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Anomalous Properties of [^{3}H]Spiperone Binding Sites in Various Areas of the Rat Limbic System

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SUMMARY

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[^{3}H]Spiperone, a neuroleptic/dopamine receptor ligand, binds with high affinity (K_d 0.15 nM) to a single specific site on rat corpus striatum membranes. The "specific" binding represents about 80% of the total binding and is displaced by dopamine, apomorphine, and stereospecifically by neuroleptics such as butaclamol and flupenthixol. However, in contrast to the striatum, only 30-40% of the binding of [^{3}H]spiperone to limbic forebrain membranes is displaced stereospecifically by butaclamol or flupenthixol, whereas dopamine and certain spiperone analogues compete with high affinity for about 70% of the labeled sites. These additional sites are saturable, reversible, and of high affinity. Kinetic analysis of association and dissociation rates yields a K_d value (1.5 nM) in agreement with equilibrium saturation data for these sites. They also possess a precise distribution, with high amounts being found in the hippocampus, septum, and nucleus accumbens, but they are completely absent in areas such as the corpus striatum, olfactory tubercles and hypothalamus. Moreover, these [^{3}H]spiperone binding sites show strict structure-affinity relationships in that only spirodecanone butyrophenone derivatives and dopamine are capable of displacing this binding with relatively high affinities.
The results emphasize the complex nature of neuroleptic/dopamine "receptor" binding sites in brain and the need for precise definition of such "specific" binding sites.

INTRODUCTION

The recent use of radiolabeled neuroleptics has assisted our understanding of the characteristics of putative dopamine receptors in the central nervous system (1-3). Although the first studies to clearly identify high affinity saturable dopaminergic binding sites utilized [^{3}H]haloperidol and [^{3}H]-dopamine as ligands (1), very recent work has emphasized the more favorable properties of [^{3}H]spiperone (3, 4).

Specific binding of these ligands has been assessed as either the binding that is displaced by the pharmacologically active (+) but not the (−) isomer of the neuroleptic butaclamol, or the binding that is inhibited by a large excess of dopamine. The former assessment of specific binding to dopamine receptors has been questioned since it has been recently reported that [^{3}H]spiperone apparently binds to serotonin receptors in the rat frontal cortex (although not in the striatum) and can be stereospecifically displaced from these sites by butaclamol (5, 6). Moreover, in recent work from these laboratories we have ob-

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served that although [^{3}H]haloperidol and [^{3}H]spiperone labeled equal numbers of sites in most areas of the rat brain, dopamine displaceable [^{3}H]spiperone binding was significantly greater than [^{3}H]haloperidol binding in the limbic forebrain (4). In the present paper we have examined this latter anomaly in some detail and describe apparent high affinity, saturable binding of [^{3}H]spiperone to sites of unknown pharmacological type in several regions of the rat brain.

MATERIALS AND METHODS

[^{3}H]Spiperone (23 Ci/mmole) and [^{3}H]-haloperidol (13 Ci/mmole) were obtained from New England Nuclear Co. All other drugs were obtained from commercial sources.

Male Wistar rats (120–150 g) were decapitated and the brain was dissected on ice, basically according to Glowinski and Iverson (7) with the exception of the limbic forebrain section used in certain experiments. This was dissected as described by Horn and Phillipson (8) and comprised olfactory tubercles and tracts, medial forebrain bundle, the caudal part of the accumbens nuclei and the fornix and septal nuclei, but not the cingulate cortex.

Tissue preparation and binding assays were carried out as described previously (4).

RESULTS

The characteristics of "specific" [^{3}H]spiperone binding (defined as that displaced by 0.30 mM dopamine) to rat corpus striatum membranes were similar to those described previously (4) with a K_d (equilibrium dissociation constant) of 0.14 nM and a B_{max} (maximal number of binding sites) of 0.70 pmol/mg protein. This binding was stereospecifically displaced by the isomers of butaclamol (and flupenthixol) and also by other drugs such as unlabeled spiperone and apomorphine (Fig. 1). In the corpus striatum, therefore, dopamine (0.30 mM) displaced the same amount of [^{3}H]spiperone binding as 1 μM (+) butaclamol and 1 μM α-flupenthixol. This represents approximately 80% of the total binding. The remainder can be defined as residual or nonspecific binding since it can be displaced only by very high concentrations of neuroleptics. This effect is not stereospecific and probably relates to the membrane effects of these agents (9).

In contrast to the striatum, only about 50% of the binding displaced by 0.3 mM dopamine in the limbic forebrain was stereospecifically displaced by butaclamol or flupenthixol, and apomorphine at concentrations up to 0.3 mM only displaced this same fraction (Fig. 2). Although spiperone appeared to be of slightly lower affinity than seen in the striatum, it was nevertheless very potent and unlike the other neuroleptics totally inhibited dopamine displaceable binding. Thus, in the limbic forebrain, [^{3}H]spiperone binds to three sites: Site "A"—binding displaced stereospecifically by neuroleptics, by apomorphine (and by dopamine); Site "B"—binding displaced by dopamine and spiperone but not stereospecifically by butaclamol and flupenthixol or by apomorphine; Site "C"—residual or nonspecific binding (Fig. 2).

The limbic forebrain section used comprised several distinct anatomical areas (see METHODS) and a more detailed dissection was made of this region and the remainder of the brain in order to examine the distribution of binding sites "A" and "B" (Fig. 3). The greatest proportion of site "B" was found in the hippocampus where it constituted over 75% of the total specific binding ("A" plus "B"). Appreciable amounts of this binding also occurred in the commissura fornicis ventralis, septum, nucleus accumbens and substantia nigra.

In view of the large proportion of site "B" in the hippocampus, the detailed characteristics of this binding site were examined in this region. The binding of [^{3}H]spiperone to site "B" on hippocampal membranes was saturable and of high affinity, Scatchard analysis revealing a K_d of 1.5 nM and B_{max} of 0.92 pmol/mg protein (Fig. 4a and b). Binding to site "A" was also saturable with a K_d of 0.2 nM and B_{max} of 0.19 pmol/mg protein (Fig. 4a and b), while nonspecific binding (site "C") increased linearly up to at least 14 nM. The association of [^{3}H]spi-

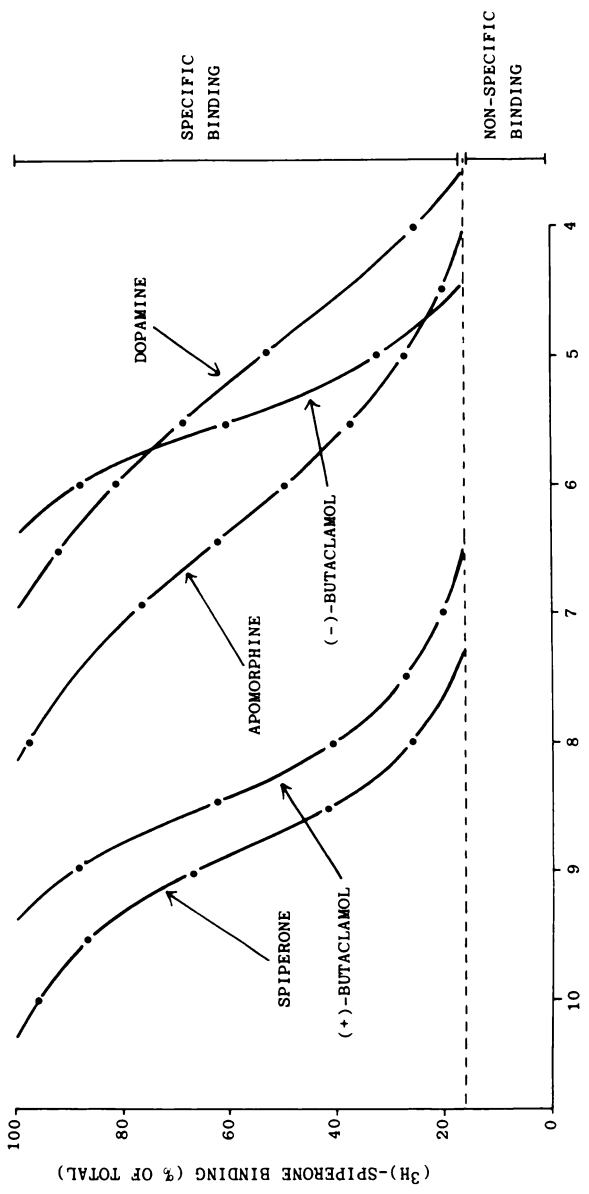

FIG. 1. *Displacement of [^{3}H]spiperone binding to rat corpus striatum membranes*
Membranes were incubated with increasing concentrations of unlabeled drugs (0.5–0.8 nM [^{3}H]spiperone) (see METHODS). Each point is the mean of duplicate determinations and each experiment was carried out on at least three occasions.

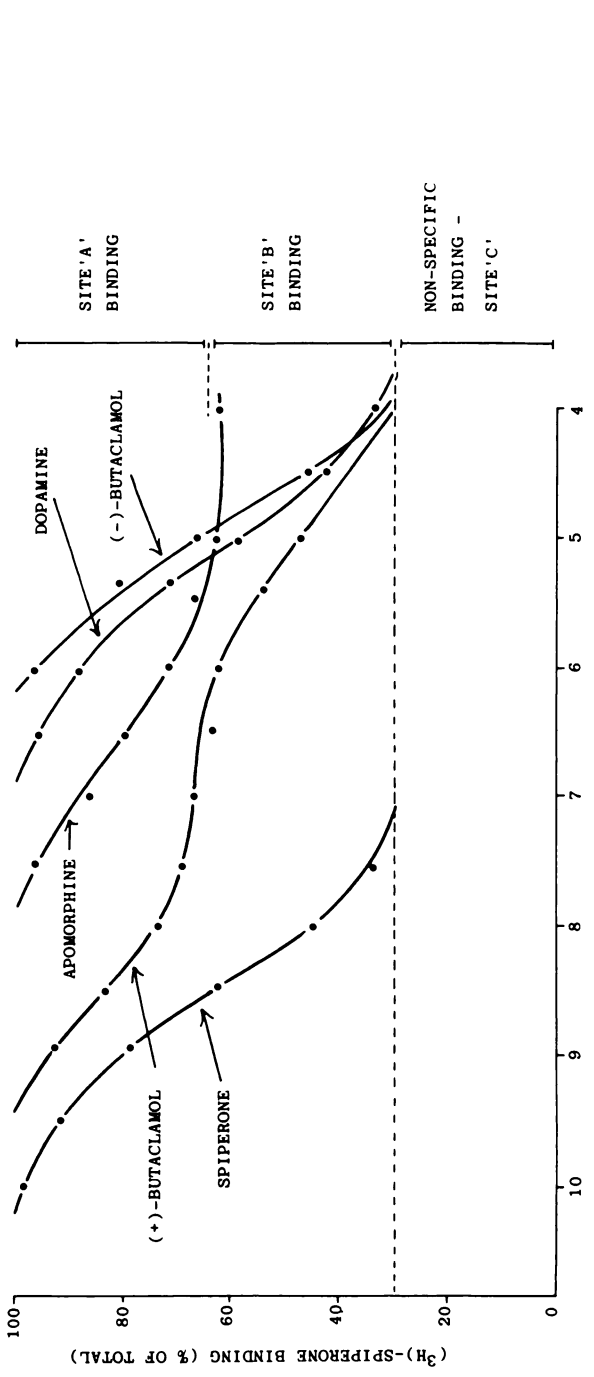

FIG. 2. *Displacement of [^{3}H]spiperone binding to rat limbic forebrain membranes*

Membranes were incubated with increasing concentrations of unlabeled drugs (0.5–0.8 nM [^{3}H]spiperone) (see METHODS). Each point is the mean of duplicate determinations from a typical experiment. Each experiment was carried out on at least three occasions. See text for definition of binding sites "A," "B" and "C."

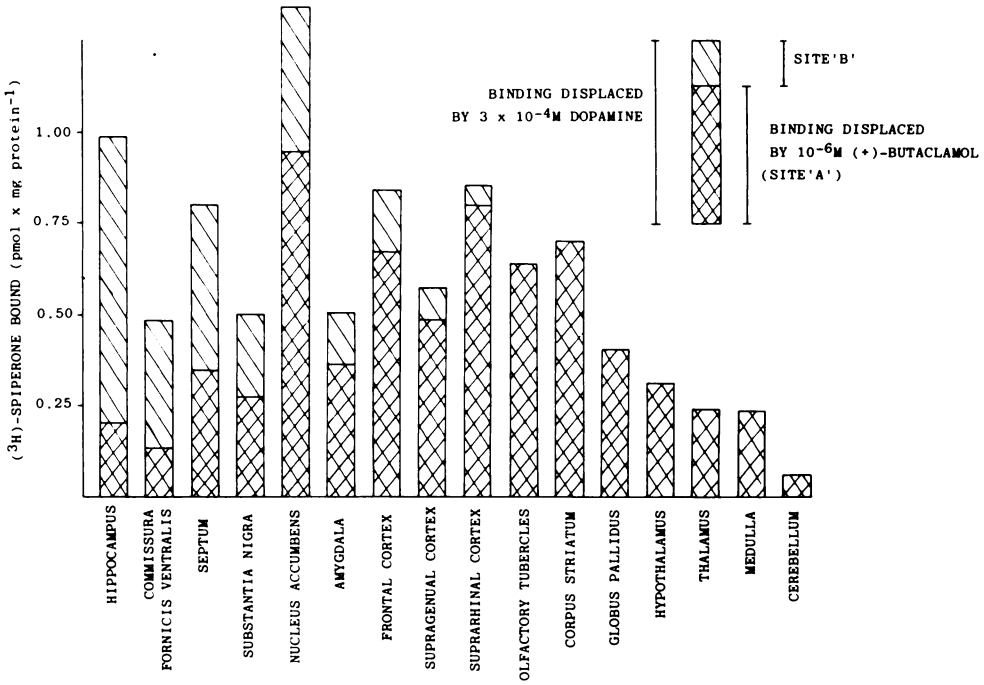

FIG. 3. *Regional distribution of [^{3}H]spiperone binding sites on rat cerebral membranes*
Tissue was incubated with approximately 3.0 nM [^{3}H]spiperone in the absence of any drug (total binding) or in the presence of either 1 μM (+)-butaclamol or 0.3 mM dopamine. Results are means of triplicate determinations.

perone to site "B" reached equilibrium by 8 min, with half maximal binding being attained at 2 to 3 min. This binding was reversible and the dissociation of bound [^{3}H]spiperone by 0.3 mM dopamine under these conditions had a half life at 37° of about 4 min. The K_d, as determined by the ratio of the dissociation to association rate constants, was 1.8 nM (± 0.3) (3 experiments), which agrees well with the value obtained from the equilibrium data. The hippocampal binding displaced by 0.3 mM dopamine was not additive with the butaclamol sterospecific binding; a residual amount of nonspecific binding (about 30%) was always apparent. Thermal inactivation, accomplished by preincubating the membrane preparation at 80° for 15 min, resulted in a complete loss of both "A" and "B" binding sites, but not the residual nonspecific binding.

In the hippocampus, only about 20% of the total binding was sterospecifically displaced by butaclamol (Fig. 5) and flupenthixol, i.e., binding to site "A." Spiperone and spirilene were among a number of butyrophenone derivatives which displaced from both sites "A" and "B" with high affinity, although others such as benperidol only displaced from site "A" with high affinity (Fig. 5). The same was true for many other dopamine agonists and antagonists (Table 1). The IC_{50} values for the inhibition of binding to hippocampus site "A" were in close agreement with those found for the striatum, for all drugs examined (Table 1). However, only butyrophenone derivatives of the spirodecanone type (spiperone, spiramide, spirilene and fluspirilene) displaced the site "B" binding with affinities close to those found against binding to site "A". Other butyrophenone derivatives were very much weaker with IC_{50} values ranging from 3 μM for benperidol to greater than 0.1 mM for droperidol and pipamperone. Dopamine was the only agonist showing similar affinities for both sites "A" and "B." Apomorphine, bromocryptine and dihydroergotamine, and a variety of other compounds, were all inactive at concentrations up to 0.1

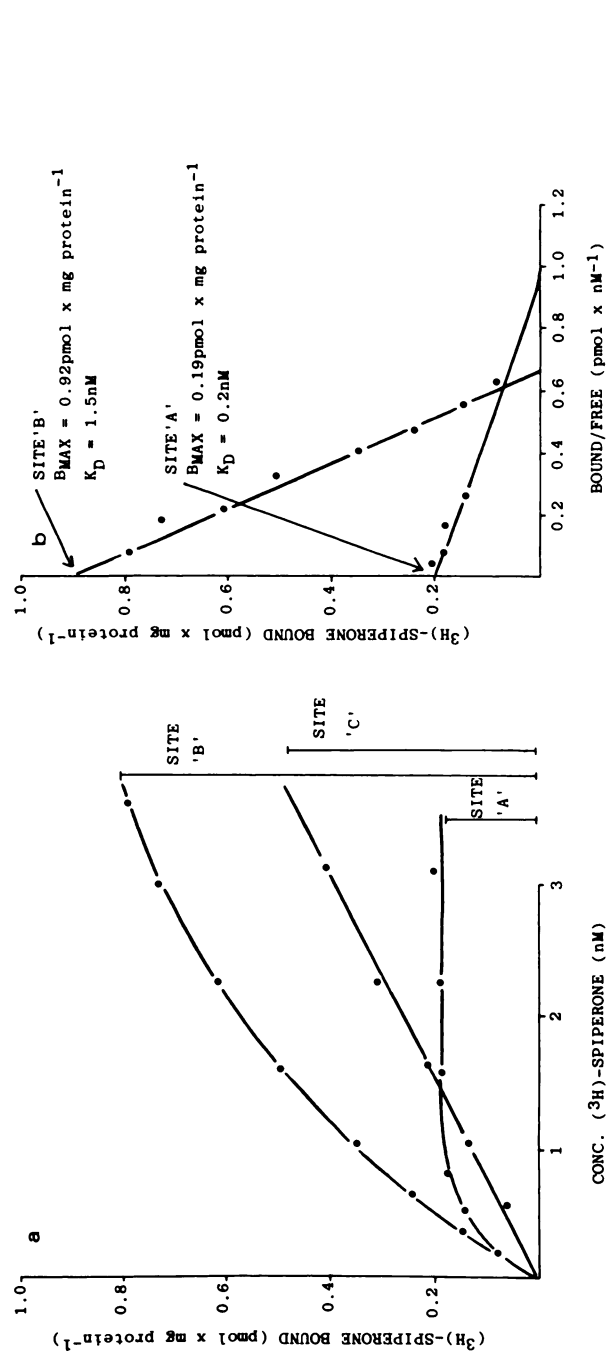

FIG. 4. *Binding of [^{3}H]spiperone to rat hippocampal membranes*

Panel A. Saturation data obtained by incubating membranes with increasing concentrations of [^{3}H]spiperone in the presence or absence of 1 μM (+)-butaclamol and/or 0.3 mM dopamine. Site "A" represents total binding minus the binding in the presence of 1 μM (+)-butaclamol; site "B", binding in presence of 1 μM (+)-butaclamol minus binding in the presence of 0.3 mM dopamine; Site "C", binding in the presence of 0.3 mM dopamine.

Panel B. Scatchard analysis of [^{3}H]spiperone binding to sites "A" and "B."

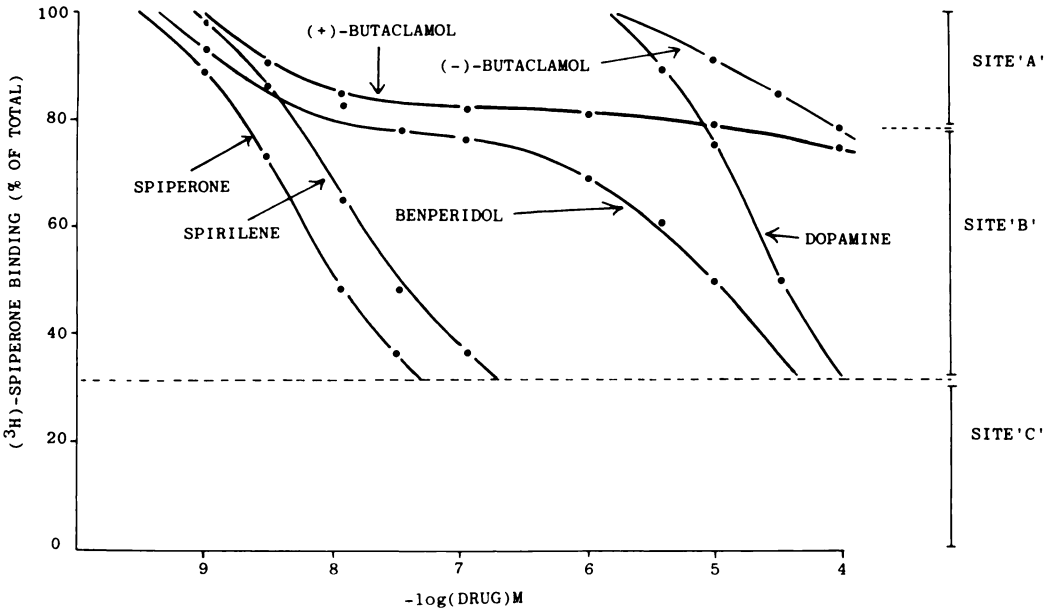

FIG. 5. *Displacement of [^{3}H]spiperone binding to rat hippocampal membranes*
Details of incubation procedure and binding site definition as for Figure 2.

TABLE 1

Drug inhibition of [^{3}H]spiperone binding to rat striatal and hippocampal membranes

Membranes were incubated with 0.5–0.8 nM [^{3}H]spiperone and at least five concentrations of drug. Results are means ± standard errors of at least three experiments.

	IC_{50}		
	Corpus striatum	Hippocampus	
		Site A	Site B
	(nM)	*(nM)*	*(nM)*
Spiperone	0.8 ± 0.1	0.9 ± 0.2	10 ± 2
Benperidol	0.9 ± 0.1	1.3 ± 0.4	3,000 ± 570
Spirilene	2.2 ± 0.3	2.0 ± 0.4	18 ± 5
(+)-Butaclamol	2.3 ± 0.3	2.1 ± 0.3	*
α-Flupenthixol	2.6 ± 0.3	2.0 ± 0.2	*
Spiramide	2.7 ± 0.2	2.0 ± 0.4	20 ± 4
Droperidol	2.8 ± 0.3	3.2 ± 0.4	*
Fluspirilene	3.1 ± 0.6	4.5 ± 0.8	30 ± 5
Haloperidol	6.5 ± 0.6	7.0 ± 1.1	3,500 ± 800
Fluphenazine	7.3 ± 0.8	8.1 ± 1.1	*
Moperone	9.0 ± 0.7	8.5 ± 1.0	10,000 ± 1,300
Fluanisone	55 ± 12	25 ± 7	*
β-Flupenthixol	160 ± 31	220 ± 57	*
Apomorphine	720 ± 90	700 ± 130	*
(−)-Butaclamol	1,700 ± 300	2,350 ± 470	*
Dopamine	4,700 ± 555	3,600 ± 480	5,200 ± 700

* Denotes IC_{50} > 100,000 nM. Also applies to site "B" binding for (+) and (−) propranolol, phentolamine, naloxone, clonidine, dihydroergotamine, bromocryptine, cyproheptadine, 5-hydroxytryptamine, pimozide, sulpiride, (+)-amphetamine, benztropine, histamine, metiamide, diphenhydramine, noradrenaline, adrenaline, tyramine, diazepam, atropine and acetycholine.

mM, against binding to site "B."

Studies with [^{3}H]haloperidol did not produce differences between butaclamol and dopamine displaceable binding. In the limbic forebrain, 1 μM (+) butaclamol and 0.3 mM dopamine both displaced the same percentage of [^{3}H]haloperidol binding giving a K_d of 2.1 nM and B_{max} of 0.23 pmol/mg protein.

DISCUSSION

The specific binding of [^{3}H]neuroleptic ligands has been defined usually as that displaced either by 0.3 mM dopamine (1, 4) or by 1 μM (+) but not 1 μM (−) butaclamol (3, 5). In the present studies, both methods gave similar numbers of binding sites for [^{3}H]spiperone in rat corpus striatum while in certain areas of the limbic system, particularly the hippocampus, commissura fornicis ventralis, septum and nucleus accumbens, but also in the substantia nigra, we have found considerable differences. This apparent nonrandom distribution of [^{3}H]spiperone binding to site "B" suggests that it may be associated with some relatively discrete structural organization within the rat brain, although unlike site "A", it clearly does not relate to the dopaminergic innervation of these structures.

On the other hand, evidence has been provided that suggests that [^{3}H]spiperone binding to site "B" possesses characteristics indicative of a specific recognition site. Thus, it is reversible, saturable, of high affinity and is clearly destroyed by heating. Moreover, this site demonstrates strict structural requirements for occupation, since the only compounds capable of inhibiting site "B" binding with relatively high affinity are dopamine and the spirodecanone butyrophenone derivatives. Whereas alterations in the butyrophenone chain have little effect on affinity, substitution of the spirodecanone group (in the case of spiperone) to form haloperidol or benperidol results in a 1000 fold or greater drop in potency. Although there are no apparent structural similarities between the spirodecanone butyrophenone derivatives and dopamine, the latter catecholamine is the only other compound tested that demonstrates relatively high affinity. Compounds structurally closely related to dopamine such as noradrenaline, tyramine and amphetamine were completely inactive at 0.1 mM. Moreover, the dopamine agonist apomorphine was also inactive at this site, which suggests that site "B" is not "classically" dopaminergic. These observations relate clearly to our previous findings (4) that dopamine displaced more [^{3}H]spiperone than [^{3}H]haloperidol binding in the limbic forebrain since haloperidol only shows high affinity for site "A," while spiperone can occupy both sites "A" and "B" at nanomolar concentrations.

Recent investigations in the rat hippocampus and frontal cortex have shown that [^{3}H]spiperone can bind to serotoninergic receptors (5, 6). However, it should be emphasized that these workers examined butaclamol-displaceable binding to these structures and since in the present studies cyproheptadine and 5-hydroxytryptamine had IC_{50} values of greater than 0.1 mM, site "B" binding sites cannot be associated with serotonin receptors. We have, however, verified that site "A" binding to the hippocampus contains a serotoninergic component (unpublished observations).

In conclusion, therefore, although we have demonstrated the presence of specific "spirodecanone binding sites" in certain precise areas of the rat brain, it remains to be established whether they represent significant neurochemical "receptors" or biologically unimportant "acceptors." Certainly, the relative potencies of the compounds examined show no correlation with any known pharmacological effect at dopaminergic or other receptor sites (see 10). However, although it can be argued that future studies should be directed toward the identification of putative ligands for these sites, the present studies underline the importance of defining the parameters of specific binding sites and emphasize the complex interaction that exists between neuroleptic and dopamine recognition sites in cerebral tissue.

Since the completion of this work, similar preliminary observations concerning [^{3}H]-spiperone binding to rat frontal cortex have been reported (11).

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