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Multiple Serotonin Receptors: Differential Binding of [^{3}H]5-Hydroxytryptamine, [^{3}H]Lysergic Acid Diethylamide and [^{3}H]Spiroperidol

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SUMMARY

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[^{3}H]5-Hydroxytryptamine (5-HT), [^{3}H]lysergic acid diethylamide (LSD) and [^{3}H]spiroperidol bind to membranes from the rat frontal cerebral cortex in a manner indicating a selective interaction with serotonin receptors. Differential drug potencies in competing for [^{3}H]5-HT and [^{3}H]spiroperidol binding sites suggest that these two [^{3}H]ligands respectively label two distinct populations of receptors, while [^{3}H]LSD labels both the [^{3}H]5-HT and [^{3}H]spiroperidol sites. After incubation of brain membranes with 30 nM spiroperidol, drug specificity of the residual [^{3}H]LSD binding resembles that of receptors labeled by [^{3}H]5-HT. Conversely, drug effects on [^{3}H]LSD binding in the presence of 300 nM 5-HT resemble effects with [^{3}H]spiroperidol. We propose that [^{3}H]5-HT and [^{3}H]-spiroperidol label distinct populations of serotonin receptors in rat brain, designated 5-HT_1 and 5-HT_2 receptors, respectively. [^{3}H]LSD appears to bind to both receptors to a similar extent.

INTRODUCTION

Binding techniques have permitted the differentiation of multiple neurotransmitter receptors. Distinct subtypes of muscarinic cholinergic (1) and dopamine (2–4) receptors can be identified by the differential binding properties of various [^{3}H]ligands. Beta-noradrenergic receptors are distinguished as β_1 and β_2 on the basis of both physiologic (5) and binding studies (6–8).

Alpha-noradrenergic receptors have been labeled by a variety of [^{3}H]ligands in both the periphery and the central nervous system. The agonists [^{3}H]clonidine, [^{3}H]norepinephrine and [^{3}H]epinephrine label postsynaptic α-receptors (9, 10) whose pharmacologic properties resemble those of α-adrenergic autoreceptors (α_2-receptors). The antagonist [^{3}H]WB-4101 (10) binds to postsynaptic α-receptors with a different pharmacologic specificity and these are referred to as α_1-sites. The ergot [^{3}H]dihydroergokryptine labels both α_1- and α_2-receptors to a similar extent (11–14). The different drug specificities of α_1- and α_2-postsynaptic α-adrenergic receptors correspond closely with differential physiological properties of these two sites in peripheral organs (15).

Serotonin (5-HT) receptors have been labeled in the brain with [^{3}H]5-HT and [^{3}H]LSD (16–21). It has recently been reported that [^{3}H]spiroperidol also labels ser-

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otonin receptors (22, 23). Though the relative potencies of various drugs indicate that all three ligands bind to postsynaptic 5-HT receptors, differences in the absolute potencies of various drugs have been reported (18, 22, 24). In earlier reports we have suggested that these different drug effects reflected distinct but interconverting states of one 5-HT receptor (18). In the present study we present evidence that [^{3}H]5-HT and [^{3}H]spiroperidol label distinct noninterconverting populations of 5-HT receptors and that [^{3}H]LSD labels both sites to a similar extent.

MATERIALS AND METHODS

Frontal cerebral cortices were dissected from freshly decapitated adult male Sprague-Dawley rats (150–200 g). The tissue was homogenized in 10 volumes of 0.32 M sucrose using a motor driven pestle. The homogenate was centrifuged at 700 × *g* for 10 min in a Sorvall RC2-B centrifuge. The supernatant fluid was decanted from the crude nuclear pellet (P1) and centrifuged at 50,000 × *g* for 10 min. The sedimented material (P2) was resuspended in 10 volumes of 50 mM Tris-HCl buffer (pH 7.5 at 25°) using a Brinkmann Polytron for 10 sec. The tissue suspension was incubated at 37° for 10 min and then centrifuged again at 50,000 × *g* for 10 min. The pellet was resuspended in the standard assay buffer which consisted of 50 mM Tris-HCl (pH 7.7 at 25°), 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% ascorbic acid. The final tissue suspension was incubated for 15 min at 37° and then stored on ice until used for no longer than 4 hr.

Incubation tubes received 100 μl of [^{3}H]ligands, 100 μl of various drugs and 0.8 ml of tissue suspension during standard binding assays. All assays were performed in triplicate. The concentrations of labeled ligands were 2.0 nM [^{3}H]5-HT, 4.25 nM [^{3}H]LSD, and 0.26 nM [^{3}H]spiroperidol. The final tissue concentration of rat frontal cerebral cortex was 10 mg/ml. The tubes were incubated at 37° (10 min for [^{3}H]5-HT and [^{3}H]LSD; 15 min for [^{3}H]spiroperidol) and then rapidly filtered under vacuum through Whatman GF/B filters with three 5 ml rinses of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°). The filters were counted by liquid scintillation spectrometry in 9 ml of Formula 947 (New England Nuclear) after 18 hr extraction at 4° at efficiencies of 38–40%.

Specific binding of the [^{3}H]ligands was defined as the excess over blank values obtained in the presence of 1 μM *d*-LSD. Generally, 70–80% of total binding was specific for [^{3}H]5-HT and [^{3}H]LSD while 55–65% of [^{3}H]spiroperidol binding was specific. [^{3}H]5-HT (28.2 Ci/mmole) and [^{3}H]-spiroperidol (23.6 Ci/mmole) were purchased from New England Nuclear and [^{3}H]LSD (10.7 Ci/mmole) was obtained from Amersham. All [^{3}H]ligands were diluted in standard assay buffer immediately before use. All drugs were dissolved in distilled water and diluted as necessary in standard assay buffer. Drugs were obtained from the following sources. 5-HT, 5-methoxytryptamine, and dopamine from Sigma; *d*-LSD and 2-bromo-LSD from the National Institute on Drug Abuse; 5,6 dihydroxytryptamine and bufotenine from Regis; methysergide and clozapine from Sandoz; cyproheptadine from Merck, Sharpe and Dohme; mianserin from Organon; cinanserin from Squibb; spiroperidol from Janssen; haloperidol from McNeil; promethazine from Wyeth. ADTN was the generous gift of Dr. R. Pinder.

RESULTS

Displacement of [^{3}H]ligands by 5-HT, spiroperidol and LSD. 5-HT, spiroperidol and LSD all mutually compete for their respective [^{3}H]ligands (Figs. 1–3). If the three [^{3}H]ligands label the same population of receptors, then 5-HT, spiroperidol and LSD should have the same potencies and displacement slopes in competing for all [^{3}H]ligands. However, there are marked differences among the three agents. For instance, 5-HT is almost a thousand times more potent in reducing [^{3}H]5-HT than in competing for [^{3}H]spiroperidol binding (Fig. 1). Hill coefficients for 5-HT inhibition of these two [^{3}H]ligands are close to 1.0. In contrast, 5-HT displacement of [^{3}H]LSD binding is intermediate in potency when compared to its effects on [^{3}H]5-HT and [^{3}H]spiroperidol binding. Moreover, the

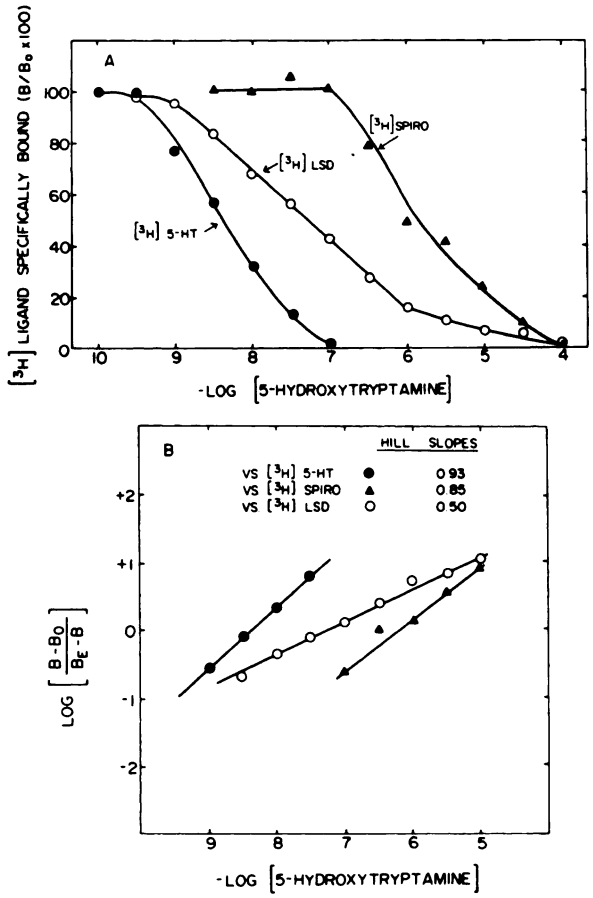

FIG. 1. *Inhibition of [^{3}H]ligand binding to serotonin receptors by 5-hydroxytryptamine*

[^{3}H]Ligand binding assays were performed as described in MATERIALS AND METHODS. Data shown are the means of triplicate assays in a single experiment. The [^{3}H]ligands studied are [^{3}H]5-HT (●), [^{3}H]spiroperidol (▲) and [^{3}H]LSD (○). A. Displacement of [^{3}H]ligands in rat frontal cerebral cortex by 5-hydroxytryptamine. B. Hill plots of 5-hydroxytryptamine displacement curves.

displacement curve of [^{3}H]LSD by 5-HT is quite shallow, with a Hill coefficient of 0.5.

An inverse type of pattern occurs when spiroperidol competes for each of the [^{3}H]ligands (Fig. 2). Thus, spiroperidol is a thousand-fold less potent in reducing [^{3}H] 5-HT binding than in competing for [^{3}H] spiroperidol binding, while [^{3}H]LSD is displaced with an intermediate potency. Spiroperidol displacement of [^{3}H]spiroperidol has a Hill coefficient of 1.1. Displacement of [^{3}H]5-HT binding by spiroperidol has a more shallow slope and a Hill coefficient of 0.64. The most shallow displacement occurs in the competition of spiroperidol for [^{3}H]-LSD binding with a Hill coefficient of 0.30. Moreover, in repeated experiments a biphasic curve is obtained with a plateau for inhibition of [^{3}H]LSD binding between 10 and 30 nM spiroperidol. The difference in apparent K_i values for spiroperidol displacement of [^{3}H]5-HT and [^{3}H]spiroperidol is fifteen hundred-fold. A plateau is, therefore, consistent with biphasic displacement of [^{3}H]LSD from two sites with more than three orders of magnitude difference in affinity. No plateau was consistently noted in 5-HT displacement of [^{3}H]LSD, where only a seven hundred-fold difference exists between 5-HT affinity for [^{3}H]5-HT and [^{3}H]spiroperidol binding.

In marked contrast to the behavior of 5-

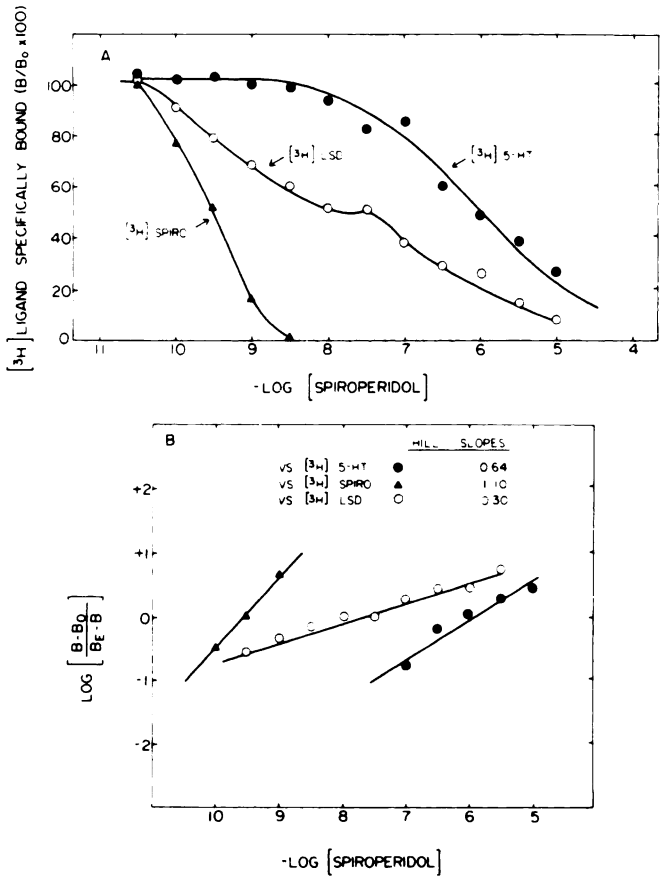

FIG. 2. *Inhibition of [^{3}H]ligand binding to serotonin receptors by spiroperidol*

[^{3}H]Ligand binding assays were performed as described in MATERIALS AND METHODS. Data shown are the means of triplicate assays in a single experiment. The experiment was replicated five times. [^{3}H]Ligands studied are [^{3}H]5-HT (●), [^{3}H]spiroperidol (▲) and [^{3}H]LSD (○). A. Displacement of [^{3}H]ligands in rat frontal cerebral cortex by spiroperidol. B. Hill plots of spiroperidol displacement curves.

HT and spiroperidol, LSD itself has identical potencies in reducing either [^{3}H]5-HT, [^{3}H]spiroperidol or [^{3}H]LSD binding (Fig. 3). The slopes of the three displacement curves are essentially the same with Hill coefficients of approximately 1.0. This type of displacement behavior suggests that LSD has similar affinity for receptors labeled by the three [^{3}H]ligands.

Saturation of [^{3}H]5-HT, [^{3}H]spiroperidol and [^{3}H]LSD as influenced by 5-HT and spiroperidol. The markedly discrepant potencies of 5-HT and spiroperidol in competing for [^{3}H]5-HT and [^{3}H]spiroperidol respectively suggest that these two [^{3}H]ligands label different sites. The intermediate potencies of 5-HT and spiroperidol in lowering [^{3}H]LSD binding and the comparative shallowness of the displacement curves suggest that [^{3}H]LSD may be labeling both the sites to which [^{3}H]5-HT and [^{3}H]spiroperidol respectively bind. To further test this possibility we examined the saturation properties of [^{3}H]5-HT, [^{3}H]spiroperidol and [^{3}H]LSD (Figs. 4–7).

All three [^{3}H]ligands display monophasic saturation with a single component in evidence on Scatchard analysis. The maximal number of [^{3}H]5-HT (Figure 4) and [^{3}H]-spiroperidol (Fig. 5) binding sites are both slightly less than 10 pmoles/g wet weight tissue. The amount of [^{3}H]LSD (Fig. 6)

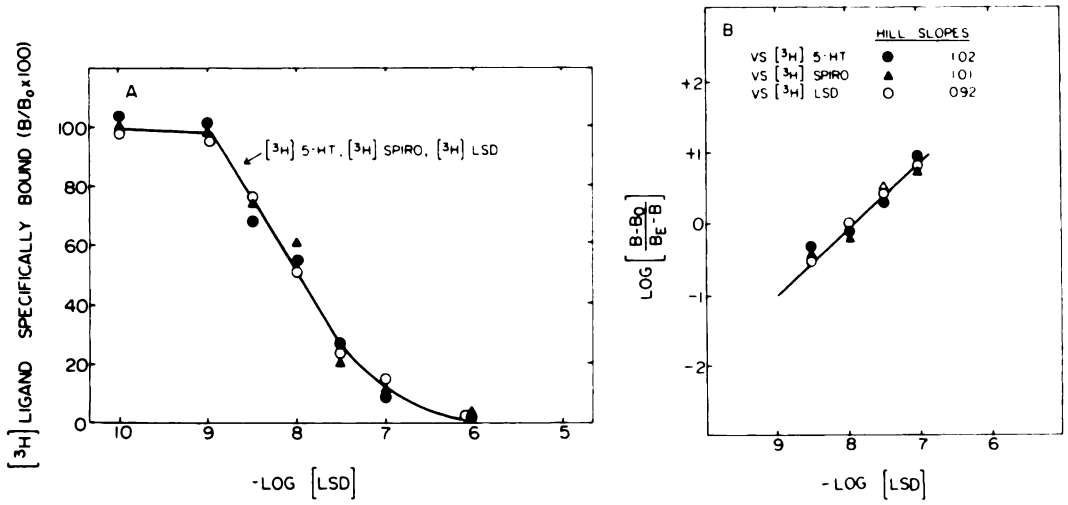

FIG. 3. *Inhibition of [^{3}H]ligand binding to serotonin receptors by LSD*

[^{3}H]Ligand binding assays were performed as described in MATERIALS AND METHODS. Data shown are the means of triplicate assays in a single experiment. The experiment was replicated three times. [^{3}H]Ligands studied are [^{3}H]5-HT (●), [^{3}H]spiroperidol (▲) and [^{3}H]LSD (○). A. Displacement of [^{3}H]ligands in rat frontal cerebral cortex by LSD. B. Hill plots of LSD displacement curves.

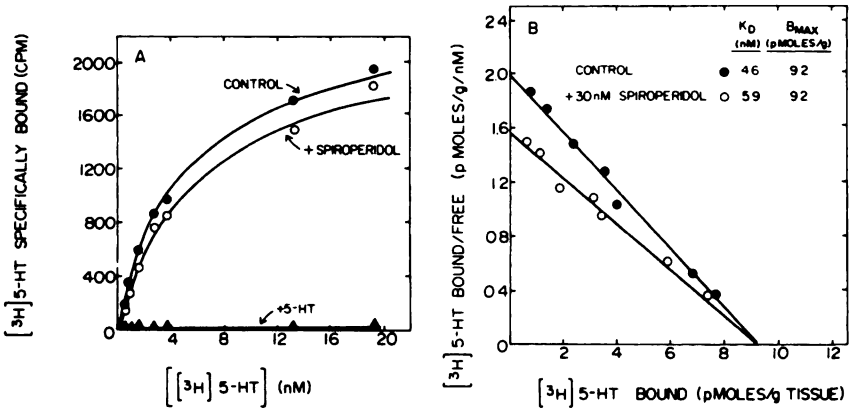

FIG. 4. *Saturation of [^{3}H]5-HT binding in rat frontal cerebral cortex*

Rat frontal cerebral cortex membranes were incubated for 10 min at 37° as described in MATERIALS AND METHODS. At each [^{3}H]5-HT concentration, total binding was measured in the absence of inhibitor (●), in the presence of 30 nM spiroperidol (○) and the in the presence of 300 nM 5-HT (▲). Specific binding was determined by subtracting from total binding that portion of binding not inhibited by the combination of 30 nM spiroperidol and 300 nM 5-HT. Points shown are the means of triplicate assays performed in a single experiment. The experiment was replicated three times with values that varied less than 20%. A. Specific [^{3}H]5-HT binding. B. Scatchard analysis of data in A.

bound, however, is greater than 20 pmoles/g tissue. This finding confirms our earlier observation that the maximal number of [^{3}H]LSD binding sites is approximately double the number of [^{3}H]5-HT sites in rat frontal cerebral cortex (18).

If [^{3}H]5-HT and [^{3}H]spiroperidol label in part the same sites, then a concentration of spiroperidol which suffices to occupy all the [^{3}H]spiroperidol sites (i.e., 30 nM) should have some effect on [^{3}H]5-HT binding. However, in the presence of 30 nM spiroperidol, the B_{max} of [^{3}H]5-HT binding is not changed and only a slight increase in the

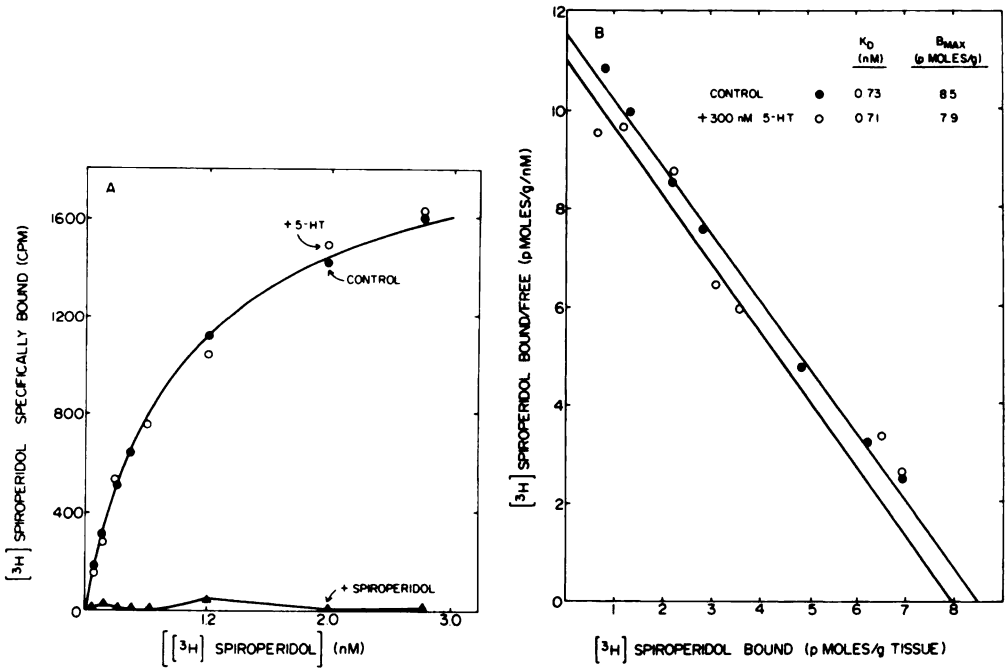

FIG. 5. *Saturation of [^{3}H]spiroperidol binding in rat frontal cerebral cortex*

Rat frontal cerebral cortex membranes were incubated for 15 min at 37° as described in MATERIALS AND METHODS. At each [^{3}H]spiroperidol concentration, total binding was measured in the absence of inhibitor (●), in the presence of 300 nM 5-HT (○) and in the presence of 30 nM spiroperidol (▲). Specific binding was determined by subtracting from total binding that portion of binding not inhibited by the combination of 300 nM 5-HT and 30 nM spiroperidol. Points shown are the means of triplicate assays performed in a single experiment. The experiment was replicated three times with values which varied less than 25%. A. Specific [^{3}H]spiroperidol binding. B. Scatchard analysis of data in A.

K_D is observed (Fig. 4). Similarly, if [^{3}H]5-HT and [^{3}H]spiroperidol label the same sites a concentration of 5-HT which occupies all the [^{3}H]5-HT sites (*i.e.*, 300 nM) should reduce [^{3}H]spiroperidol binding. Strikingly, 300 nM 5-HT does not affect either the K_D value or B_{max} value for [^{3}H]spiroperidol binding (Fig. 5).

Interestingly, yet another pattern of inhibition is observed when [^{3}H]LSD binding is analyzed. Both 30 nM spiroperidol and 300 nM 5-HT inhibit [^{3}H]LSD binding by about 50%. When [^{3}H]LSD saturation curves are examined in the presence of either of these concentrations of inhibitors, the K_D value is unchanged while the B_{max} is reduced by 50% (Fig. 6). This finding again suggests that [^{3}H]5-HT and [^{3}H]spiroperidol label distinct receptor populations while [^{3}H]LSD labels both sites to a similar extent.

If 30 nM spiroperidol completely eliminates [^{3}H]LSD binding to one of two 5-HT receptors, then further increases in spiroperidol concentration should competitively inhibit [^{3}H]LSD binding to the remaining receptor site. An increase in the K_D without change in the B_{max} would be predicted. A similar argument would apply to the reduction of [^{3}H]spiroperidol by 5-HT. As spiroperidol concentration is increased from 30 nM to 1,000 nM the reduction of [^{3}H]LSD binding occurs by an increase in the K_D value with no change in the B_{max} value (Fig. 7A). Similarly, increasing the 5-HT concentration from 300 nM to 3,000 nM is associated with an increase in the K_D value with no change in the B_{max} (Fig. 7B).

Influence of drugs on [^{3}H]5-HT, [^{3}H]-spiroperidol and [^{3}H]LSD binding. To further examine the apparent labeling of different sites by the [^{3}H]ligands, we eval-

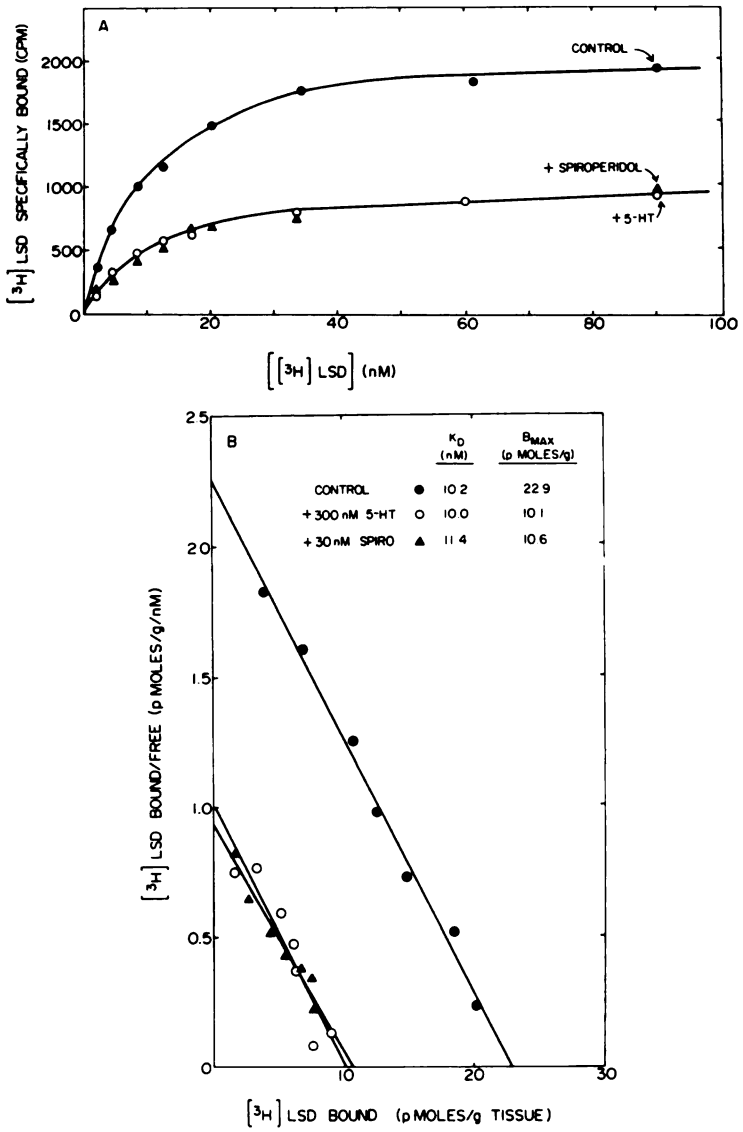

FIG. 6. *Saturation of [^{3}H]LSD binding in rat frontal cerebral cortex.*

Rat frontal cerebral cortex membranes were incubated for 10 min at 37° as described in MATERIALS AND METHODS. At each [^{3}H]LSD concentration, total binding was measured in the absence of inhibitor (●), in the presence of 300 nM 5-HT (○) and in the presence of 30 nM spiroperidol (▲). Specific binding was determined by subtracting from total binding that portion of binding not inhibited by the combination of 300 nM 5-HT and 30 nM spiroperidol. Points shown are the means of triplicate assays performed in a single experiment. The experiment was replicated four times with values which varied less than 20%. A. Specific [^{3}H]LSD binding. B. Scatchard analysis of data in A.

uated the effects of various drugs on the binding of [^{3}H]5-HT, [^{3}H]spiroperidol and [^{3}H]LSD (Table 1). As observed previously, tryptamines are substantially more potent in reducing [^{3}H]5-HT binding than [^{3}H]spiroperidol binding, while their effects on [^{3}H]LSD are intermediate in potency (18, 21, 22). By contrast, classical serotonin antagonists and neuroleptics are much more potent in reducing [^{3}H]spiroperidol than [^{3}H]5-HT binding with intermediate influences on [^{3}H]LSD binding. *d*-LSD is unique

in that it has similar potency in competing for all three [^{3}H]ligands, while bromo-LSD and methysergide are more potent on [^{3}H]spiroperidol binding than [^{3}H]5-HT with, again, intermediate effects on [^{3}H]-LSD binding. The K_i values of each of these drugs against [^{3}H]5-HT and [^{3}H]spiroperidol are not correlated (Fig. 8).

For all drugs examined, the K_i value vs. [^{3}H]LSD is intermediate between those for [^{3}H]5-HT and [^{3}H]spiroperidol. If [^{3}H]LSD labels both the [^{3}H]5-HT and [^{3}H]spiroperidol sites, then a "predicted" K_i value against [^{3}H]LSD can be computed as the arithmetic mean between the logarithms of K_i values for the inhibition of [^{3}H]5-HT and [^{3}H]spiroperidol binding. These "predicted" K_i values agree well with those observed experimentally (Table 1; columns C and D), indicating that [^{3}H]LSD binds to a similar extent to the sites labeled independently by [^{3}H]5-HT and [^{3}H]spiroperidol.

Additionally, we evaluated drug influences on [^{3}H]LSD binding in the presence of 30 nM spiroperidol or in the presence of 300 nM 5-HT. Since 30 nM spiroperidol completely blocks [^{3}H]spiroperidol binding and reduces [^{3}H]LSD binding by 50%, then the residual [^{3}H]LSD binding should involve the same sites as those labeled by [^{3}H]5-HT. When [^{3}H]LSD binding is assayed in the absence of spiroperidol no significant correlation is found between K_i values against [^{3}H]LSD and [^{3}H]5-HT (Fig. 9A). However, when [^{3}H]LSD binding is assayed in the presence of 30 nM spiroperidol the potencies of all drugs closely resemble their effects on [^{3}H]5-HT binding with a correlation coefficient of 0.98 (Fig. 9C).

Conversely, the binding of [^{3}H]LSD in the presence of 300 nM 5-HT should represent labeling of the same sites as [^{3}H]spiroperidol. While some correlation does exist between drug effects on [^{3}H]spiroperidol and [^{3}H]LSD binding ($r = 0.71$), resembling results of Leysen *et al.* (22), the addition of

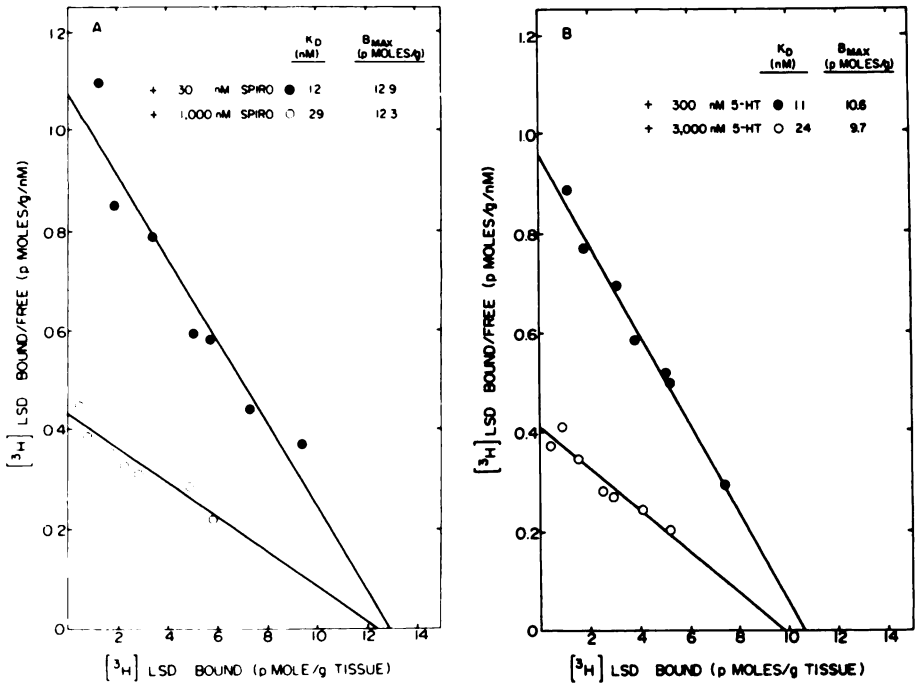

FIG. 7. *Scatchard plots of [^{3}H]LSD binding saturation in rat fronal cerebral cortex in the presence of spiroperidol (A) and 5-HT (B)*

Experiments were performed as described in Figure 6. Points shown are the means of triplicate assays done in a single experiment. A. (●), 30 nM spiroperidol; (○), 1,000 nM spiroperidol. B. (●), 300 nM 5-HT; (○), 3,000 nM 5-HT.

TABLE 1

Drug effects on [^{3}H]5-HT, [^{3}H]spiroperidol and [^{3}H]LSD binding to rat frontal cerebral cortex membranes

Rat frontal cerebral membranes were incubated with 2.0 nM [^{3}H]5-HT, 0.26 nM [^{3}H]spiroperidol or 4.25 nM [^{3}H]LSD under standard assay conditions as described in MATERIALS AND METHODS together with four concentrations of unlabeled drugs. [^{3}H]LSD assays were also performed in the presence of 30 nM spiroperidol or 300 nM 5-HT. IC_{50} values were determined by log-probit analysis and apparent K_i values were calculated from the equation $K_i = IC_{50}/(1 + [^3H]\text{-ligand}/K_D)$. The K_D values were obtained from the data in Figures 4–6. Values given are the means ± standard errors of three or more experiments, each performed in triplicate.

Drugs	Apparent K_i					
	[^{3}H]5-HT	[^{3}H]LSD + 30 nM SPIRO	[^{3}H]LSD	"PREDICTED" [^{3}H]LSD	[^{3}H]LSD + 300 nM 5-HT	[^{3}H]SPIRO
			(nM)			
Tryptamines						
5-HT	3.8 ± 0.55	17 ± 2.9	110 ± 16	100	1,500 ± 200	2,700 ± 420
5-Methoxytryptamine	11 ± 2.5	21 ± 5.0	210 ± 77	170	1,600 ± 590	2,700 ± 500
5,6 Dihydroxytryptamine	300 ± 3.8	680 ± 85	3,700 ± 650	2,500	23,000 ± 5,100	22,000 ± 2,300
Bufotenine	37 ± 4.8	83 ± 47	220 ± 70	180	2,600 ± 810	840 ± 60
LSD Analogues						
D-LSD	10 ± 0.37	13 ± 3.1	7.3 ± 1.3	11	13 ± 2.4	13 ± 2.4
2-Bromo-LSD	89 ± 25	75 ± 22	13 ± 4.3	15	1.9 ± 0.38	2.5 ± 0.41
Methysergide	88 ± 9.4	110 ± 22	8.4 ± 1.3	15	2.8 ± 0.14	2.6 ± 0.89
Serotonin Antagonists						
Cyproheptadine	1,500 ± 150	480 ± 92	90 ± 37	56	1.7 ± 0.36	2.0 ± 0.19
Mianserin	860 ± 110	1,500 ± 240	33 ± 9.4	64	5.7 ± 2.0	4.8 ± 2.0
Cinanserin	1,800 ± 540	2,000 ± 590	130 ± 39	180	13 ± 2.1	18 ± 2.6
Neuroleptics						
Spiroperidol	730 ± 71	630 ± 210	18 ± 1.1	19	0.76 ± 0.30	0.51 ± 0.084
Haloperidol	16,000 ± 2,200	13,000 ± 3,300	960 ± 270	820	36 ± 5.7	42 ± 17
Promethazine	10,000 ± 4,500	13,000 ± 3,200	2,000 ± 190	1,300	190 ± 31	170 ± 62
Clozapine	1,000 ± 150	720 ± 91	79 ± 8.8	110	24 ± 4.0	13 ± 0.84
Other Drugs						
Dopamine	20,000 ± 2,700	46,000 ± 4,600	51,000 ± 15,000	38,000	89,000 ± 17,000	75,000 ± 5,500
ADTN	70,000 ± 10,000	150,000 ± 71,000	110,000 ± 28,000	77,000	160,000 ± 40,000	83,000 ± 21,000

300 nM 5-HT to the [^{3}H]LSD assay markedly improves the correlation (r = 0.99) (Fig. 9D). In addition, the slope of the linear regression line is 1.01 which suggests that the sites labeled by [^{3}H]spiroperidol are identical to those labeled by [^{3}H]LSD in the presence of 300 nM 5-HT. The much lesser potency of dopamine and ADTN (28) than 5-HT at [^{3}H]spiroperidol sites confirms earlier reports (26–28) that in rat frontal cerebral cortex [^{3}H]spiroperidol binding involves 5-HT rather than dopamine receptors.

DISCUSSION

The major finding of the present study is that [^{3}H]5-HT and [^{3}H]spiroperidol label distinct populations of apparent 5-HT receptors, while [^{3}H]LSD appears to label both of these sites. Evidence supporting this conclusion includes the following items: (1) potencies of various 5-HT agonists and antagonists differ by factors of up to a thousand-fold in competing differentially for [^{3}H]5-HT and [^{3}H]spiroperidol binding; (2) incubation of brain membranes with concentrations of 5-HT sufficient to occupy all the [^{3}H]5-HT binding sites fails to alter [^{3}H]spiroperidol binding. Conversely, incubation of brain membranes with enough spiroperidol to occupy all [^{3}H]spiroperidol binding sites fails to alter significantly [^{3}H]5-HT binding; (3) the number of [^{3}H]LSD binding sites equals the sum of [^{3}H]5-HT and [^{3}H]spiroperidol binding sites; (4) no correlation exists between drug potencies in competing for [^{3}H]5-HT and [^{3}H]spiroperidol binding; (5) [^{3}H]LSD binding can be made to display drug specificity essentially identical to that of [^{3}H]5-HT or [^{3}H]spiroperidol if brain membranes are simultaneously incubated with 30 nM spiroperidol or 300 nM 5-HT respectively; (6) in an accompanying paper in this issue we report that guanine nucleotides affect agonist interactions with [^{3}H]5-HT but not [^{3}H]spiroperidol sites while [^{3}H]LSD binding is influenced in an intermediate manner (24). Since 5-HT and other 5-HT agonists and antagonists are substantially more potent in competing for binding of both [^{3}H]-5-HT and [^{3}H]spiroperidol than are agents associated with other neurotransmitter systems, we conclude that both [^{3}H]5-HT and [^{3}H]spiroperidol label apparent 5-HT receptors.

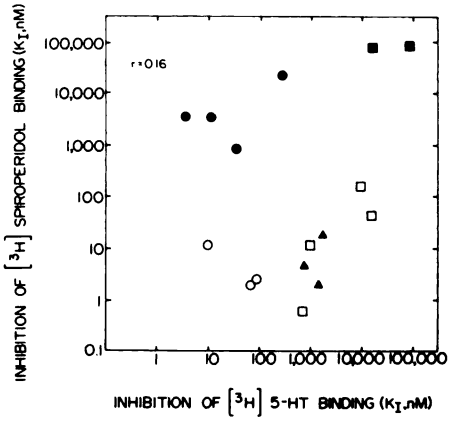

FIG. 8. *Correlation between drug affinities for [^{3}H]5-HT and [^{3}H]spiroperidol binding sites in rat frontal cerebral cortex*

K_i values are taken from Table 1. The correlation coefficient r = 0.16 is not statistically significant. The drug classes studied include tryptamines (●), LSD analogues (○), serotonin antagonists (▲), neuroleptics (□), and others (■).

We propose that these two ligands bind to two distinct 5-HT receptors in contrast to our earlier suggestion that [^{3}H]5-HT and [^{3}H]LSD label distinct interconvertible states of one receptor (18). Earlier studies (18, 19) had noted the subtle differences in the binding characteristics of [^{3}H]5-HT and [^{3}H]LSD. By analogy to similar differences in the opiate and dopamine systems, an interconvertible receptor model was proposed to explain these data. The recent availability of [^{3}H]spiroperidol as a label for serotonin receptors has provided new data that is not consistent with the earlier model but fits well with the existence of two distinct 5-HT receptors. We suggest that the receptors labeled by [^{3}H]5-HT be designated 5-HT$_1$ receptors, while those labeled by [^{3}H]spiroperidol be referred to as 5-HT$_2$ receptors.

Are there any physiological correlates of the two apparent 5-HT receptors? The drug specificity of the 5-HT receptors generally resembles that of the 5-HT sensitive adenylate cyclase (25, 26). The inhibition of [^{3}H]5-HT binding by guanine nucleotides follows a pattern closely similar to that of

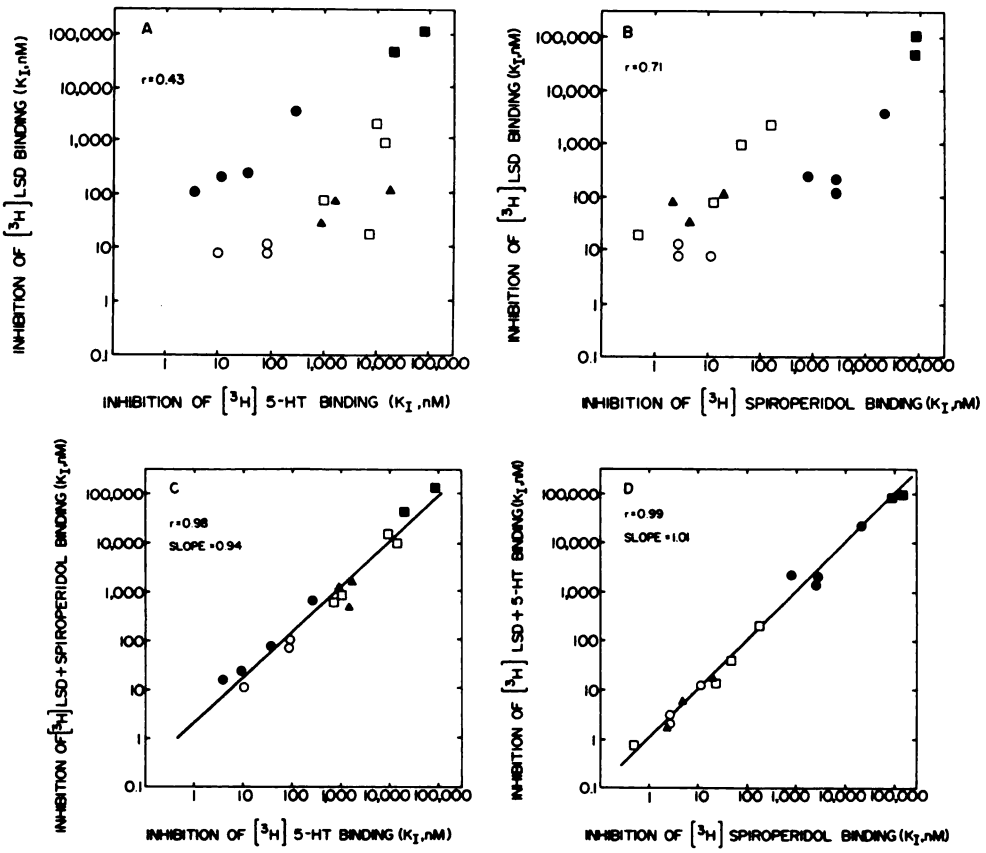

FIG. 9. *Correlations between drug affinities for [^{3}H]ligands in rat frontal cerebral cortex*

K_i values are taken from Table 1. The drug classes studied include tryptamines (●), LSD analogues (○), serotonin antagonists (▲), neuroleptics (□), and others (■). A. Correlation between affinities for [^{3}H]5-HT and [^{3}H]LSD. The correlation coefficient $r = 0.43$ was not significant. B. Correlation between affinities for [^{3}H]spiroperidol and [^{3}H]LSD. The correlation coefficient $r = 0.71$ was significant at $p < 0.05$. C. Correlation between affinities for [^{3}H]5-HT and [^{3}H]LSD in the presence of 30 nM spiroperidol. The correlation coefficient $r = 0.98$ is highly significant ($p < 0.001$). D. Correlation between affinities for [^{3}H]spiroperidol and[^{3}H]LSD in the presence of 300 nM 5-HT. The correlation coefficient $r = 0.99$ is highly significant ($p < 0.001$).

the regulation of receptor binding of neurotransmitters whose effects are linked to an adenylate cyclase (24). These findings indirectly suggest that [^{3}H]5-HT binds to 5-HT receptors that may be linked to an adenylate cyclase.

Recently, McCall and Aghajanian (27) reported differential neurophysiological actions of 5-HT at different sites in the brain. In the midbrain and forebrain nuclei of rat, 5-HT action on neurons is inhibitory and this effect is not blocked by classical antagonists such as methysergide (28, 29). In contrast, in the facial nucleus and the reticular formation 5-HT facilitates neuronal transmission and this effect is antagonized by methysergide (27, 30). Based on these neurophysiological findings, McCall and Aghajanian conclude that at least two types of 5-HT receptors exist in the rat central nervous system (27). The limited numbers of drugs evaluated in these neurophysiological studies preclude direct comparisons with distinct 5-HT receptors described in this study.

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