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Binding Interactions of Lysergic Acid Diethylamide and Related Agents with Dopamine Receptors in the Brain

DAVID R. BURT,[1] IAN CREESE,[2] AND SOLOMON H. SNYDER[3]

Departments of Pharmacology and Experimental Therapeutics and of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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SUMMARY

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In most brain regions saturable binding of *d*-[^{3}H]lysergic acid diethylamide ([^{3}H]LSD) appears to involve postsynaptic serotonin receptors. In calf caudate, however, a portion of [^{3}H]LSD binding involves postsynaptic dopamine receptors. Thus, in the hippocampus of calf brain, dopamine competes for [^{3}H]LSD binding sites with a single low-affinity component, while in the caudate dopamine competition for [^{3}H]LSD binding displays both high- and low-affinity components. The high-affinity component, accounting for 15-20% of [^{3}H]LSD binding, displays a K_i value for dopamine of about 30 nM, similar to the K_D for the binding of [^{3}H]dopamine itself to postsynaptic dopamine receptors in the calf caudate. Addition of serotonin to the incubations increases the proportion of [^{3}H]LSD binding in the caudate competed for by dopamine with high affinity, presumably by occupying serotonin receptors. *d*-LSD competes stereospecifically for [^{3}H]dopamine binding in the caudate, consistent with the conclusion that LSD binds to dopamine receptors. Of numerous serotonin agonists and antagonists examined, several ergot alkaloids have high affinity for [^{3}H]dopamine receptor binding in calf caudate, with K_i values similar to that of *d*-LSD.

INTRODUCTION

d-Lysergic acid diethylamide is a potent serotonin antagonist in several smooth muscle systems (1-3) and may have weak mixed agonist-antagonist activity at postsynaptic serotonin receptors in the brain (4-6). Its psychedelic actions have been ascribed primarily to its ability to inhibit the firing of serotonin neurons through actions on presynaptic receptors which are distinct from the postsynaptic serotonin receptor sites (4, 7-10). Recent behavioral observations suggest that LSD[4] can stimulate dopamine receptors in the brain. In intact animals high doses of LSD elicit stereotyped behavior (11, 12), and, after unilateral nigrostrial lesions, LSD provokes circling behavior like that produced by dopamine receptor stimulants (13, 14). In studies of the dopamine-sensitive adenylate cyclase, LSD behaves as a mixed

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[4] The abbreviation used is: LSD, lysergic acid diethylamide.

agonist-antagonist (15–17), blocking dopamine stimulation but itself enhancing enzyme activity.

Like LSD, ergot alkaloids are potent serotonin as well as *alpha* adrenergic antagonists in smooth muscle (2, 18). In invertebrates, ergots block ganglionic dopamine influences (19). In mammals, ergots, like LSD, elicit dopamine-like actions on prolactin release (13) and locomotor and turning behavior (20–23).

Recently direct binding of [^{3}H]dopamine to its postsynaptic receptor sites in the brain has been reported (24–28). The binding of [^{3}H]LSD to brain membranes appears to involve primarily the postsynaptic serotonin receptor (29, 30). Here we describe the influence of LSD, ergots, and related agents upon specific receptor binding of [^{3}H]dopamine and in addition demonstrate that a portion of [^{3}H]LSD binding in the caudate nucleus is to dopamine receptors.

METHODS

Methods were essentially those described previously (24, 27). Caudate regions of fresh or frozen calf brains were homogenized in 30–40 volumes of ice-cold 50 mM Tris buffer, pH 7.7, at 25°, with a Brinkmann Polytron PT-10. Binding results were the same for fresh brains and brains frozen for up to 3 weeks. The homogenate was centrifuged twice at 50,000 $\times$ *g* for 10 min, with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was homogenized in 90 volumes of cold, freshly prepared 50 mM Tris buffer, pH 7.6, at 25°, to which had been added 0.1% ascorbic acid, 10 μM pargyline, and ions as follows: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. This tissue suspension was placed in a 37° bath for 5 min and returned to ice.

[*ethyl*-1-^{3}H*(N)*]Dopamine, 8.4 Ci/mmole, was obtained from New England Nuclear Corporation and stored under nitrogen at 4°. *d*-[2*(N)*-^{3}H]Lysergic acid diethylamide, 20 or 26 Ci/mmole, was obtained from Amersham/Searle or New England Nuclear and stored at −20° in ethanol. The two radioactive drugs were diluted in 0.1% ascorbic acid to concentrations of 100 nM and 40 nM, respectively, on the day of use.

Incubation tubes in triplicate received 100 μl of diluted [^{3}H]dopamine or [^{3}H]LSD, 100 μl of various concentrations of drugs dissolved in 0.1% ascorbic acid, and 1.8 ml of caudate suspension. The tubes were incubated at 37° for 10 min ([^{3}H]dopamine) or 15 min ([^{3}H]LSD), and their contents were rapidly filtered under vacuum through Whatman GF/B filters with two 5-ml rinses of ice-cold 50 mM Tris buffer, pH 7.7, at 25°. The filters were counted in liquid scintillation counters (Packard or Nuclear-Chicago) in 12 ml of Hydromix (Yorktown Research) at efficiencies of 39–46%.

Saturable or specific binding of [^{3}H]dopamine was measured as the excess over blanks taken in the presence of 1 μM dopamine or 10 μM (+)-butaclamol. Blanks for [^{3}H]LSD binding were taken in the presence of 1 μM *d*-LSD. Specificity of binding sites was demonstrated by the lack of additivity of maximal displacements by various drugs, including dopamine, butaclamol, LSD, and apomorphine.

The sources of the drugs were as follows: dopamine, Sigma Chemical Compnay; apomorphine, Merck; (+)-butaclamol, Ayerst; fluphenazine, Squibb; serotonin and other tryptamine compounds, Regis Chemical Company; LSD analogues, psilocin, and psilocybin, the NIMH-FDA Committee on Scheduled Substances; 2,5-dimethoxy-4-ethylamphetamine isomers, Charles Barfknecht; bromo-LSD and ergots, Sandoz; mianserin, Organon; cyproheptadine, Smith Kline & French; and methiothepin, Roche.

RESULTS

d-LSD has substantial affinity for [^{3}H]dopamine binding sites (Table 1 and Fig. 1). Its IC_{50} value of 38 nM indicates an affinity more than half that of dopamine itself. The competition of LSD for dopamine binding is stereospecific, with the *l* isomer being less than 0.1% as potent as *d*-LSD. 2-Bromo-LSD is similar in potency to *d*-LSD, while *d*-lysergic acid amide and *d*-isolysergic acid amide are substantially less potent than *d*-LSD. The affinities of these LSD analogues for [^{3}H]dopamine sites resemble their affinities for [^{3}H]LSD

TABLE 1

Inhibition of [³H]dopamine binding in calf caudate by LSD and related agents

The values listed are the mean concentrations of each drug required to inhibit the specific binding of 5 nM [³H] dopamine by 50%. Specific binding was defined as the excess over that measured in the presence of 1 μM dopamine or 10 μM (+)-butaclamol. Each IC_{50} value was determined from a log probit plot, using three or more concentrations of drug in triplicate. Numbers are means ± standard errors for the indicated number of experiments.

Drug	IC_{50} nM
Lysergic acid derivatives	
d-LSD	38 ± 4 (9)
l-LSD	70,000 ± 17,000 (4)
2-Bromo-LSD	68 ± 9 (6)
d-Lysergic acid amide	226 ± 29 (3)
d-Isolysergic acid amide	243 ± 29 (3)
Ergots	
Ergotamine	37 ± 9 (3)
Ergocornine	45 ± 5 (5)
Ergocristine	65 ± 10 (5)
Ergometrine (ergobasin)	83 ± 21 (5)
α-Ergocryptine	86 ± 9 (6)
2-Bromo-α-ergocryptine (CB154)	132 ± 15 (6)
Serotonin antagonists	
Methiothepin	264 ± 35 (7)
Methysergide	366 ± 26 (5)
Cyproheptadine	1,490 ± 200 (7)
Mianserin	3,070 ± 520 (3)
Tryptamine derivatives	
5-Methoxytryptamine	13,300 ± 1,700 (3)
Tryptamine	16,800 ± 1,800 (4)
Serotonin (5-hydroxytryptamine)	17,000 ± 2,000 (7)
Bufotenine (5-hydroxy-*N*,*N*-dimethyltryptamine)	21,000 ± 1,000 (5)
Psilocin (4-hydroxy-*N*,*N*-dimethyltryptamine)	22,000 ± 2,500 (3)
N,*N*-Dimethyltryptamine	24,000 ± 1,000 (2)
Miscellaneous hallucinogens	
Mescaline	135,000 ± 35,000 (2)
R(−)-2,5-Dimethoxy-4-ethylamphetamine	153,000 ± 22,000 (3)
S(+)-2,5-Dimethoxy-4-ethylamphetamine	277,000 ± 63,000 (3)
(±)-2,5-Dimethoxy-4-methylamphetamine	300,000 ± 58,000 (3)
Psilocybin	>1,000,000
Dopamine agonists and antagonists	
Apomorphine	11 ± 1 (5)
Dopamine	21 ± 3 (10)
(+)-Butaclamol	97 ± 19 (8)
Fluphenazine	260 ± 57 (6)

binding sites in the cerebral cortex (29). Potent dopamine receptor-blocking agents such as fluphenazine and (+)-butaclamol are much less potent than *d*-LSD in competing for [³H]dopamine receptor binding.

The effect of LSD on [³H]dopamine receptor binding is probably not related to its psychedelic actions. The potent psychedelic agent psilocin is only about 0.1% as potent as LSD in competing for [³H]dopamine binding. Like psilocin, the psychedelic drug *N*,*N*-dimethyltryptamine is less than 1/1000 as potent as LSD in competing for [³H]dopamine binding, similar to the potency of serotonin itself and other tryptamines such as bufotenine, tryptamine, and 5-methoxytryptamine. Mescaline and the potent psychedelic substances 2,5-dimethoxy-4-ethyl- and -4-methylamphetamine fail to inhibit [³H]-

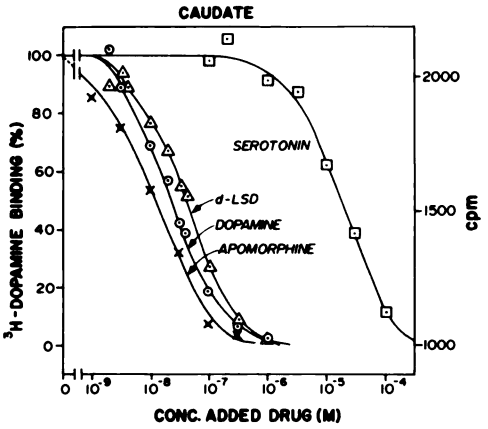

FIG. 1. *Competition by dopamine, apomorphine, d-LSD, and serotonin for specific [^{3}H]dopamine binding in membranes of calf caudate*

Tubes contained 5 nM [^{3}H]dopamine and tissue equivalent to 20 mg, wet weight, per 2 ml. For each curve, specific binding in 6–10 experiments was averaged on a percentage basis, taking binding in the presence of 1 μM dopamine or 10 μM (+)-butaclamol as a blank. Absolute standard errors of the means were less than 3%. The right-hand ordinate is based on the total mean counts per minute observed in all the experiments for the 100% and 0% points for specific binding.

dopamine binding at 0.1 mM concentrations.

The ergot alkaloids are also potent in competing for [^{3}H]dopamine binding sites. Ergotamine and ergocornine have an affinity similar to *d*-LSD for [^{3}H]dopamine binding sites, while ergocristine, ergometrine, and ergocryptine are one-third to one-half, and brom-ergocryptine about one-fourth, as potent as ergotamine.

The serotonin antagonists methiothepin and methysergide are 10–14% as potent as *d*-LSD in competing for [^{3}H]dopamine binding, while two other serotonin antagonists, mianserin and cyproheptadine, are only 1–3% as active as *d*-LSD.

Receptor binding of [^{3}H]dopamine and [^{3}H]LSD can be clearly differentiated by the influence of dopamine itself and of serotonin (see below) and by marked differences in the regional distribution of dopamine and LSD binding (Table 2). As previously reported (24), [^{3}H]dopamine binding is highly restricted to areas of the brain such as the corpus striatum, olfactory tubercle, and nucleus accumbens, which are known to be rich in dopamine nerve terminals. The caudate possesses about twice the dopamine binding of the globus pallidus, putamen, and olfactory tubercle, and 3 times the binding of the nucleus accumbens. LSD binding is also highest in the caudate, but, in contrast to the distribution of dopamine binding, substantial LSD binding is detectable in all brain regions except the cerebellum, as previously reported for rat and monkey brain (28, 29).

Previous results demonstrated that specific [^{3}H]dopamine binding appears to selectively involve postsynaptic dopamine receptors, while [^{3}H]LSD binding predominantly involves sites with characteristics expected of postsynaptic serotonin receptors. However, because LSD competes for [^{3}H]dopamine binding sites with high affinity, one might expect some [^{3}H]LSD binding to involve the dopamine receptor. In order to determine whether any detectable portion of [^{3}H]LSD binding labels postsynaptic dopamine receptors, we compared the binding of [^{3}H]dopamine and [^{3}H]LSD in the caudate, which is enriched

TABLE 2

Regional distribution of [^{3}H]dopamine and [^{3}H]LSD binding in calf brain

Fresh calf brain was dissected into regions and assayed fresh or after storage at −20° for up to 1 week. Binding measurements were conducted in triplicate with 5 nM [^{3}H]dopamine and 2 nM [^{3}H]LSD, using 10 μM (+)-butaclamol and 1 μM *d*-LSD as blanks, respectively. Regions in which apparent specific binding was less than 10% of the blank values are listed as "ND" (not detectable). Results with [^{3}H]dopamine are based on two to four experiments. Results with [^{3}H]LSD are those of a single experiment.

Region	[^{3}H]Dopamine	[^{3}H]LSD
	fmoles/mg tissue	
Caudate	6.3	20.1
Globus pallidus	3.8	12.6
Putamen	3.5	16.0
Olfactory tubercle	2.5	8.5
Nucleus accumbens	2.0	5.5
Hippocampus	ND	13.7
Hypothalamus	ND	8.4
Cerebral cortex	ND	5.1
Cerebellum	ND	ND

in both dopamine and serotonin, and in the hippocampus, which possesses substantial amounts of serotonin and [^{3}H]LSD binding but no detectable [^{3}H]dopamine binding. To the extent that [^{3}H]LSD labels dopamine receptors, one might anticipate differences in competition for [^{3}H]LSD binding by dopamine and serotonin in the caudate and hippocampus.

In the caudate (Fig. 1), specific [^{3}H]dopamine binding is inhibited with considerable potency by dopamine, apomorphine, and *d*-LSD, with IC_{50} values of 21 nM, 11 nM, and 28 nM, respectively, while serotonin has much less affinity, with an IC_{50} value of 17,000 nM. The competition curves for all four agents appear parallel. In the hippocampus (Fig. 2), the affinities of these agents for [^{3}H]LSD binding are markedly different. *d*-LSD is about 4–5 times more potent in competing for [^{3}H]LSD binding in the hippocampus than for [^{3}H]dopamine binding in the caudate. The relative potencies of serotonin and dopamine are reversed. Whereas in the caudate dopamine is 800 times more potent than serotonine in displacing [^{3}H]dopamine, in the hippocampus serotonin is 2000 times more potent than dopamine in displacing [^{3}H]LSD. Apomorphine is about 40 times more potent than dopamine in competing for [^{3}H]LSD binding in the hippocampus. Competiton curves for all four agents at hippocampal LSD binding sites are parallel. These differences between [^{3}H]dopamine binding in the caudate and [^{3}H]LSD binding in the hippocampus support other data indicating that the former involves postsynaptic dopamine receptors and the latter involves sites that may be postsynaptic serotonin receptors.

The pattern of [^{3}H]LSD binding in the caudate differs substantially from that in the hippocampus (Fig. 3). *d*-LSD appears to have similar affinity for [^{3}H]LSD binding sites in the two regions. However, the competition curve of dopamine for [^{3}H]LSD binding in the caudate in repeated experiments displays two components which appear to separate at about 1 μM dopamine, a concentration at which dopamine completely inhibits specific [^{3}H]dopamine binding in the caudate but has no influence on [^{3}H]LSD binding in the hippocampus. The high-affinity component of dopamine competition for [^{3}H]LSD binding in the caudate accounts for 15–20% of specific [^{3}H]LSD binding (Fig. 4). Half-maximal inhibition of this high-affinity component occurs at about 30–40 nM dopamine, which is slightly greater than the concentration of dopa-

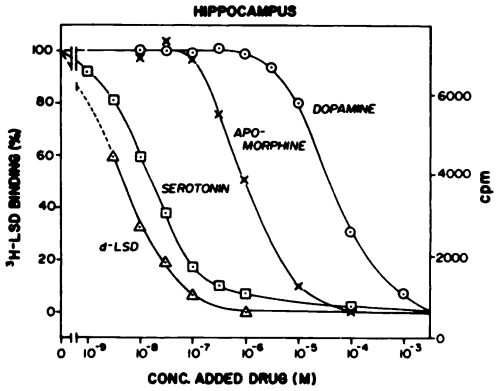

FIG. 2. *Competition by dopamine, apomorphine, d-LSD, and serotonin for specific [^{3}H]LSD binding in membranes of calf hippocampus*

Tubes contained 2 nM [^{3}H]LSD and tissue equivalent to 20 mg, wet weight, per 2 ml. Mean values of the results of three or four experiments were derived as in Fig. 1, except that blanks contained 1 μM *d*-LSD. The apomorphine curve is based on a single experiment. The ordinate refers to specific binding values. Standard errors were 2–4% of mean values.

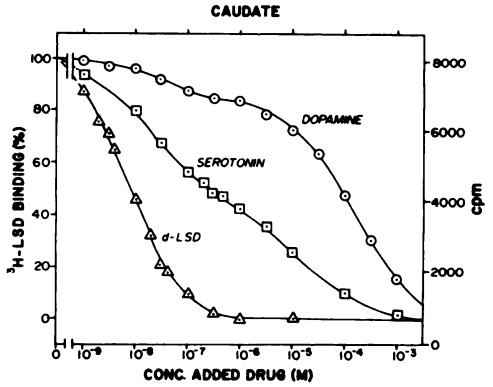

FIG. 3. *Competition by dopamine, d-LSD, and serotonin for specific [^{3}H]LSD binding in membranes of calf caudate*

Results are the means of five to seven experiments, with standard errors less than 4% of means. Conditions were the same as in Fig. 2. The ordinate refers to specific binding.

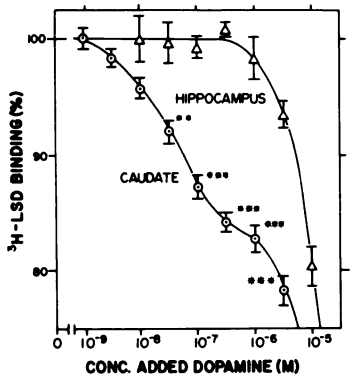

FIG. 4. *Competition by dopamine for [^{3}H]LSD binding in caudate and hippocampus*

Enlargement of data in Fig. 2 and 3. Error bars represent standard errors of the mean (n = 7, caudate; n = 4, hippocampus). Points on the two curves were significantly different as indicated. The ordinate refers to specific binding.

** $p < 0.01$.

*** $p < 0.001$.

mine that inhibits 50% of [^{3}H]dopamine binding in the caudate. By contrast, in the hippocampus, which contains no detectable postsynaptic dopamine receptors, there appears to be only one low-affinity component of dopamine competition for [^{3}H]LSD binding, with an IC_{50} value of about 40 μM. This is similar to the low-affinity component of dopamine inhibition of [^{3}H]LSD binding in the caudate, whose IC_{50} value is about 100 μM.

These data suggest that a detectable portion of [^{3}H]LSD binding in the caudate involves postsynaptic dopamine receptor sites. Presumably serotonin should have considerably lower affinity for [^{3}H]LSD binding to dopamine than to serotonin receptors. Consistent with this notion are the differences in competition curves of serotonin for [^{3}H]LSD binding in the caudate and hippocampus. Whereas serotonin displays only a single high-affinity component of competition for [^{3}H]LSD binding in the hippocampus, with an IC_{50} value of 16 nM, in the caudate serotonin displacement of [^{3}H]LSD binding exhibits an anomalous slope which we have interpreted as possibly representing competition for at least two classes of [^{3}H]LSD binding sites. If one assumes that the high- and low-affinity components of serotonin competition for [^{3}H]LSD binding in the caudate are approximately separable at about 1 μM serotonin, then the high-affinity inhibition of [^{3}H]LSD binding by serotonin has an IC_{50} of about 10–20 nM, the same as the affinity of serotonin for [^{3}H]LSD binding sites in the hippocampus.

If [^{3}H]LSD binding in the caudate involves both serotonin and dopamine sites, it might be possible to increase the relative proportion of [^{3}H]LSD binding that involves postsynaptic dopamine receptors by conducting incubations in the presence of 1 μM serotonin to reduce binding of LSD to putative serotonin receptors (Fig. 5). Without serotonin in the incubation mixture, only 15–20% of [^{3}H]LSD binding in the caudate involves the apparent high-affinity dopamine sites. By contrast, in the presence of 1 μM serotonin, the high-affinity component of dopamine competition for [^{3}H]LSD binding appears to involve about 40–45% of the total saturable [^{3}H]LSD binding. The IC_{50} for the high-affinity component of dopamine competition for [^{3}H]LSD binding is again about 35 nM. Apomorphine also appears to display two components of competition for [^{3}H]LSD binding in the caudate in the presence of 1 μM serotonin. The high-affinity component of apomorphine competition for [^{3}H]LSD binding under these conditions has an IC_{50} of about 6–10 nM.

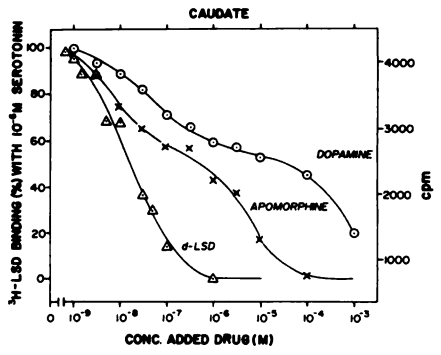

FIG. 5. *Competition by dopamine, apomorphine, and d-LSD for [^{3}H]LSD binding in the presence of 1 μM serotonin in membranes of calf caudate*

Results of two or three experiments for each curve are plotted as in Fig. 2. Standard errors were 2–4% of means.

DISCUSSION

A principal finding of the present study is that LSD and ergot alkaloids have substantial affinity for [^{3}H]dopamine receptor binding sites, thus explaining many of the previously reported behavioral and pharmacological effects of these drugs.

In the caudate we have obtained evidence that a portion of [^{3}H]LSD binding involves postsynaptic dopamine receptors, although in the hippocampus [^{3}H]LSD binding is associated almost exclusively with apparent synaptic serotonin receptors. Since destruction of presynaptic serotonin and dopamine nerve terminals, respectively, fails to lower [^{3}H]LSD and [^{3}H]dopamine binding, it is likely that these [^{3}H]ligands bind to postsynaptic rather than presynaptic sites (24, 25, 27, 28, 30). Dopamine competes with high affinity for only 15–20% of total [^{3}H]LSD binding in the caudate. However, because the affinity of LSD for dopamine receptors appears to be less than that for serotonin receptors (Figs. 1 and 2), the proportion of total [^{3}H]LSD binding sites that represents dopamine receptors is probably substantially greater than 15–20%. Scatchard analyses of [^{3}H]dopamine and [^{3}H]LSD binding in the caudate indicate that the total number of [^{3}H]LSD and [^{3}H]dopamine binding sites is 70–90 and 25–35 pmoles/g, respectively. This would suggest that perhaps one-third or more of the LSD binding sites in the caudate represent dopamine receptors. Estimates of total numbers of sites derive from saturating concentrations of [^{3}H]ligands, at which binding is 4–5 times the levels at nonsaturating concentrations (Table 2).

The biphasic competition of serotonin for [^{3}H]LSD binding in the caudate but not in the hippocampus resembles data obtained for [^{3}H]LSD binding in microsomal membrane preparations of rat cerebral cortex (30). Conceivably the factors determining this biphasic curve in the caudate, such as binding of [^{3}H]LSD to both dopamine and serotonin receptors, may be related to previous observations in the cerebral cortex. The evidence that LSD and related serotonin agonists and antagonists bind to dopamine receptors suggests that these agents may produce some of their behavioral effects directly through dopaminergic mechanisms. In any case, some of these drugs can no longer be considered "pure" serotonin agonists or antagonists.

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