyrosine	10^{-6}	5
L-Glutamate	10^{-6}	16
Ergonovine	10^{-6}	70
Spermidine	10^{-6}	18
Histamine	10^{-6}	−3
Tyramine	10^{-6}	5
d-Amphetamine	10^{-6}	5
Adrenochrome	10^{-6}	34
Morphine	10^{-5}	3
Morphine	10^{-6}	−2
Chlorpromazine	10^{-6}	55
Reserpine	10^{-6}	−12
Prostaglandin E_1	10^{-6}	−41
Cyclic AMP	10^{-6}	−34
Cyclic AMP	10^{-7}	−24
Cyclic AMP	10^{-8}	−12

* See footnote on p. 1113.

Acknowledgements—The authors are indebted to Dr. Doreen Winstanley for the electron microscopy and for many stimulating discussions of this work. This is Publication No. 884 of the Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154.

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* In all cases the LSD was $5{\cdot}4 \times 10^{-9}$ M, all as ^{3}H-LSD. Some flasks containing 10^{-4} M unlabeled LSD were included in each experiment to indicate "100 per cent inhibition" on the assumption that low affinity binding (see Fig. 1) is not involved (see text). Data are mean values from two or three synaptosome preparations. Experimental values are all within 5 per cent of the mean value, except for the following: serotonin, ergonovine, *d*-amphetamine, ± 12 per cent; L-LSD, chlorpromazine, cannabidiol, ± 15 per cent; DL-Dopa, DOM, L-tryptophan, ± 20 per cent; 5-hydroxyindoleacetic acid ± 25 per cent. Enhancement of LSD binding by prostaglandin E_1 was highly variable, 41 ± 32 per cent (S.E., $n = 6$, from four synaptosome preparations). Some of the data and sources of some of the inhibitors have been reported previously.[1]