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Characterization of Solubilized Serotonin (S_2) Receptors in Rat Brain

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SUMMARY

[^{3}H]Spiperone binding sites were solubilized from rat frontal cortex and striatum by means of the mild detergent, lysolecithin. In the frontal cortex, the binding sites were extracted from a microsomal membrane fraction which was found to be enriched in serotonin (S_2) receptors when labeled either with [^{3}H]spiperone or with [^{3}H]lysergic acid diethylamide. Although the extraction yield was relatively low, the [^{3}H]spiperone binding sites solubilized from the frontal cortex retained the high-affinity characteristics of serotonin (S_2) receptors in the original membrane: low K_D (1.4 nM); binding saturable, reversible, and stereospecific; and displaying a high affinity toward the most potent serotonin antagonists (pirenperone, pipamperone, ketanserin, methysergide, and mianserin). There was a very good correlation between the drug potencies in both soluble and membrane preparations. The molecular dispersion of the soluble extract was assessed by several criteria, including a low sedimentation coefficient in sucrose gradient. No specific binding sites could be extracted from the cerebellum. In contrast, few binding sites endowed with the characteristics of serotonin (S_2) receptors were detected in lysolecithin extracts from rat striatum. However, the S_2 sites became more apparent when a tetralin derivative was added to the incubation medium in order to prevent [^{3}H]spiperone binding on solubilized dopamine receptors. In contrast, when microsomal membranes from rat striatum were treated with digitonin, the solubilized [^{3}H]spiperone binding sites were only of a dopaminergic nature.

INTRODUCTION

5-HT[3] receptors have been identified in different brain regions by means of *in vitro* binding assays using various ligands such as [^{3}H]LSD (1, 2), [^{3}H]spiperone (3, 4), and [^{3}H]5-HT (2, 5, 6). However, they do not appear to label the same receptor site (7). In the frontal cortex, LSD and spiperone were found to bind to the same site (4), designated as the serotonin (S_2) receptor (7). The relative potency of drugs in preventing the bilateral clonic seizures induced by tryptamine (4) or the head twitches induced by 5-hydroxytryptophan (8) parallels their affinities for the S_2 site but not for the S_1 site. This S_1 site is selectively labeled by [^{3}H]5-HT; it is probably a recognition site for indole-like structures, since most of the serotonin antagonists are either weakly active or inactive (9). Recently, two populations of S_1 sites, also labeled with [^{3}H]5-HT, were identified on the basis of a low or a high affinity for neuroleptics (10). However, the physiological role of S_1 receptors or of these different [^{3}H]5-HT binding sites remains unclear.

In a preliminary communication, we showed that lysolecithin could be used to solubilize [^{3}H]spiperone binding sites from rat frontal cortex which retained the high-affinity properties of the serotonin receptor in the original membranes (11). We now report on the binding and molecular characteristics of the solubilized serotonin receptor.

MATERIALS AND METHODS

[^{3}H]Spiperone (53.4 Ci/mmole) and [^{14}C]lysopalmitoyl phosphatidylcholine ([^{14}C]lysolecithin, 0.1 mCi/mg) were obtained from New England Nuclear Corporation (Boston, Mass.). The radiochemical purity (95–98%) of [^{3}H]spiperone was controlled by thin-layer chromatography. [^{3}H]LSD (13.3 Ci/mmole) and [^{3}H]5-HT (14 Ci/mmole) were obtained from the Radiochemical Centre (Amersham, England). L-α-Lysophosphatidylcholine from egg yolk, Type 1, No. L-4129 (lysolecithin), was from Sigma Chemical Company (St. Louis, Mo.) and digitonin from Serva. R 5573, R 5260 (see ref. 11 for chemical structure), ketanserin (R 41 468), and pirenperone (R 47 465), two S_2 antagonists (9), were from Janssen Pharmaceutica.

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

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Tissue Fractionation

Wistar rats were killed by decapitation and their brains were removed; striata or frontal cortices were dissected out and homogenized in 10 volumes of 0.25 M ice-cold sucrose. The total homogenate of the frontal cortex was subjected to differential centrifugation using the five-fraction scheme previously described (12). The nuclear (N), the heavy (M), and light (L) mitochondrial fractions were successively pelleted at 1,100 × *g*, 7,600 × *g*, and 20,400 × *g* for 10 min. After a last centrifugation (178,000 × *g* for 45 min), the pellet or microsomal fraction (P) was separated from the supernatant (S). The different pellets were resuspended in 2 volumes of ice-cold sucrose and further diluted in Tris-salt buffer for membrane binding assays (4) or in various appropriate buffers for enzyme assays. The results were expressed according to de Duve *et al.* (13).

For solubilization experiments, a P fraction was prepared as above but suspended in 2 volumes of ice-cold water and kept at −16° before use.

Solubilization Procedures

The P fraction from rat frontal cortex was treated at 0° for 15 min with 0.25% lysolecithin suspended in 4 volumes of 0.25 M ice-cold sucrose containing 10 mM sodium phosphate (pH 7.2), 1 mM EDTA, and 0.01% NaN_3 (Buffer A). After centrifugation at 182,000 × *g* (r_{av}) for 60 min (SW65 Ti rotor, Spinco), the supernatant was carefully harvested at 0° and taken as the soluble extract (11).

Dopamine receptors were solubilized from a P fraction of rat striatum using 1% digitonin as previously described (14).

Binding Assays

Soluble extract (0.4 ml) was incubated at 0° for 18 hr with 1 nM [^{3}H]spiperone and various concentrations of unlabeled drugs. In order to reduce nonspecific binding, 10^{-5} M R 5573 was added to the frontal cortex extract (11) and 10^{-5} M R 5260 to the striatal extract (14). Both compounds are known to prevent [^{3}H]spiperone binding on spirodecanone sites (11, 14). When the soluble extract from frontal cortex was incubated at 30°, R 5573 was omitted. Specific [^{3}H]spiperone binding was defined as that displaceable by 10^{-6} M pipamperone (frontal cortex) or 10^{-6} M (+)-butaclamol (striatum). After incubation, the ligand-receptor complex was separated from the free ligand by means of three different procedures.

Ammonium sulfate assay. Cold saturated ammonium sulfate (0.5 ml) was added to the total incubation mixture (0.5 ml). The samples were rapidly mixed, filtered under vacuum through GF/B filters (Whatman), and washed twice with 5 ml of 50% saturated ammonium sulfate (11, 15). The filters were placed in vials with 10 ml of Instagel II (Packard) and counted for radioactivity.

Charcoal assay. Bovine serum albumin-coated charcoal (10% charcoal with 2% bovine serum albumin in water) (50 µl) was added to 0.4 ml of the incubation medium and centrifuged at 14,000 rpm for 3 min in a Microfuge (15, 16). A 0.2-ml aliquot of the supernatant was counted for radioactivity.

Sephadex G-50 assay. A portion of the incubation mixture (0.1 ml) was layered on top of a Sephadex G-50 (Pharmacia) column (13 × 0.5 cm) which had been precooled at 2° and then eluted at a constant rate with Buffer A (17). Four-drop fractions were collected in vials and counted for radioactivity.

5-HT Uptake, Marker Enzymes, and Protein Determination

Each subcellular fraction was adjusted to 1 ml by diluting it in Krebs-Henseleit solution (pH 7.4) and preincubated for 5 min at 25° with or without 10^{-6} M chlorimipramine. The incubation was then performed with 10^{-8} M [^{3}H]5-HT at 25° for 5 min and stopped by the addition of 5 ml of ice-cold Krebs-Henseleit solution. The incubation medium was immediately filtered through GF/B filters (Whatman), which were washed twice. The filters were placed in vials and counted for radioactivity. [^{3}H]5-HT uptake was defined as the difference of recovered radioactivity on filters between assays deprived of chlorimipramine and those supplemented with chlorimipramine.

Cytochrome oxidase activity was measured according to ref. 18, and 5′-nucleotidase as in ref. 19. Proteins were assayed by means of the Bio-Rad method (20).

RESULTS

Fractionation by differential centrifugation. The subcellular distribution of serotonin receptors was studied in rat frontal cortex by using an analytical tool, the five-fraction scheme. Figure 1 shows that the specific binding was mainly enriched in the microsomal (P) fraction. Identical distribution patterns were obtained with [^{3}H]spiperone and [^{3}H]LSD binding on particulate preparations. Among marker enzymes, only 5′-nucleotidase (plasma membranes) showed a high relative specific activity in the P fraction, whereas cytochrome oxidase was found in the mitochondrial (M) fraction. 5-HT uptake was enriched in both M and L fractions but not in the P fraction. The recovery from particulate [^{3}H]spiperone binding and protein averaged about 100% (Table 1). When the subcellular fractions were first subjected to lysolecithin treatment, solubilized [^{3}H]spiperone binding displayed distribution pattern similar to that of the particulate preparations (Fig. 1). Here again the recovery was about 100%, although the extraction yield was relatively low and did not exceed 6.3% (Table 1).

Comparison of different assay methods. Three different methods of measuring solubilized [^{3}H]spiperone binding in a lysolecithin extract from frontal cortex were compared (Table 2). The Sephadex G-50 filtration gave the highest amount of specific binding, but nonspecific binding was quite high.

The charcoal assay, the most reproducible, and the ammonium sulfate technique yielded 86% and 68%, respectively, of the specific binding measured by means of gel filtration. However, in contrast to the latter, nonspecific binding was much less pronounced.

Solubilization of serotonin receptors from rat striatum. A minor serotonergic component of the [^{3}H]spiperone binding was found in the membranes of rat striatum

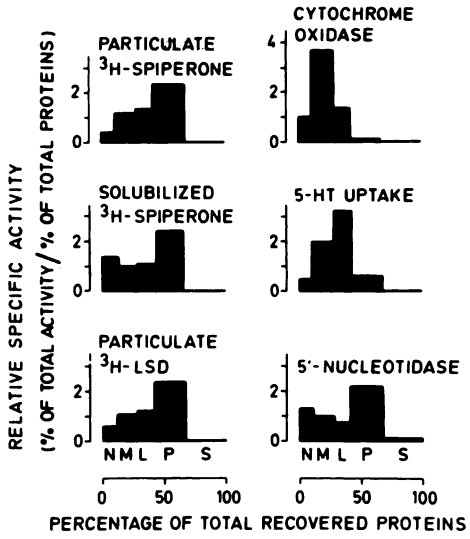

FIG. 1. *Distribution pattern of serotonin receptors in subcellular fractions of rat frontal cortex after differential centrifugation*

The nuclear fraction (N), heavy (M) and light (L) mitochondrial fractions, the microsomal fraction (P), and the supernatant (S) were obtained as described under Materials and Methods. Binding assays for particulate fractions were performed with 2×10^{-9} M ^{3}H-labeled ligand as reported in ref. 4 except that 10^{-6} M pipamperone was used for measuring nonspecific binding. The five fractions were also subjected to solubilization with 0.25% lysolecithin (see Materials and Methods). The corresponding solubilized extracts were incubated at 0° for 18 hr with 10^{-9} M [^{3}H]spiperone under standard conditions, and the binding was measured by means of the charcoal technique. All of the recoveries except that of cytochrome oxidase and 5'-nucleotidase ranged between 93% and 107%.

(4, 21, 22). After digitonin (14) or lysolecithin (11) treatment of a striatal P fraction, [^{3}H]spiperone binding was measured in both soluble extracts. Figure 2 shows that, when digitonin was used, (+)-butaclamol (IC_{50} 9 nM) was about 125 times more potent than pipamperone in displacing [^{3}H]spiperone binding. In the presence of 10^{-5} M 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin, [^{3}H]spiperone binding was strongly reduced and no longer displaceable even at high concentrations of (+)-butaclamol or pipamperone. Interestingly, the lysolecithin extract revealed other features; when compared with digitonin, the number of binding sites was lower, (+)-butaclamol

TABLE 2

Comparison of different methods for measuring [^{3}H]spiperone binding in soluble extracts from rat frontal cortex

The soluble preparation was incubated at 0° for 18 hr with 10^{-9} M [^{3}H]spiperone in the presence of 10^{-5} M R 5573. Nonspecific binding was determined with 10^{-6} M pipamperone. Values are the means of the three independent determinations (± SEM).

Assay	Total binding	Specific binding	Nonspecific binding	Recovery[a]
	fmoles/ml	*fmoles/ml*	*% of total*	%
Sephadex G-50	334.6 ± 6.9	86.5	74.1	100
Charcoal	125.3 ± 0.6	74.6	40.4	86
Ammonium sulfate	98.4 ± 8.0	58.8	40.2	68

[a] Specific [^{3}H]spiperone binding is expressed as percentage of that found with the Sephadex G-50 assay.

became 10 times less active, and pipamperone retained its low relative affinity (IC_{50} 1070 nM). Note the biphasic curve with pipamperone. In the presence of 10^{-5} M 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin, the drug potency was reversed, pipamperone becoming 20 times more active than (+)-butaclamol.

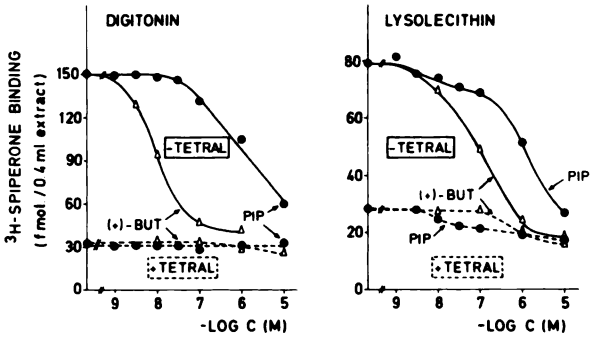

FIG. 2. *Differential solubilization of dopaminergic and serotonergic receptors from rat striatum by digitonin and lysolecithin*

Inhibition curves of pipamperone (●——●, *PIP*) and of (+)-butaclamol (△——△, *BUT*) on [^{3}H]spiperone binding were obtained in the presence (- - -) and absence (——) of 10^{-5} M 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin (*TETRAL*). Experimental conditions were as follows: 1% digitonin or 0.25% lysolecithin, 10^{-5} M R 5260 for the digitonin extract, 10^{-5} M R 5573 for the lysolecithin extract, 10^{-9} M [^{3}H]spiperone, incubation at 0° for 18 hr, charcoal assay and ammonium sulfate technique, respectively, for digitonin and lysolecithin extracts.

TABLE 1

Distribution and recovery of particulate and solubilized [^{3}H]spiperone binding in different subcellular fractions obtained by differential centrifugation

	Fraction					% Recovery[a]
	N	M	L	P	S	
Particulate [^{3}H]spiperone binding						
pmoles/g tissue	0.77	1.77	1.08	5.8	0.03	107
protein (mg/g tissue)	10.4	18.2	13.4	28.1	48.2	99
fmoles/mg protein (a)	74.5	97.3	80.8	206.6	0.6	—
Solubilized [^{3}H]spiperone						
fmoles/g tissue	42.4	54.1	43.1	201.1	10.9	104
fmoles/mg protein (b)	4.3	4.5	4.1	13.0	0.4	—
Extraction yield, b/a (%)	5.8	4.6	5.1	6.3	—	—

[a] Recovery (R) is equal to the sum of activity found in all of the fractions divided by the activity in the starting material [nuclear (N) fraction + cytoplasmic (CY) fraction]: R = (N + M + L + P + S/N + CY) × 100.

TABLE 3

Comparison of IC_{50} values for [^{3}H]spiperone binding in soluble extract from frontal cortex and striatum

Binding was carried out by using the lysolecithin extract from the frontal cortex and the digitonin extract from the striatum. After incubation (18 hr at 0°), [^{3}H]spiperone binding was measured by means of the ammonium sulfate technique (A) and the charcoal method (B and C).

	IC_{50}			Ratio, C/A
	Frontal cortex		Striatum (C)	
	A	B		
		nM		
Pirenperone	3.2	3.0	280	87
Spiperone (SPIP)	6.3	—	6.3	1
Ketanserin (R)	10	8.9	6,310	631
Pipamperone (PIP)	11.8	13.9	1,260	107
Benperidol (BENP)	14.1	—	2.2	0.15
Methysergide (METHY)	25.1	10	1,580	63
Cyproheptadine (CYPRO)	39.8	—	891	22
Pizotifen (PIZO)	52.5	—	631	12
LSD	56.2	—	1,410	25
Mianserine (MIAN)	66	45	6,310	95
Chlorpromazine (CPZ)	224	—	141	0.63
(+)-Butaclamol [(+)BUT]	316	355	12.3	0.04
Phentolamine	1,580	—	—	—
Bufotenin (BUFO)	1,860	—	35,500	19
5-HT	3,160	5,100	178,000	56
Quipazine	10,000	3,200	—	—
(−)-Butaclamol	41,700	—	8,910	0.21
Atropine (ATROP)	44,700	—	—	—
2-(*N,N*-Dipropyl)amino-5,6-dihydroxytetralin (TETRAL)	158,000	—	178	0.001
Dopamine (DA)	251,000	—	28,200	0.11
Naloxone, tubocurarine, alprenolol, flunitrazepam, mepyramine, γ-aminobutyric acid, prazosin, clonidine, citalopram	≥10,000			

Drug potency in frontal cortex and striatum. Antagonists and agonists belonging to different chemical classes were tested on [^{3}H]spiperone binding in solubilized extracts from frontal cortex and striatum; their IC_{50} values are summarized in Table 3. Serotonin agonists and antagonists were much more active in the frontal cortex than in the striatum. The reverse was true for dopaminergic drugs. In both systems, [^{3}H]spiperone binding revealed a pronounced stereospecific effect for (+)- and (−)-butaclamol. Among the serotonergic antagonists, pirenperone displayed the highest potency in the frontal cortex and ketanserin the largest dissociation (striatum versus frontal cortex). The IC_{50} values were similar when the charcoal assay and the ammonium sulfate technique were used.

Figure 3 reveals a very good correlation between the IC_{50} values obtained in [^{3}H]spiperone binding using soluble and membrane preparations ($r = 0.97$; $p < 0.001$; $n = 16$).

Solubilization criteria for lysolecithin-extracted serotonin receptors. The [^{3}H]spiperone binding sites solubilized from rat frontal cortex were not sedimentable at 182,000 × *g* (r_{av}) for 60 min; they were retained on GF/B filters only after previous ammonium sulfate precipitation. The lysolecithin extract passed entirely through 0.22-nm pore size filters (GSWP, Millipore), which are known to trap very small membrane particles (24).

Among the most important criteria are the sedimentation properties of soluble binding sites through sucrose gradients. Figure 4 shows that different sedimentation profiles were obtained using either soluble or membrane preparations. A peak of soluble receptor was repeatedly obtained in the first fractions of the gradient, whereas the microsomal fraction was recovered in the bottom. As compared with the soluble starting material, a 2.6-fold enrichment in specific [^{3}H]spiperone binding was obtained in Fraction 6. Proteins and [^{14}C]lysolecithin pre-

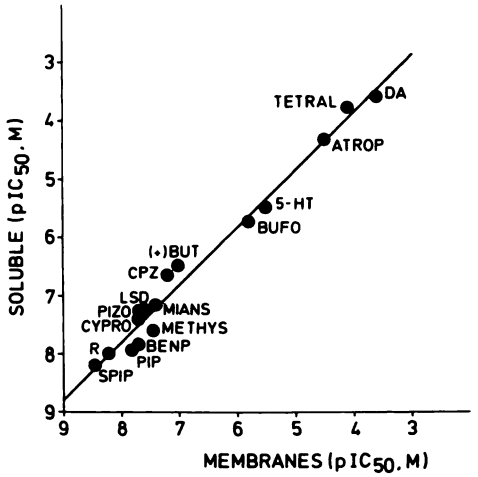

FIG. 3. *Correlation between drug affinities for [^{3}H]spiperone binding sites in soluble and membrane preparations from rat frontal cortex*

Compounds and assay conditions were as described in Table 2 and under Materials and Methods; values for membranes were taken from refs. 11 and 23. Spearman rank correlation coefficient: $r = 0.976$ ($n = 16$, $p < 0.001$).

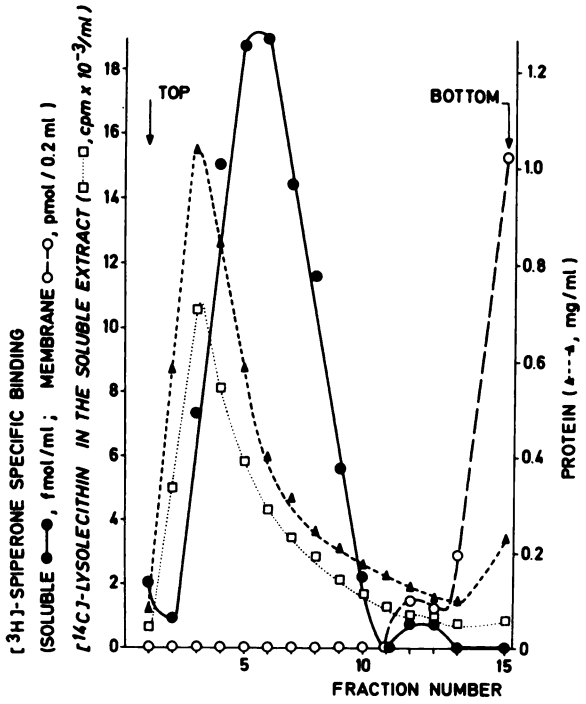

FIG. 4. *Sedimentation profile of a soluble extract from rat frontal cortex in a sucrose gradient*

Soluble extract (●——●) (1 ml) or a microsomal membrane fraction (○- - -○) (1 ml) was layered on an 11.3-ml sucrose gradient (15–30%), buffered with 10 mM phosphate buffer (pH 7.2) containing 1 mM EDTA, and centrifuged at 152,000 × *g* (r_{av}) for 18 hr at 2° (SW 40 Ti rotor). After the run, binding assays were performed in each fraction with 10^{-9} M [^{3}H]spiperone in the presence or absence of 10^{-6} M pipamperone. After incubation (20 min at 30° for the soluble extract and 10 min at 37° for the membranes), binding was measured by means of the charcoal (soluble extract) or the filtration technique (membrane; see ref. 25. In a control experiment (□- - -□), a soluble extract was obtained by treating membranes with 0.25% [^{14}C]lysolecithin, and then applied on a gradient. The radioactivity was measured in each fraction.

sented profiles which differed from that of [^{3}H]spiperone binding.

Another criterion for the soluble state is provided by comparing the thermal stability of soluble and membrane preparations (Fig. 5). At 56°, heat denaturation occurred more rapidly in the lysolecithin extract than in membrane preparations, whereas at 30° the binding sites from both preparations remained practically unaffected.

Binding properties of solubilized serotonin receptors. Figure 6 shows that, when increasing concentrations of ^{3}H-labeled ligand were used, the binding reached a plateau at 4 nM [^{3}H]spiperone. On the other hand, nonspecific binding increased linearly. At 1 nM, specific binding represented 50–60% of the total binding. Scatchard analysis provided an apparent straight line with a K_D value of 1.4 nM and a B_{max} value of 0.9 pmole of receptor per gram of tissue (43.5 fmoles of receptor per milligram of protein).

In order to demonstrate the reversibility of [^{3}H]spiperone binding on soluble receptors, association and dissociation curves were established (Fig. 7). At 30°, association was faster ($t_{1/2}$ ~ 1 min) than dissociation in the presence of 10^{-6} M pipamperone ($t_{1/2}$ ~ 2 min). However,

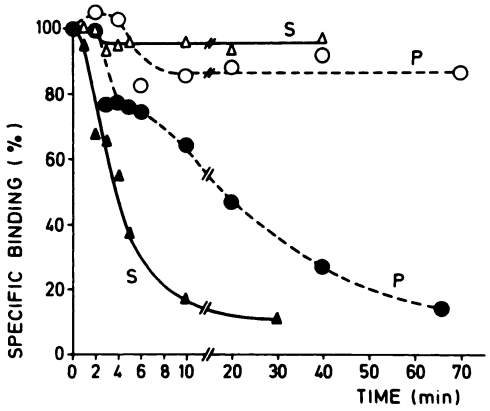

FIG. 5. *Effect of temperature on soluble (S) or membrane (P) preparations from frontal cortex*

Microsomal membranes (○- - -○) (●- - -●) or soluble extract (△——△) (▲——▲) were treated, under similar conditions, for various times at 30° (*open symbols*) or 56° (*closed symbols*), then cooled at 0°. The samples were incubated at 0° for 18 hr with 10^{-9} M [^{3}H]spiperone as described under Materials and Methods, except that R 5573 was omitted. Binding on membranes was measured by means of the filtration technique, whereas the charcoal method was used for the soluble extract.

the dissociation did not follow a first-order reaction plot (log C drug-receptor complex versus time) but provided two straight lines with a rapid component and a slow component.

DISCUSSION

The foregoing results provide evidence that [^{3}H]spiperone binding sites solubilized by lysolecithin from rat

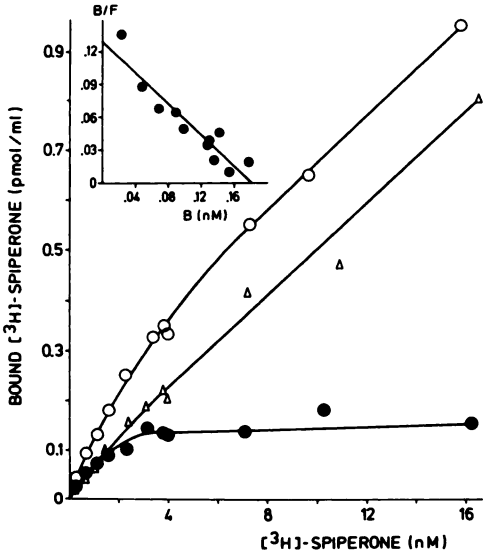

FIG. 6. *Binding in soluble extract from frontal cortex with increasing concentrations of [^{3}H]spiperone*

The extracts were incubated for 18 hr at 0° with various concentrations of [^{3}H]spiperone (0.22–16.57 nM) in the presence of 10^{-5} M R 5573. Specific [^{3}H]spiperone binding (●——●) represents the difference between total (○——○) and nonspecific binding (△——△) occurring in the presence of 10^{-6} M pipamperone. The ligand concentration was controlled by measuring the radioactivity in each incubation medium. Bound [^{3}H]spiperone was assayed by means of the ammonium sulfate technique. *Inset*, Scatchard plot of specific binding.

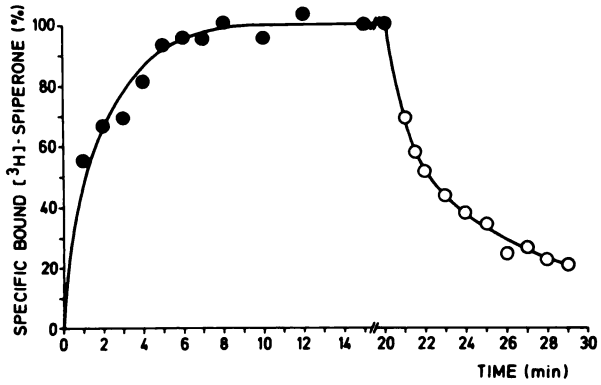

FIG. 7. *Association (●——●) and dissociation (○——○) curves for specific [^{3}H]spiperone binding in a soluble extract from rat frontal cortex*

Samples were incubated with 10^{-9} M [^{3}H]spiperone for various times at 30°. After 20 min (steady total association), 50 µl of pipamperone were added (dissociation) in order to obtain a 10^{-6} M final concentration. The reactions were stopped by chilling the media rapidly to 0°; binding was measured by means of the charcoal technique.

frontal cortex possess the high-affinity characteristics of S_2 receptors. Since such receptors, when labeled either with [^{3}H]LSD or [^{3}H]spiperone, were mainly found in the microsomal (P) fraction, this particulate fraction was used throughout the present work. The fact that lysolecithin could extract [^{3}H]spiperone binding sites in all of the subcellular fractions, giving rise to a distribution pattern similar to that obtained for the membrane-bound S_2 receptors, suggests that both solubilized and membrane-bound receptors possess the same features in common. This was confirmed by the appearance of a single peak of solubilized binding sites in sedimentation gradients. 5-HT uptake did not occur in the microsomal fraction but was found in the (M + L) mitochondrial fraction. This strengthens the idea that the nerve endings which show 5-HT uptake are not associated with the postsynaptic membranes where the 5-HT receptors are localized (19).

In rat striatum, digitonin solubilized only [^{3}H]spiperone binding sites endowed with the high-affinity characteristics of the dopamine receptor (14, 26). However, the use of lysolecithin enabled us to solubilize serotonin receptors together with dopamine receptors from rat striatum. Indeed, after blockade of dopamine receptors with a potent dopamine agonist, a tetrahydronaphthalene derivative (4), [^{3}H]spiperone binding sites solubilized by lysolecithin were displaceable by pipamperone in a way similar to those from the frontal cortex (cf. Table 3); in contrast, pipamperone was much less active in the digitonin extract; the reverse was true for (+)-butaclamol. Recently, the use of [^{3}H]ketanserin to label solubilized serotonin receptors (27) enabled us to confirm the presence in striatum of a small number of serotonergic sites labeled with [^{3}H]spiperone (21, 22).

Most of the solubilization criteria (16) were fulfilled; the serotonin (S_2) receptors solubilized by lysolecithin from rat frontal cortex displayed in sucrose gradients a much lower sedimentation coefficient than did the original membranes; this indicates that the physical state of solubilized receptors markedly differed from that of the membrane-bound sites.

Soluble [^{3}H]spiperone binding sites are saturable, with a K_D value (1.4 nM) corresponding to that (1 nM) reported from membrane preparations (22, 28). Therefore, the solubilization by lysolecithin of serotonin (S_2) receptors did not modify the high-affinity properties of the receptor. The binding was found to be reversible and gave rise to a biphasic dissociation curve, as previously obtained from membrane preparations (29). As found for the dopamine receptor (26), the membrane-bound receptor was more resistant to thermal inactivation than was the solubilized receptor; this may be considered an additional criterion for assessing the solubilization of the receptor.

Although the extraction yield was relatively low (Table 1), [^{3}H]spiperone binding sites solubilized from frontal cortex and measured with either the ammonium sulfate technique or the charcoal method were of a serotonergic nature; all of the serotonergic compounds, namely the potent S_2 antagonists pirenperone, ketanserin, pipamperone, methysergide, and mianserin, were much more active on [^{3}H]spiperone binding sites solubilized by lysolecithin from frontal cortex than by digitonin from striatum; the reverse was true for dopamine antagonists and agonists. Various compounds belonging to different pharmacological classes (e.g., anticholinergic, *alpha-* and *beta*-adrenergic, antihistamine, and benzodiazepine) were inactive or were active only at very high concentrations. There was a good correlation in the potency of drugs for both soluble and membrane preparations. One has to remember that the affinity of numerous drugs in [^{3}H]spiperone binding on cortical membranes was closely correlated with that obtained in [^{3}H]LSD binding, which in turn was correlated with their potency to antagonize pharmacological effects such as tryptamine-induced clonic seizures (4, 30), 5-hydroxytryptophan-induced head twitches in rats (8), and 5-HT-induced contractions in rat caudal artery (31).

Recently, [^{3}H]ketanserin was found to label selectively serotonin (S_2) receptors solubilized by lysolecithin from dog and rat brains (27); by using this ligand we were able to obtain a much higher solubilization yield (40%) in receptors than that obtained with [^{3}H]spiperone. However, the lysolecithin-solubilized binding sites measured with both ligands are endowed with the same high-affinity characteristics of the membrane-bound serotonin (S_2) receptors.

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