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σ opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF 10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes

(dopamine receptor/striatum/spiperone/phenothiazine)

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ABSTRACT **The relationship between binding of antipsychotic drugs and σ psychotomimetic opiates to binding sites for the σ agonist (+)-[^{3}H]SKF 10,047 (*N*-allylnormetazocine) and to dopamine D_2 sites was investigated. In guinea pig brain membranes, (+)-[^{3}H]SKF 10,047 bound to a single class of sites with a K_d of 4 × 10^{-8} M and a B_{max} of 333 fmol/mg of protein. This binding was different from μ, κ, or δ opiate receptor binding. It was inhibited by opiates that produce psychotomimetic activities but not by opiates that lack such activities. Some antipsychotic drugs inhibited (+)-[^{3}H]SKF 10,047 binding with high to moderate affinities in the following order of potency: haloperidol > perphenazine > fluphenazine > acetophenazine > trifluoperazine > molindone ≧ pimozide ≧ thioridazine ≧ chlorpromazine ≧ triflupromazine. However, there were other antipsychotic drugs such as spiperone and clozapine that showed low affinity for the (+)-[^{3}H]SKF 10,047 binding sites. Affinities of antipsychotic drugs for (+)-[^{3}H]SKF 10,047 binding sites did not correlate with those for [^{3}H]spiperone (dopamine D_2) sites. [^{3}H]-Haloperidol binding in whole brain membranes was also inhibited by the σ opiates pentazocine, cyclazocine, and (+)-SKF 10,047. In the striatum, about half of the saturable [^{3}H]haloperidol binding was to [^{3}H]spiperone (D_2) sites and the other half was to sites similar to (+)-[^{3}H]SKF 10,047 binding sites.**

Three types of opiate receptors have been proposed to account for the different pharmacological effects of opiates in chronic spinal dogs (1). These receptors are named after their prototype drugs: μ (morphine), κ (ketocyclazocine), and σ (SKF 10,047). The existence of δ (enkephalin) receptors has also been demonstrated *in vitro* (2). The "σ agonists" SKF 10,047, cyclazocine, and pentazocine produced delirium in the dog (1) and psychotomimetic effects in man that include dysphoria and hallucinations (3–5).

Because SKF 10,047 and cyclazocine have been found to bind to the phencyclidine (PCP) binding site, although with low affinity (6), it has been suggested that the σ activities of opiates may be mediated through the PCP receptor (7). Recently, an etorphine-inaccessible [^{3}H]SKF 10,047 binding site in guinea pig brain (8) and a (+)-[^{3}H]SKF 10,047 binding site in the rat central nervous system (9) have been identified. These binding sites (8, 9) have different drug selectivity compared with the PCP binding site (6). The (+)-[^{3}H]SKF 10,047 binding site also shows different regional distribution than the PCP binding site (9). Thus, the σ agonists may be acting at the PCP receptor as well as at a separate binding site.

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It has been suggested that the σ agonist activity of SKF 10,047 may involve a dopaminergic mechanism (1). The purpose of this study was to investigate the relationship of the binding of σ agonists and antipsychotic drugs to the σ (+)-[^{3}H]SKF 10,047 binding site and to the dopamine receptor.

MATERIALS AND METHODS

(+)-[^{3}H]SKF 10,047 (34.0 Ci/mmol; 1 Ci = 37 GBq), [^{3}H]spiperone (22.8 Ci/mmol), and [^{3}H]haloperidol (14.7 Ci/mmol) were from New England Nuclear.

Brains were dissected from 250- to 300-g male Hartley guinea pigs (Charles River Breeding Laboratories) that were sacrificed by decapitation. Brain membranes were prepared as described (9) and then suspended to about 1.0 mg of protein/ml of 50 mM Tris·HCl buffer (pH 7.4).

In the binding assays, 1-ml aliquots of membrane preparation were incubated with unlabeled drugs, radiolabeled ligands, and Tris buffer in a final volume of 2 ml in 50 mM Tris·HCl (pH 7.4). Samples were incubated at room temperature for 45 min, filtered through Whatman GF/C glass-fiber filters under reduced pressure, and rapidly washed three times with ice-cold Tris·HCl (5 ml). Nonspecific binding of (+)-[^{3}H]SKF 10,047 was determined in the presence of 10 μM SKF 10,047. Nonspecific binding of [^{3}H]spiperone was determined in the presences of either 0.1 μM (+)-butaclamol or 1 μM spiperone, depending on the protocol. Nonspecific binding of [^{3}H]haloperidol was determined in the presence of either 0.1 μM (+)-butaclamol or 10 μM haloperidol, depending on the protocol.

RESULTS

(+)-[^{3}H]SKF 10,047 Binding. The σ agonist (+)-[^{3}H]SKF 10,047 bound to specific sites in the guinea pig brain membranes. A typical experiment with 2 nM (+)-[^{3}H]SKF 10,047 gave 1400 dpm of total binding and 330 dpm of nonspecific binding. There was no saturable or displaceable binding to filters without brain membranes. Binding was linearly proportional to the amount of membrane protein (0.3–3.0 mg) and was time dependent. Maximum binding was reached in 20 min at room temperature. The Scatchard plot was linear, suggesting a class of binding site with a K_d of 4 × 10^{-8} M and a B_{max} of 333 fmol/mg protein (Fig. 1). The (+)-[^{3}H]SKF 10,047 binding site is highly stereoselective because (−)-SKF 10,047 was 1/38th as potent as (+)-SKF 10,047 (Table 1).

(+)-[^{3}H]SKF 10,047 bound to a site clearly different from the opiate μ, δ, or κ sites, because the potent μ, δ, and κ antagonist naloxone had no effect on its binding even at very high concentrations (Table 1). Opiates of the benzomorphan

Abbreviation: PCP, phencyclidine.

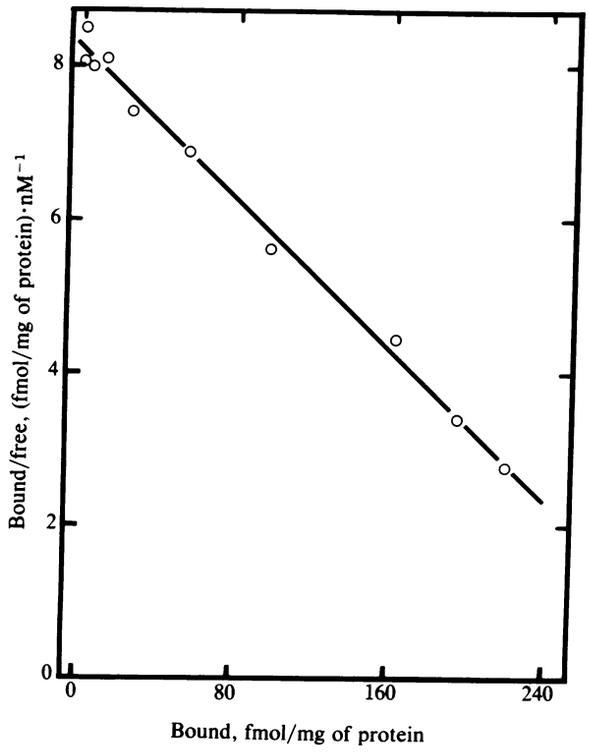

FIG. 1. Scatchard plot of binding of (+)-[^{3}H]SKF 10,047 to guinea pig brain membranes. The concentration range was 0.1 to 80 nM. Binding was carried out in the presence of 100 mM NaCl. The line was determined by linear regression. Data represent the mean of triplicate determinations of two experiments.

series such as pentazocine, cyclazocine, and (+)-SKF 10,047 bound to the (+)-[^{3}H]SKF 10,047 binding site with relatively high affinity. These opiates produce psychotomimetic effects such as dysphoria and hallucinations (5). Opiates that do not produce apparent σ psychotomimetic effects did not bind to the (+)-[^{3}H]SKF 10,047 binding site (ref. 9; unpublished data).

Surprisingly, several chemical classes of antipsychotic drugs bound to the σ agonist (+)-[^{3}H]SKF 10,047 binding site with high to moderate affinities. These antipsychotic drugs included the butyrophenone haloperidol, the benzimidazolinone pimozide, the tetrahydroindolone molindone, and phenothiazines such as perphenazine, fluphenazine, acetophenazine, trifluoperazine, chlorpromazine, thioridazine, and triflupromazine (Table 1). However, there are other antipsychotic drugs such as spiperone, (+)-butaclamol, thiothixene, loxapine, and clozapine that bound to the (+)-[^{3}H]SKF 10,047 binding site with low affinity. The stereoselective dopamine receptor blocker (+)-butaclamol was 1/34th as potent as (−)-butaclamol in inhibiting (+)-[^{3}H]SKF 10,047 binding, and apomorphine and dopamine had no effect. Unlike the known effect of Na^+ on μ opiate binding (10), (+)-[^{3}H]SKF 10,047 binding was not affected by the presence of 100 mM NaCl (unpublished data). Its displacement by drugs was also not affected by NaCl (Table 1).

[^{3}H]Spiperone Binding. To compare (+)-[^{3}H]SKF 10,047 binding with dopamine receptor binding, dopamine D_2 receptor binding studies were performed using striatum membranes and [^{3}H]spiperone. Nonspecific binding was measured in the presence of 0.1 μM (+)-butaclamol. The σ opiates (+)-SKF 10,047 and cyclazocine had very low affinity for [^{3}H]spiperone (D_2) binding sites, whereas the antipsychotics displaced [^{3}H]spiperone with high to moderate affinity (Table 1). Clearly, the binding of the drugs to the (+)-[^{3}H]SKF 10,047 binding site is different than the binding to [^{3}H]spiperone (D_2) sites. Because (+)-[^{3}H]SKF 10,047 binding was carried out with whole brain membranes, [^{3}H]-spiperone binding to whole brain membranes was studied for comparison. In this case, excess unlabeled spiperone was used to measure nonspecific binding to allow detection of all displaceable spiperone binding sites, which include dopamine and serotonin sites (11). Although affinities in the whole brain assay differ slightly from that found in the striatum assay, the [^{3}H]spiperone binding sites were different from the (+)-[^{3}H]SKF 10,047 binding site (Table 1).

[^{3}H]Haloperidol Binding. Haloperidol bound to the (+)-[^{3}H]SKF 10,047 binding site with high affinity (Table 1). Because haloperidol is a dopamine receptor antagonist, it was important to show that the σ opiates pentazocine, cyclazocine, and (+)-SKF 10,047 can also compete with [^{3}H]haloperidol for binding. As shown in Fig. 2, [^{3}H]haloperidol binding in whole brain membranes was strongly inhibited by haloperidol and perphenazine, which had high affinity for both dopamine and (+)-[^{3}H]SKF 10,047 binding sites. The σ agonists pentazocine, cyclazocine, and (+)-SKF 10,047 were also effective in inhibiting [^{3}H]haloperidol binding. The high-affinity dopamine receptor antagonists spiperone and (+)-butaclamol were less effective. The rank order of potency was haloperidol > perphenazine > pentazocine > (−)-butaclamol > (+)-SKF 10,047 ≧ cyclazocine > spiperone > (+)-butaclamol > (−)-SKF 10,047. This rank order is similar to that in inhibiting (+)-[^{3}H]SKF 10,047 binding. Serotonin and methylsergide were not effective in inhibiting [^{3}H]haloperidol binding ($IC_{50} > 50$ μM), suggesting that [^{3}H]haloperidol bound mostly to sites other than serotonin-binding sites. The results were similar when membranes prepared from brain from which the dopamine receptor-rich striatum region had been removed were used in the [^{3}H]haloperidol binding studies. Thus, the majority of sites labeled by [^{3}H]haloperidol in guinea pig brain membranes were similar to the (+)-[^{3}H]SKF 10,047 binding site.

To further compare the effects of antipsychotic drugs and σ opiates on dopamine D_2 sites and (+)-[^{3}H]SKF 10,047 binding sites, [^{3}H]haloperidol binding in striatum was studied. The dopamine receptor blockers spiperone, loxapine, and (+)-butaclamol showed biphasic displacement curves in the binding of [^{3}H]haloperidol to striatal membranes (Fig. 3). At a concentration (0.1 μM) commonly used to block specific binding to dopamine receptors, (+)-butaclamol blocked only about 50% of total binding. Perphenazine and fluphenazine, which have similar affinity for both the [^{3}H]spiperone (D_2) site and the (+)-[^{3}H]SKF 10,047 binding site, did not show biphasic displacement. The σ opiates also showed biphasic displacement.

In the next experiment, binding of [^{3}H]haloperidol to striatum was carried out in the presence of 0.1 μM (+)-butaclamol to block dopamine D_2 binding, to investigate whether the σ opiates were binding to sites different from the dopamine D_2 sites. After dopamine D_2 sites were blocked, perphenazine, (−)-butaclamol, and the σ opiates pentazocine, cyclazocine, and (+)-SKF 10,047 were effective in inhibiting [^{3}H]haloperidol binding (Fig. 4). (−)-SKF 10,047 was much less effective than (+)-SKF 10,047, which is consistent with binding to (+)-[^{3}H]SKF 10,047 binding sites in brain membranes.

DISCUSSION

Racemic SKF 10,047 produces analgesia and psychotomimetic subjective effects in man (3) and delirium in dogs (1, 12). The analgesic activity of SKF 10,047 resides primarily in its (−)-isomer (13). (−)-SKF 10,047 binds with high affinity to both μ and κ receptors and produces predominantly κ agonist activity in β-funaltrexamine-treated guinea pig ileum

Table 1. Effect of opiates, antipsychotics, and other drugs on specific binding of (+)-[^{3}H]SKF 10,047 and [^{3}H]spiperone to guinea pig brain and striatal membranes

	(+)-[^{3}H]SKF 10,047, $K_i \times 10^9$ M		[^{3}H]Spiperone, $IC_{50} \times 10^9$ M	
	+ NaCl	− NaCl	Striatum*	Brain†
Opiate				
Morphine	>100,000	>100,000	>100,000	>100,000
[D-Ala^2, D-Leu^5]-Enkephalin	>100,000	>100,000	>10,000	>10,000
Pentazocine	18 ± 4	20 ± 1		
Cyclazocine	36 ± 7	47 ± 2	14,000 ± 1100	>10,000
(+)-SKF 10,047	48 ± 2	48 ± 4	29,000 ± 400	>10,000
(−)-SKF 10,047	1,800 ± 293	1,300 ± 90	14,000 ± 500	>10,000
(−)-Naloxone	>100,000	>100,000	>100,000	>100,000
(+)-Naloxone	>100,000			
Antipsychotics and others				
Haloperidol	4 ± 1	3 ± 0.4	1.9 ± 0.2	22 ± 3
Perphenazine	12 ± 1	8 ± 2	4.0 ± 0.3	13 ± 1
Fluphenazine	17 ± 2	13 ± 5	6.5 ± 0.4	19 ± 2
(−)-Butaclamol	38 ± 2	40 ± 2	2,200 ± 130	
Acetophenazine	54 ± 8	36 ± 0.1		17 ± 2
Trifluoperazine	67 ± 11	54 ± 3		28 ± 3
Molindone	124 ± 13	194 ± 25		697 ± 162
Pimozide	144 ± 11	139 ± 4	3.4 ± 0.3	18 ± 2
Thioridazine	174 ± 2	130 ± 10	16 ± 1	35 ± 7
Chlorpromazine	180 ± 4	146 ± 14	9.1 ± 0.8	17 ± 2
Triflupromazine	214 ± 26	154 ± 4		7 ± 1
Spiperone	1,090 ± 246	1,200 ± 140	0.8 ± 0.08	2 ± 0.1
(+)-Butaclamol	1,300 ± 240	1,300 ± 190	2.7 ± 0.2	
Thiothixene	1,400 ± 280	2,000 ± 50	3.6 ± 0.4	55 ± 18
Loxapine	1,600 ± 307	1,700 ± 490	12 ± 0.6	32 ± 6
Clozapine	11,400 ± 4040	8,600 ± 420		194 ± 49
Apomorphine‡	>31,000			
(+)-Amphetamine	>10,000			
Dopamine‡	>100,000			

Binding of 2 nM (+)-[^{3}H]SKF 10,047 was measured in brain only in the presence and absence of NaCl. Binding of 0.15 nM [^{3}H]spiperone was measured in brain and in striatum in the absence of NaCl. Values represent mean ± SEM of two to four experiments in triplicate.
*Nonspecific binding was measured in 0.1 μM (+)-butaclamol.
†Nonspecific binding was measured in 1 μM spiperone.
‡Assayed in 1 mM (−)-ascorbic acid.

(unpublished data). On the other hand, (+)-SKF 10,047 has very low affinity for μ and κ receptors and is primarily a σ receptor ligand.

The present study has demonstrated a binding site for the σ agonist (+)-[^{3}H]SKF 10,047 in guinea pig brain membranes. This site binds the σ opiates (+)-SKF 10,047, cyclazocine, and pentazocine but has very low affinity for μ,

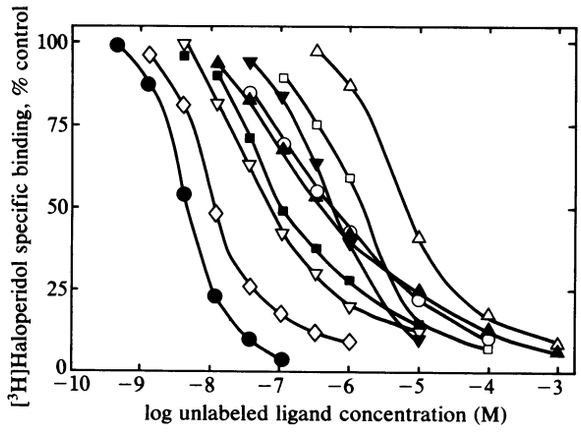

FIG. 2. Competitive inhibition of specific binding of 1 nM [^{3}H]haloperidol in guinea pig brain membranes by haloperidol (●), perphenazine (◇), pentazocine (▽), (−)-butaclamol (■), (+)-SKF 10,047 (▲), cyclazocine (○), spiperone (▼), (+)-butaclamol (□), and (−)-SKF 10,047 (△). Nonspecific binding was determined in the presence of 10 μM haloperidol. Data represent means of triplicate determinations of three experiments.

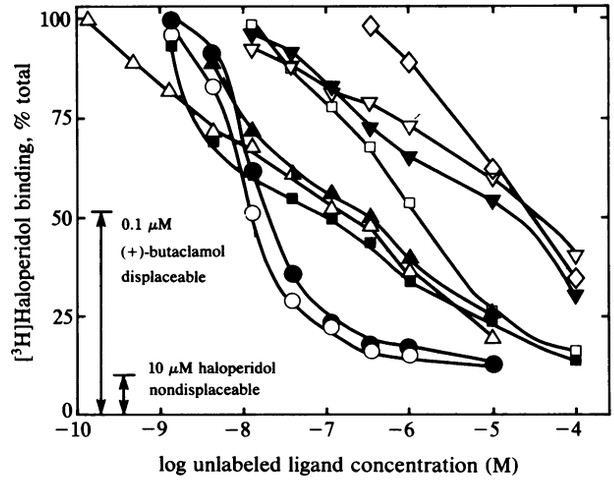

FIG. 3. Competitive inhibition of total binding of 1 nM [^{3}H]haloperidol in guinea pig striatal membranes by spiperone (△), perphenazine (○), fluphenazine (●), (+)-butaclamol (■), loxapine (▲), (−)-butaclamol (□), cyclazocine (▼), (+)-SKF 10,047 (▽), and (−)-SKF 10,047 (◇). Data represent means of duplicate determinations of three or four experiments.

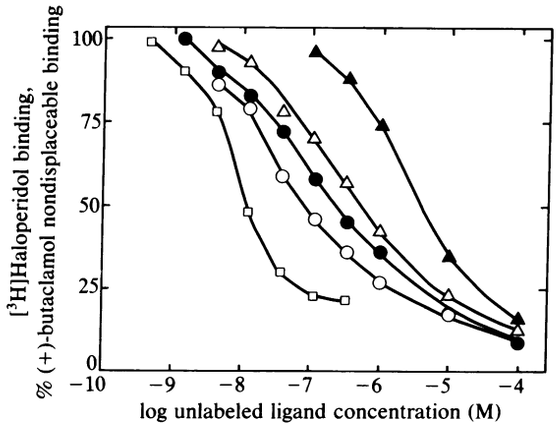

FIG. 4. Competitive inhibition of 0.1 μM (+)-butaclamol-nondisplaceable [^{3}H]haloperidol binding in guinea pig striatal membranes. Binding of 1 nM [^{3}H]haloperidol in the presence of 0.1 μM (+)-butaclamol was inhibited by perphenazine (□), pentazocine (○), cyclazocine (●), (+)-SKF 10,047 (△), and (−)-SKF 10,047 (▲). Data represent means of duplicate determinations of three experiments.

δ, or κ opioids such as morphine, naloxone, the enkephalins, and the dynorphins. Similar binding sites have been identified in guinea pig brain by using racemic [^{3}H]SKF 10,047 in the presence of excess unlabeled etorphine (8) and in the rat central nervous system by using racemic [^{3}H]ethylketocyclazocine in the presence of excess unlabeled naloxone or (+)-[^{3}H]SKF 10,047 (9). The regional distribution of the (+)-[^{3}H]SKF 10,047 binding site is different from that of the μ, δ, and PCP receptors in the rat brain (9) and that of the μ, δ, and κ receptors in guinea pig brain (unpublished data).

Interestingly, a number of antipsychotic drugs representing several chemical classes were found to bind to the (+)-[^{3}H]SKF 10,047 binding site with high to moderate affinity. These drugs include a butyrophenone, a benzimidazolinone, a tetrahydroindolone, and all the phenothiazine antipsychotics tested. The rank order of binding potency is haloperidol > perphenazine > fluphenazine > acetophenazine > trifluoperazine > molindone ≧ pimozide ≧ thioridazine ≧ chlorpromazine ≧ triflupromazine. However, there are other antipsychotic drugs such as spiperone, thiothixene, loxapine, and clozapine that had much lower affinity for the (+)-[^{3}H]SKF 10,047 binding site. So, there is no direct relationship between the affinity of these antipsychotic drugs for this site and for the [^{3}H]spiperone binding site. The affinity of the antipsychotic drugs for the [^{3}H]spiperone (D_2) site in guinea pig brain membranes was similar to literature values reported? for the rat (14).

Binding studies with [^{3}H]haloperidol to membranes prepared from whole guinea pig brain showed that the order of drug potency for opiates and antipsychotics in inhibiting [^{3}H]haloperidol binding is similar to that in inhibiting (+)-[^{3}H]SKF 10,047 binding. In the striatum, which is rich in dopamine receptors, about half of the displaceable [^{3}H]haloperidol binding could be blocked by 0.1 μM (+)-butaclamol and therefore was supposedly to the dopamine D_2 site. The other half of the displaceable [^{3}H]haloperidol binding to striatal membranes could not be blocked by 0.1 μM (+)-butaclamol and thus is not to the dopamine D_2 site. These sites are generally referred to as nonspecific but saturable sites (14). Interestingly, the antipsychotic drug perphenazine and the σ opiate agonists (+)-SKF 10,047, cyclazocine, and pentazocine were effective in inhibiting 0.1 μM (+)-butaclamol-nondisplaceable saturable [^{3}H]haloperidol binding. Furthermore, the order of potency and stereoselectivity in inhibiting this binding is similar to the order of potency and stereoselectivity in inhibiting σ (+)-[^{3}H]SKF 10,047 binding. Therefore, the results suggest that the 0.1 μM (+)-butaclamol-nondisplaceable saturable [^{3}H]haloperidol binding sites and the (+)-[^{3}H]SKF 10,047 binding sites are similar if not identical.

The findings that the majority of sites in the brain that are labeled by [^{3}H]haloperidol are similar to the (+)-[^{3}H]SKF 10,047 binding site and that some psychotomimetics as well as a number of antipsychotic drugs bind to these sites raise important questions. Are the (+)-[^{3}H]SKF 10,047 binding sites pharmacologically relevant? Although a definitive answer is not possible, the characteristics of this binding site are consistent with the hypothesis that it is pharmacologically relevant. These characteristics include a protein moiety (unpublished data), stereoselectivity for ligands, large number of sites showing regional distribution in brain, binding of opiates that produce dysphoric or psychotomimetic effects, and binding of several chemical classes of antipsychotic drugs but not binding of known neurotransmitters or selective ligands for other known receptors (ref. 9; unpublished data). Are there endogenous "psychotomimetic" ligands for this binding site? Such ligands have not been found. Various endogenous psychoactive compounds have been identified. For example, α-endorphin has been reported to have some activity common with amphetamine and des-Tyr-γ-endorphin has been reported to have neuroleptic activity (15). These two neuropeptides do not appear to act through this binding site, because their affinity is very low. With the present selective binding assay, it should be possible to determine whether endogenous ligands for this binding site exist. Some antipsychotic drugs may act therapeutically by antagonizing both this site and dopamine receptors whereas others may be specifically antagonizing dopamine receptors only.

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