MOLECULAR PHARMACOLOGY, 14, 50-59

High-Affinity Binding of [^{3}H]5-Hydroxytryptamine to Brain Synaptosomal Membranes: Comparison with [^{3}H]Lysergic Acid Diethylamide Binding

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(Received February 18, 1977)
(Accepted September 6, 1977)

SUMMARY

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[^{3}H]5-Hydroxytryptamine ([^{3}H]5-HT) binds to crude brain membrane preparations at two different sites (K_d = 1-3 nM and 10-30 nM). These two sites are present in a limited number as saturable populations and selectively bind 5-HT and related structures. In the same crude membrane preparations, lysergic acid diethylamide (LSD) also binds at two different sites (K_d = 3-4 nM and 20-30 nM). 5-HT binding is found mostly in fractions enriched in synaptosomal and microsomal membranes; fractions rich in mitochondria or in synaptic vesicles have a low binding capacity. The two serotoninergic sites are physically separable; only high-affinity binding sites are found on purified synaptosomal membranes, whereas both types of sites are present in fractions enriched in microsomal membranes. The interaction between LSD and 5-HT shows that high-affinity binding sites for 5-HT are not identical with those for LSD, since the inhibition of binding of one substance by the other is complex.

INTRODUCTION

That 5-hydroxytryptamine possesses a neurotransmitter function has been suggested on the basis of neuropharmacological and electrophysiological evidence (1-10). Furthermore, there is considerable evidence that lysergic acid diethylamide and 5-HT[1] act at a common site (1, 5, 9, 10). To test this hypothesis on a molecular basis, several workers have studied the binding of these two substances to various membrane preparations.

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

The sites first described for 5-HT had a low affinity (K_d = 0.1-1 μM) (11). More recently, high-affinity sites (K_d = 10 nM) have been found for LSD (12-15). Bennett and Snyder (16) reported binding of 5-HT involving specific sites in rat brain homogenates, with a dissociation constant of 8-15 nM. Previously we reported, in rat brain purified synaptosomal membrane fractions, 5-HT-specific binding with a higher affinity constant (K_d close to 1 nM) (17).

In this work we have studied the binding of 5-HT and LSD to various subcellular fractions of bovine brain and shown that 5-HT binding involves two different classes of sites with dissociation constants

close to 2 nM and 20 nM. In comparative assays, LSD binding has been studied and some relationships between the binding of the two substances have been examined.

MATERIALS AND METHODS

Several types of preparations were used for binding tests. They were prepared using either a slightly modified method described by Whittaker *et al.* (18) or the method of Cotman and Matthews (19).

Bovine brains were collected at a local slaughterhouse 20–30 min after the death of the animals and rapidly dissected on ice. In some assays horse brains were used. Cerebral tissues (usually striatum) were homogenized in 15 volumes of ice-cold sucrose (0.32 M) buffered to pH 7.4 with Tris-HCl containing phenylmethylsulfonyl fluoride (10 mM), using a motor-driven Teflon pestle in a glass homogenizer. The homogenate was first centrifuged at 1000 × *g* for 10 min, and then fractions were prepared by one of the following two methods.

1. The fractions were prepared according to Whittaker *et al.* (18) as follows. P_2L, a crude mitochondrial fraction, was lysed in Tris-HCl buffer (5 mM, pH 8.2) for 1.5 hr at 0°, centrifuged, and resuspended in Tris-HCl buffer (50 mM, pH 7.4). This fraction was subfractionated on a discontinuous sucrose gradient according to the nomenclature of Whittaker *et al.*: fractions D (synaptic vesicles, occasional microsomes), E (microsomes, some synaptic vesicles, occasional myelin fragments), F (synaptosome ghosts, membrane fragments, nonvesicular membrane fragments), G (synaptosome ghosts, membrane fragments), H (damaged synaptosomes), and I (small mitochondria, some shrunken synaptosomes).

2. The technique of Cotman and Matthews (19) was used to prepare purified synaptosomal membranes. Synaptosomes were isolated from a crude mitochondrial fraction using a discontinuous Ficoll gradient (7–13% Ficoll in 0.32 M sucrose) and lysed, and synaptosomal membranes were isolated on a discontinuous sucrose gradient (25%, 32.5%, 35%, and 38%). The brain synaptosomal membrane fraction was collected on the 32.5% sucrose. It was rich in pre- and postsynaptic neuronal membranes as described by Cotman and Matthews and as indicated by electronmicroscopic controls we previously performed in rat brain (17). This fraction consisted mainly of membranes. Some of them showed thickening, possibly related to the synaptic cleft, and a few of them still contained some attached neuronal vesicles; no dense material or mitochondria were observed. A fraction called C_1, corresponding to the material collected on the 7% Ficoll, was lysed, centrifuged on the same discontinuous sucrose gradient, and collected on the 25% sucrose layer; it was also tested for comparison with purified synaptosomal membranes.

Binding assays were conducted as follows: 2-ml aliquots (in some cases 1 ml) of tissue suspension in Tris-HCl buffer (50 mM, pH 7.4) containing 100 μM pargyline were usually incubated for 15 min at 37° with the radioactive ligand in the presence and absence of the antagonist. The conditions of steady-state binding were determined by studying kinetics at different temperatures. Equilibrium was reached at 37°, 22°, and 0° for 5-HT after 2–5 min, 20–30 min, and 60 min, respectively, and for LSD after 5–10 min, 45–60 min, and 2 hr, respectively.

The tubes were then cooled for 3 min on ice, and the contents were filtered under vacuum (glass fiber filter, Whatman GF/B). After washing with 15 ml of ice-cold buffer, the radioactivity left on the filter was measured by liquid scintillation spectrometry in 10 ml of scintillator (Triton X-100, 250 ml; 2,5-diphenyloxazole, 7.3 g; 1,4-bis[2-(5-phenyloxazoyl)]benzene, 0.167 g; and toluene up to 1 liter). Under these conditions the counting efficiency varied from 34% to 40%.

Degeneration of the raphe-striatal serotoninergic system was accomplished in rat brains by stereotaxic injections of 5,6-dihydroxytryptamine (10 mg/ml in isotonic NaCl containing 0.02% ascorbic acid): 50 μg into the anterior ventricles and 70 μg in the third ventricle. Rats were killed after 12–14 days, and [^{3}H]5-HT uptake was measured in synaptosomes isolated from

treated and control rats to determine the efficacy of the treatment, using aliquots of unlysed crude mitochondrial fractions incubated at 37° for 10 min with 10 nM [^{3}H]5-HT in buffered Krebs-Ringer solution containing 10 mM glucose, 0.2% ascorbic acid, and 100 μM pargyline. Each fraction was then cooled to 0° for 3 min, filtered on a Whatman GF/B filter, washed, and dried, and the radioactivity left on the filter was counted as usual. Control uptake, determined at 37° for 5 min was 2.9–3.6 pmoles of 5-HT per milligram of protein per minute, whereas in treated animals it varied from 0.8 to 1.4 pmoles/mg of protein per minute, representing 50–80% reduction of uptake.

Proteins were measured by the method of Lowry *et al.* (20). [^{3}H]5-HT (10.5 and 14.2 Ci/mmole) and [^{3}H]LSD (22 Ci/mmole) were purchased from the Radiochemical Centre.

RESULTS

Binding of [^{3}H]5HT and [^{3}H]LSD to brain membrane preparations. The reversible binding of 5-HT was determined by subtracting the radioactivity left after addition of 10 μM nonradioactive 5-HT from the total bound radioactivity in the presence of increasing concentrations of tritiated amine (Fig. 1). Two different populations of binding sites were observed in lysed crude mitochondrial fractions and in fractions enriched in synaptosome ghosts [fractions F and G according to Whittaker *et al.* (18)] (Fig. 2); the K_d values were 2.5 ± 1.05 nM for the high-affinity binding and 31 ± 15 nM [means and standard errors for nine experiments calculated by Scatchard (21) plots] for the low-affinity binding (Fig. 3).

A single population of high-affinity binding sites was observed in purified synaptosomal membranes, with a K_d of 2.6 ± 0.94 nM (seven experiments), which corresponded to a binding capacity of 0.4–0.5 pmole of 5-HT per milligram of protein. Comparable values were found using horse brain preparations.

The affinity of 5-HT for its binding sites was also determined studying displacement of the radioactive by the nonradioactive ligand. Analysis of the inhibition curves indicated the existence of competitive inhibition, with K_i values similar to the K_d values determined by direct binding studies; i.e., K_i = 0.9–2.2 nM for the first inhibition component and 8–45 nM for the second (Fig. 4).

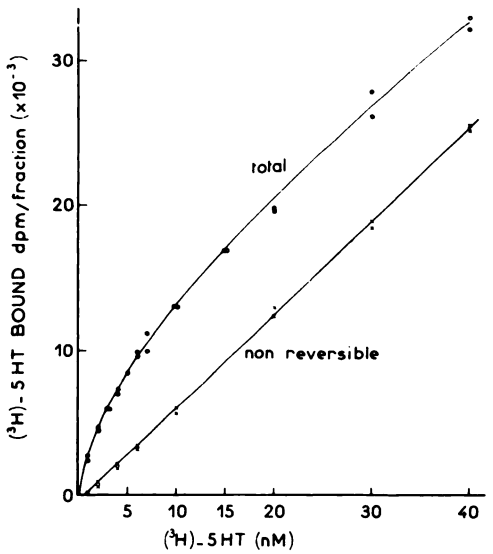

FIG. 1. *Binding of [^{3}H]5-HT as a function of increasing concentrations of [^{3}H]5-HT*

Aliquots (2 ml containing 880 μg of protein) of the purified synaptosomal membrane preparation from bovine striatum were incubated at 37° for 15 min with various concentrations of [^{3}H]5-HT (10.5 Ci/mmole) in Tris-HCl buffer (50 mM, pH 7.4), then transferred to an ice bath for 3 min and filtered as described in MATERIALS AND METHODS. The upper curve represents total binding, and the lower curve shows nonsaturable binding in the presence of 10 μM nonradioactive 5-HT. The difference between the curves corresponds to reversible binding. Each point was measured in duplicate.

[^{3}H]LSD binding was studied in the same preparations as 5-HT. Two populations of sites were involved in crude mitochondrial fractions (Fig. 5); the K_d values were 4.1 ± 1.2 nM for the high-affinity sites and 20 ± 13 nM for the low-affinity sites (means ± standard errors for six experiments) (Fig. 6). In fractions enriched in synaptosome ghosts, most of the binding appeared to involve high-affinity sites (K_d = 4.5 ± 0.8 nM), although in some of those fractions Scatchard (21) or

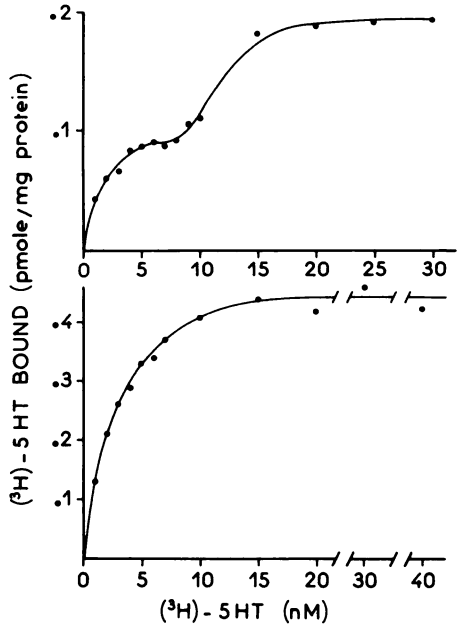

FIG. 2. *Variation of [³H]5-HT specific binding with concentration of [³H]5-HT in incubation medium*

The upper curve represents binding to the lysed crude mitochondrial fraction isolated from bovine striatum, and the lower curve shows binding to purified synaptosomal membranes. The results were reproduced nine times for the crude mitochondrial fraction and six times for purified synaptosomal membranes. Each point was measured in duplicate.

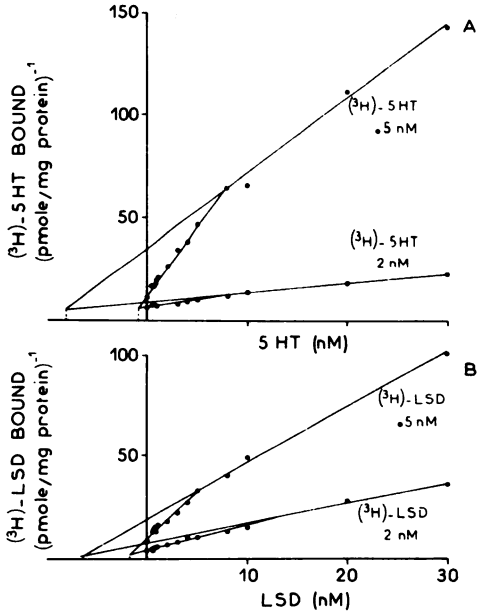

FIG. 4. *Dixon plots of inhibition of [³H]5-HT binding by increasing concentrations of nonradioactive 5-HT (A) and of [³H]LSD binding by increasing concentrations of nonradioactive LSD (B)*

Competitive inhibition was observed, with K_i values of 1 and 9 nM for 5-HT and 1.5 nM and 9 nM for LSD. The experiments were performed using the lysed crude mitochondrial fraction as described in MATERIALS AND METHODS. Results were reproduced three times. Each point is the mean of duplicate determinations.

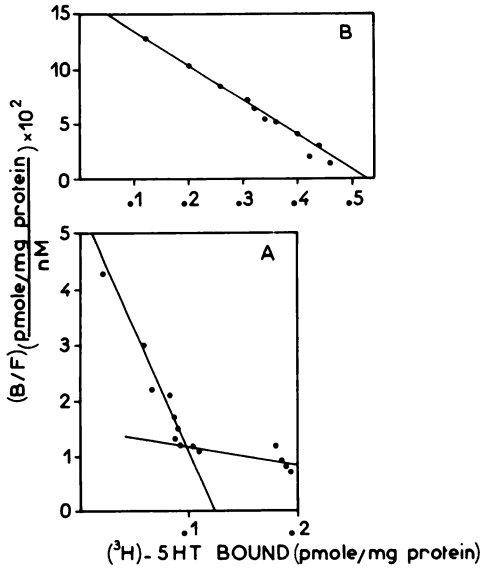

FIG. 3. *Scatchard plots of [³H]5-HT binding to lysed crude mitochondrial fraction (A) and purified synaptosomal membranes (B)*

Membrane preparations, prepared as described in MATERIALS AND METHODS, were incubated for 15 min at 37° with increasing concentrations of [³H]5-HT (1–40 nM).

double-reciprocal plots of the binding curves indicated the presence of a small percentage of binding to sites with low affinity (K_d = 20 nM). Purified synaptosomal membranes contained only a single population of sites, corresponding to high-affinity binding (K_d = 3.8 nM), with a binding capacity of 0.15–0.35 pmole of LSD per milligram of protein (Figs. 5 and 6). Similar results were obtained using horse brain preparations.

As it was possible that [³H]5-HT or [³H]LSD might bind to an enzymatic degradative system and that the observed

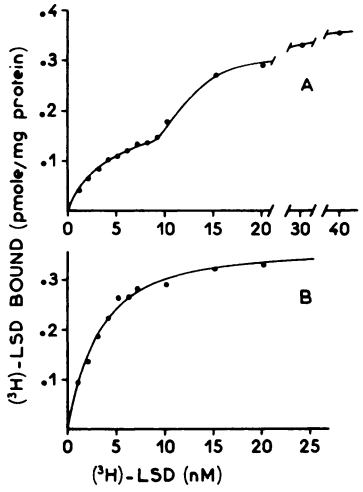

FIG. 5. *Variation of [³H]LSD specific binding with concentration of [³H]LSD in incubation medium*

A. [³H]LSD binding to the lysed crude mitochondrial fraction from bovine striatum. Experiments were reproduced six times. B. Binding to purified synaptosomal membranes from bovine striatum. Experiments were reproduced five times. Each point was measured in duplicate.

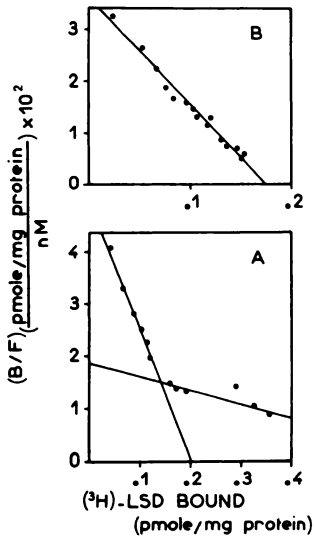

FIG. 6. *Scatchard plots of [³H]LSD binding to lysed crude mitochondrial fraction (A) and purified synaptosomal membrane preparation (B)*

Membrane preparations, prepared as described in MATERIALS AND METHODS, were incubated for 15 min at 37° with increasing concentrations of [³H]LSD (1-40 nM)

radioactivity would not correspond to the original ligand, assays were performed to characterize the bound radioactivity. After incubation of synaptosomal membranes or crude mitochondrial fractions with [³H]5-HT or [³H]LSD, the bound radioactive substance was displaced by a high concentration (5 μM) of the corresponding nonradioactive form and analyzed by thin-layer chromatography using two solvent systems. The migration of the displaced radioactivity was identical with that of the original ligand, indicating that no degradation had occurred.

The binding of 5-HT and LSD was also studied in crude brain preparations isolated from rats treated as described in MATERIALS AND METHODS to degenerate the raphe-striatal serotoninergic system. Using lysed crude mitochondrial fractions isolated from control and treated animals, no significant modification of the reversible binding of 5-HT and LSD was observed for cortex, striatum, or hippocampus (Table 1).

Subcellular localization. The binding of [³H]5-HT was studied in several subcellular fractions obtained as described in MATERIALS AND METHODS (Table 2). In crude fractions, binding was mainly of low affinity (K_d = 10–30 nM); high-affinity binding was estimated as less than 10% of the total reversible binding in homogenates and as 25–55% in synaptosome ghost frac-

TABLE 1

Binding capacity of rat brain regions

Values are the means of two series of assays, each performed in triplicate. [³H]5-HT and [³H]LSD were added at a 5 nM concentration to a lysed crude mitochondrial fraction of the brain region being studied. Treated rats received stereotaxic injections of 5,6-dihydroxytryptamine as described in MATERIALS AND METHODS.

Region	Control		Treated	
	5-HT	LSD	5-HT	LSD
	pmoles/mg protein			
Striatum	0.23	0.20	0.25	0.19
Hippocampus	0.20	0.16	0.19	0.17
Frontal cortex	0.14	0.08	0.15	0.07
Raphe	0.06	0.05		

TABLE 2

Subcellular distribution of 5-HT binding sites in bovine brain

Fractions were prepared as described in MATERIALS AND METHODS, using (a) the technique of Whittaker *et al.* (18) to prepare the homogenate, lysed crude mitochondrial fraction, vesicular fraction, microsomal fraction, synaptosomal ghost fraction, and mitochondrial fraction, and (b) the technique of Cotman and Matthews (19) to prepare purified synaptosomal membranes and fraction C_1. This last fraction corresponds to the layer collected on the 7% Ficoll, centrifuged on a sucrose gradient, and collected on the 25% sucrose layer. Values represent the means of three to five experiments. The ratio total reversible binding to total binding was measured at a 5-HT concentration corresponding to half the maximal reversible binding.

Fraction	[^{3}H]5-HT binding constant		Total reversible binding	Estimated high-affinity reversible binding	Ratio of total reversible to total binding	Ratio of high-affinity to total reversible binding
	K_{d_1}	K_{d_2}				
	nM	*nM*	*pmole/mg protein*		%	%
Homogenate	3.17 ± 2.4	30 ± 17.3	0.45 ± 0.35	0.02–0.04	20	5–10
Lysed crude mitochondrial fraction	2.17 ± 0.91	14.3 ± 5.1	0.24 ± 0.14	0.05–0.08	32	20–35
Vesicular fraction		12.3 ± 4.6	0.16 ± 0.10		15	–[a]
Microsomal fraction	2.90 ± 1.65	19 ± 8	0.23 ± 0.19	0.02–0.05	41	10–20
Synaptosomal ghost fraction	2.43 ± 0.90	26.7 ± 10	0.36 ± 0.12	0.10–0.20	48	25–55
Mitochondrial fraction		31.0 - 16.5	0.15 ± 0.11		16	–[a]
Purified synaptosomal membranes	2.66 ± 0.56		0.46 ± 0.14	0.48 ± 0.14	75	100
Fraction C_1	1.2	36	0.30	0.03	30	16

[a] Negligible.

tions. In the purified synaptosomal fraction, the observed binding was totally of the high-affinity type. The estimated amount of high-affinity binding sites increased from 0.03 pmole/mg of protein in homogenates to 0.05–0.08 in the lysed crude mitochondrial fraction, 0.10–0.20 in synaptosome ghost fractions, and 0.4–0.5 in purified synaptosomal membranes.

Low-affinity binding represented the majority of the binding in homogenates, corresponding to 0.3–0.4 pmole/mg of protein, and decreased in synaptosomal ghost fractions (0.15–0.30 pmole/mg of protein) and in the microsomal fraction (0.10–0.40 pmole/mg of protein). It was not observed in synaptosomal membranes.

5-HT binding to fractions rich in synaptic vesicles or in mitochondria was low (0.10–0.15 pmole/mg of protein); in both cases it appeared to involve solely low-affinity binding sites. Binding determined in fraction C_1 (see MATERIALS AND METHODS), which might correspond to a microsomal fraction, involved sites with high (K_d = 1.2 nM) and low (K_d = 36 nM) affinity.

Regional distribution of 5-HT and LSD binding sites within the brain. The distributions of the binding sites for [^{3}H]5-HT and for [^{3}H]LSD studied in the lysed crude mitochondrial fraction isolated from bovine brain were very similar for both ligands; their variations were parallel in the areas considered. The highest binding capacity was found in striatum hippocampus frontal cortex raphe (Table 2).

Dissociation constants were determined using synaptosomal ghost fractions (fractions F and G) isolated from striatum, hippocampus, frontal cortex, and superior colliculus. Results showed the existence of two types of binding sites for all regions studied, with K_d values close to 2 nM for the first type and 15 nM for the second. The number of high-affinity binding sites was very low in cerebral cortex and did not allow precise determination of the corresponding K_d; the K_d was 12 nM for low-affinity binding. For other regions, the K_d

values were, respectively, 2 nM and 40 nM in striatum, 1.1 nM and 15 nM in hippocampus, and 2 nM and 15 nM in superior colliculus.

Displacement of [³H]5-HT and [³H]LSD by analogues and other drugs. Various drugs were tested for their ability to inhibit the reversible high-affinity binding of [³H]5-HT and [³H]LSD. Assays were performed using various batches of purified synaptosomal membrane preparations, in which [³H]5-HT binding was tested; under these conditions Scatchard analysis indicated the presence of a single type of high-affinity binding site.

The results (Table 3) indicated, first, that high-affinity binding of [³H]5-HT was preferentially inhibited by compounds structurally related to 5-HT. Other amines or drugs of different structure showed either weak or no inhibition. Second, 5-HT was about 100 times more efficient in displacing [³H]5-HT than [³H]LSD; the 5-HT derivatives (bufotenine, methoxytryptamine, and *N,N*-dimethyltryptamine) were also more selective (7–14 times) toward [³H]5-HT binding. Likewise, LSD derivatives (bromo-LSD, methysergide) or compounds possessing antiserotoninergic properties (cyproheptadine, cinanserine, methiothepin) were as potent or more potent as inhibitors of the high-affinity binding of [³H]LSD than that of [³H]5-HT.

LSD had a complex inhibitory effect on [³H]5-HT binding; 30–50% of the binding was inhibited by low concentrations of LSD (1–15 nM) whereas the remaining binding required much higher concentrations (100–1000 times) of LSD to be displaced. Scatchard plots (Fig. 7) indicated that the mechanisms involved in the inhibition of [³H]5-HT binding by low concentrations of LSD and that of [³H]LSD by 5-HT were predominantly of a noncompetitive type. The inhibitory effects of methiothepin appeared to be the result of a noncompetitive mechanism for [³H]5-HT binding and a mixed type for [³H]LSD binding (Fig. 8).

TABLE 3

Displacement of specifically bound [³H]5-HT and [³H]LSD from purified synaptosomal membrane fraction

Purified synaptosomal membrane fractions were incubated at 37° for 15 min with 5 nM [³H]5-HT or [³H]LSD. The nonradioactive inhibitor was added simultaneously with the radioactive ligand; the rest of the procedure was performed as described in MATERIALS AND METHODS. IC_{50} values were measured by log probit analysis of the inhibition curves, which were constructed from four to six values, each determined in triplicate, and are the means of two to four assays for each substance.

Substance	IC_{50}		Potency ratio 5-HT:LSD
	[³H]5-HT	[³H]LSD	
	nM	*nM*	
5-Hydroxytryptamine	5	200	40
5-Hydroxy-*N,N*-dimethyltryptamine	35	500	14
N,N-Dimethyltryptpamine	850	3,500	4
5-Methoxytryptamine	120	300	2.5
Tryptamine	>10,000	>10,000	
5-Hydroxyindoleacetic acid	>10,000	>10,000	
LSD	8	7	0.9
Methiothepin	80	75	0.9
Cyproheptadine	2,000	1,000	0.5
Cinanserine	9,500	2,000	0.2
2-Bromo-LSD	150	30	0.2
Methysergide	1,000	170	0.17
Dopamine	70,000	70,000	
Norepinephrine	>10,000	70,000	
Octopamine	>10,000	>10,000	
Haloperidol	25,000	30,000	
Pimozide	>20,000	16,000	
Pipamperone	10,000	3,000	
Chlorpromazine	35,000	4,500	
Chlorimipramine	10,000	40,000	
Dihydroergotamine	10,000	4,500	
Phenoxybenzamine	15,000	16,000	
Morphine	>30,000	>10,000	

DISCUSSION

The binding of [³H]5-HT was previously studied by Bennett and Snyder (16), who reported the existence of a single type of low-affinity binding (K_d = 8–15 nM) in a crude brain membrane preparation and postulated that the corresponding site represented the serotoninergic receptor.

In this work, we report that 5-HT bind-

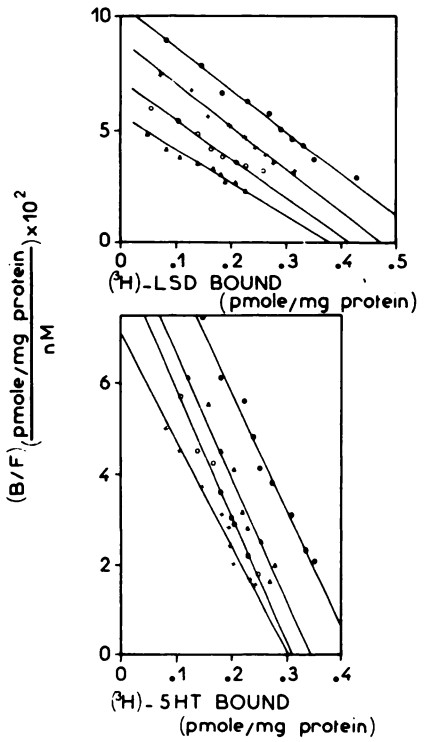

FIG. 7. *Scatchard plots of binding to purified synaptosomal membrane fraction*

Upper: [³H]LSD binding. ●——●, control; X——X, in the presence of 10 nM nonradioactive 5-HT; O——O, 30 nM 5-HT; Δ——Δ, 100 nM 5-HT. Lower: [³H]5-HT binding. ●——●, control; Δ——Δ, in the presence of 3 nM nonradioactive LSD; O——O, 8 nM LSD; X——X, 12 nM LSD. Each experiment was repeated three times. Points are the means of duplicate determinat ions.

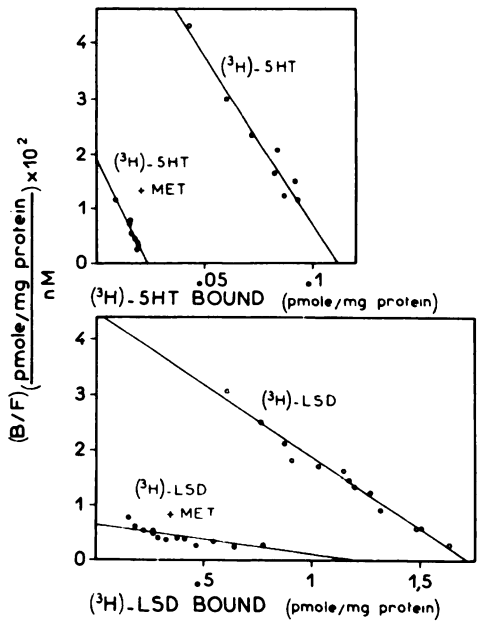

FIG. 8. *Inhibition by methiothepin of binding of [³H]5-HT and [³H]LSD to purified synaptosomal membrane fraction.*

O——O, controls; ●——●, in the presence of 10 nM methiothepin (MET). Each experiment was repeated twice, using duplicate determinations.

ing involves two different sites, which correspond to (a) high-affinity binding (K_d = 2–3 nM), which we have already described in rat brain synaptosomal membranes in a preliminary paper (17), and (b) lower-affinity binding (K_d = 20–30 nM). Both types of binding involve sites that represent saturable populations and are located on membranes, as the ratio of reversible to total binding increases in parallel with the enrichment of the fraction in membranes; it is close to 20% in the homogenate, 30% in the lysed crude mitochondrial fraction, and 40% in the microsomal fraction, and reaches almost 50% in the synaptosome ghost fraction. Moreover, synaptic vesicles and the mitochondrial fraction have a low binding capacity, and the corresponding ratio of reversible to total binding is low (10–20%); this suggests that neither storage nor binding to an enzymatic degradative system is involved in the observed binding. Furthermore, no change in chromatographic characteristics was observed after the binding of 5-HT (or LSD).

We found that these two types of binding sites are physically separable. The purified synaptosomal fraction, which is assumed to represent a large proportion of pre- and postsynaptic membranes (see MATERIALS AND METHODS), contains high-affinity binding sites for 5-HT, corresponding to a single homogeneous population, whereas low-affinity binding sites for 5-HT are absent; this indicates that only high-affinity sites are located on synaptosomal membranes. The low-affinity binding sites are present in fractions rich in nonsynaptosomal membranes (axonal, glial, cytoplasmic, etc.), such as the crude mitochondrial fraction or fraction C_1 (see

MATERIALS AND METHODS). The lack of low-affinity binding sites does not seem to be due to artifactual modifications during the experimental procedure; fraction C_1, which contains microsomal and synaptosomal membranes and was prepared by a procedure similar to that used for the purified synaptosomal membranes, binds 5-HT with high and low affinity. Thus these two binding sites, which have different subcellular distributions, appear independent. However, the hypothesis that the two affinities might correspond to different conformations of the same site cannot be totally excluded, as the physical characteristics of the various membrane preparations might favor one or the other conformation of the site.

Several authors (12–14) have reported the presence of a single high-affinity binding for [^{3}H]LSD in rat brain homogenates. Recently Lovell and Freedman (15) reported LSD binding involving two sites (K_{d1} = 4 nM and K_{d2} = 25 nM) in crude brain membrane preparations. Our findings are in agreement with their results, since we also found two LSD binding sites in crude bovine brain membrane preparations (K_d = 4.1 nM and 20 nM); in addition, we have shown that these two binding site populations are probably independent, since their subcellular distributions are different.

It was previously reported by Bennett and Aghajanian (13) and Bennett and Snyder (14) that the binding capacities for 5-HT and LSD in different regions of the brain (especially the striatum) were not modified after raphe lesions. Our experiments using rat brain are in agreement with their results, since no change was observed in the binding of 5-HT or LSD after destruction of the raphe area by 5,6-dihydroxytryptamine injections. If the binding observed in such experiments corresponds to synaptic receptors involved in neurotransmission, the lack of modification of binding after presynaptic fiber degeneration would indicate that these receptors are probably postsynaptic, as has been suggested previously (14, 16). In the light of the current results, however, the use of a crude brain preparation might be expected to give specific binding derived predominantly from nonsynaptosomal, low-affinity sites, which would not be expected to be significantly modified by presynaptic lesions. Thus the lack of modification observed after such lesions does not permit one to distinguish among the various possibilities. However, recently reported results obtained in neuronally depleted rat striata (22) and human basal ganglia (24) suggest that [^{3}H]5-HT and [^{3}H]LSD binding involves postsynaptic sites on nerve cells, although presynaptic binding to nerve endings of non-5-HT neurons is not excluded.

Displacement studies performed on preparations that contained a single type of high-affinity binding sites showed that 5-HT itself, 5-HT derivatives, or compounds structurally related to 5-HT are the most effective in displacing [^{3}H]5-HT, whereas other amines or substances structurally different from 5-HT are ineffective. This binding thus seems to correspond to a site specific for 5-HT.

In the concentration range studied, noncompetitive effects were observed for the majority of the inhibition of [^{3}H]5-HT binding by LSD or of [^{3}H]LSD binding by 5-HT, indicating that the high-affinity binding sites for 5-HT are not identical with those for LSD. The finding that serotoninergic agonists act preferentially on 5-HT binding whereas antagonists or LSD derivatives act preferentially on LSD binding suggests that a serotoninergic site might be present in two conformations, one that binds 5-HT or tryptamine derivatives and another that preferentially binds serotoninergic antagonists or LSD derivatives. The first type would correspond to the agonist conformation of the serotoninergic site, and the second, to the antagonist conformation, as suggested for other sites (16, 24).

In conclusion, the high-affinity binding of 5-HT observed in our experiments is saturable, reversible, and 5-HT-specific; the distribution of the corresponding sites in various brain regions resembles that of 5-HT terminals. These sites are probably located on synaptosomal membranes and might represent a serotoninergic re-

ceptor involved in neurotransmission. A functional role of these sites is supported by preliminary results obtained using the same material, which indicated the presence of 5-HT-sensitive adenylate cyclase in the membrane fractions (25).

ACKNOWLEDGMENTS

This investigation was supported in part by "Direction des Recherches et Moyens d'Essais (Contract No. 76/507) and Centre National de la Recherche Scientifique (A.T.P. No. 1803). We wish to thank Dr. Bocquet R., veterinaire inspecteur en chef, for his kind help in supplying horse brains.

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