MOLECULAR PHARMACOLOGY, 12, 800-812

Properties of [^{3}H]Haloperidol and [^{3}H]Dopamine Binding Associated with Dopamine Receptors in Calf Brain Membranes

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(Received March 8, 1976)
(Accepted May 5, 1976)

SUMMARY

BURT, DAVID R., CREESE, IAN & SNYDER, SOLOMON H. (1976) Properties of [^{3}H]haloperidol and [^{3}H]dopamine binding associated with dopamine receptors in calf brain membranes. *Mol. Pharmacol.* 12, 800-812.

[^{3}H]Haloperidol and [^{3}H]dopamine bind in saturable fashion to membranes from calf brain with high affinity and other characteristics indicating an association with postsynaptic dopamine receptors. Kinetic analysis of rates of association and dissociation yields K_D values in agreement with equilibrium measurements. Regional variations in [^{3}H]dopamine and [^{3}H]haloperidol binding are parallel and correspond to regional differences in dopaminergic innervation. Drug specificity does not appear to differ between limbic and striatal areas. The relative potencies of various agonists and antagonists on the binding of the two ligands parallel their pharmacological actions at dopamine receptor sites. Dopamine agonists have 6-38 times more affinity for [^{3}H]dopamine than [^{3}H]haloperidol binding sites. By contrast, dopamine antagonists have 20-12,000 times more affinity for [^{3}H]haloperidol than [^{3}H]dopamine binding sites. Ergot derivatives, including *d*-lysergic acid diethylamide, and other serotonin antagonists have substantial affinity for both types of binding.

INTRODUCTION

Membranes of rat and calf brain bind [^{3}H]haloperidol and [^{3}H]dopamine with characteristics indicating an association with postsynaptic dopamine receptors (1-9). Regional variations in the distribution of binding sites parallel regional differences in forebrain dopamine innervation (1, 6, 7). The relative affinities of drugs for these binding sites are similar to their relative pharmacological potencies in affecting behavior presumably mediated via dopamine receptors (1, 10). We now describe detailed properties of the binding of [^{3}H]haloperidol and [^{3}H]dopamine to membranes of calf brain.

METHODS

Methods were those described previously (1, 3-5). Caudate or other regions of fresh or frozen calf brains were homogenized in 40 volumes of ice-cold 50 mM Tris buffer, pH 7.7, at 25°, with a Brinkmann Polytron PT-10 (setting 6, 5 sec). The homogenate was centrifuged twice at 50,000 × *g* for 10 min (Sorvall RC2-B, 20,000 rpm,

This research was supported by United States Public Health Service Grants MH-18501 and DA-00266 and by the John A. Hartford Foundation.

[1] Recipient of United States Public Health Service Fellowship NS-01654.

[2] Recipient of United States Public Health Service Fellowship DA-05328.

[3] Research Scientist Career Development Awardee of the United States Public Health Service (MH-33128).

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rotor SS-34 or SM-24), with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was homogenized in 90 volumes of cold, freshly prepared 50 mM Tris buffer containing 0.1% ascorbic acid, 10 μM pargyline, and ions as follows: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, to give a final pH of 7.1 at 37°. The tissue suspension (11 mg/ml) was placed in a 37° bath for 5 min and returned to ice.

Freezing tissue for periods of up to 2 weeks produced little or no effect on binding. Addition of approximately physiological concentrations of ions lowered blank values for both ligands but did not alter specific binding.

[*ethyl*-1-^{3}H(*N*)]Dopamine, 8.4 Ci/mmole, was obtained from New England Nuclear. [^{3}H]Haloperidol, 0.86 Ci/mmole, tritiated for us by New England Nuclear and purified by thin-layer chromatography (1), was used for almost all experiments reported here. Very recently [^{3}H]haloperidol, 9.6 Ci/mmole, was kindly donated by Janssen Pharmaceutica and has yielded very similar results. On the day of use, each radioactive drug was diluted with 0.1% ascorbic acid to a concentration of 100 nM ([^{3}H]dopamine) or 20–40 nM ([^{3}H]haloperidol).

Incubation tubes in duplicate or triplicate received 100 μl of diluted [^{3}H]-dopamine or [^{3}H]haloperidol, 100 μl of various concentrations of drugs dissolved in 0.1% ascorbic acid, and 1.8 ml of tissue suspension. The tubes were incubated at 37° for 10 min and rapidly filtered under vacuum through Whatman GF/B filters with two 5-ml rinses of ice-cold 50 mM Tris buffer, pH 7.7, at 25°. The filters were counted by liquid scintillation spectrometry in 10–12 ml of Hydromix (Yorktown Research) at efficiencies of 37–44%.

Saturable or specific binding of [^{3}H]-dopamine was measured as the excess over blanks taken in the presence of 1 μM dopamine or 10 μM (+)-butaclamol. Blanks for [^{3}H]haloperidol binding were taken in the presence of 100 μM dopamine or 0.1 μM (+)-butaclamol. Bound radioactivity from [^{3}H]haloperidol migrated with authentic haloperidol on thin-layer chromatograms, using the same solvents as in the original purification (1). Bound radioactivity from [^{3}H]dopamine was previously found to migrate with authentic dopamine (3). Total membrane-bound radioactivity was less than 10% of that added to the tubes for [^{3}H]haloperidol and less than 3% for [^{3}H]dopamine. Specific binding to caudate membranes was about 40% of the total for [^{3}H]haloperidol and about 60% of the total for [^{3}H]dopamine. The latter value represents a considerable improvement over previous results with the centrifugation method (3).

Butyrophenone and similar drugs were dissolved in a minimal volume of glacial acetic acid (less than 1%, final volume) and brought up to 2 mM with hot 0.1% ascorbic acid. Other drugs were dissolved with 0.1% ascorbic acid, and all drugs were diluted further with 0.1% ascorbic acid. Proteins were determined by the method of Lowry *et al.* (11).

The sources of drugs were as follows: dopamine, Sigma Chemical Company; haloperidol and benperidol, McNeil Laboratories; apomorphine, Merck and Company; *d*- and *l*-butaclamol, Ayerst Laboratories; *cis*- and *trans*-thiothixene, Pfizer; α- and β-flupenthixol, *cis*- and *trans*-clopenthixol, chlorprothixene, piflutixol, and teflutixol, H. Lundbeck and Company; 2-amino-6,7-dihydroxy-(1, 2, 3, 4)-tetrahydronaphthalene, a gift from Dr. L. L. Iversen; azaperone, bromoperidol, clofluperol, droperidol, fluanisone, fluspirilene, moperone, spiroperidol (spiperone), penfluridol, pimozide, pipamperone, and trifluperidol, Janssen Pharmaceutica; epinine, serotonin, and other tryptamine compounds, Regis Chemical Company; 2-bromo-LSD,[4] ergot alkaloids, and thioridazine, Sandoz; LSD analogues, psilocin, and psilocybin, the NIMH-FDA Committee on Scheduled Substances; 2,5-dimethoxy-4-ethylamphetamine isomers, Charles Barfknecht; mianserin, Organon; chlorpromazine and cyproheptadine, Smith Kline & French; methiothepin, Roche; fluphenazine, triflu-

[4] The abbreviations used are: LSD, lysergic acid diethylamide; ADTN, 2-amino-6,7-dihydroxy-(1,2,3,4)-tetrahydronaphthalene.

promazine, and trifluoperazine, E. R. Squibb and Sons; promazine and promethazine, Wyeth; molindone, Endo.

RESULTS

Saturability and kinetics of specific [^{3}H]haloperidol binding. Competition by nonradioactive haloperidol for [^{3}H]haloperidol binding gives a continuous curve over many orders of magnitude of drug concentration, suggesting the presence of multiple classes of [^{3}H]haloperidol binding sites (Fig. 1A). The potent neuroleptic butaclamol exists as two optical isomers, with essentially all pharmacological potency residing in the (+) isomer (12). Thus the extent to which butaclamol isomers differ in their inhibition of haloperidol binding should provide a measure of the portion of [^{3}H]haloperidol binding which is specifically associated with dopamine receptors. (+)-Butaclamol displays two clearly distinct components in inhibiting [^{3}H]haloperidol binding. The high-affinity component elicits half-maximal effects at about 1 nM, while the low-affinity component is only apparent at concentrations above 1 μM. By contrast, (−)-butaclamol lacks the high-affinity component of inhibition of [^{3}H]haloperidol binding, while its lower-affinity inhibition of [^{3}H]haloperidol binding resembles the low-affinity influence of (+)-butaclamol. Accordingly we have taken the stereospecific portion of competition by (+)-butaclamol for [^{3}H]haloperidol binding to represent "specific" [^{3}H]haloperidol binding, presumably associated with dopamine receptors. Dopamine maximally reduces [^{3}H]haloperidol binding to the same extent as the high-affinity component of (+)-butaclamol inhibition. [^{3}H]Haloperidol binding is reduced to the same extent by 0.1 μM (+)-butaclamol, by 100 μM dopamine, and by the combination of 0.1 μM (+)-butaclamol and 100 μM dopamine. This result indicates that both dopamine and (+)-butaclamol compete for the same class of [^{3}H]haloperidol binding sites. We have routinely defined specific [^{3}H]haloperidol binding as the amount of binding inhibited by either 0.1 μM (+)-butaclamol or 100 μM dopamine.

As observed in preliminary studies, apomorphine is more potent in competing for

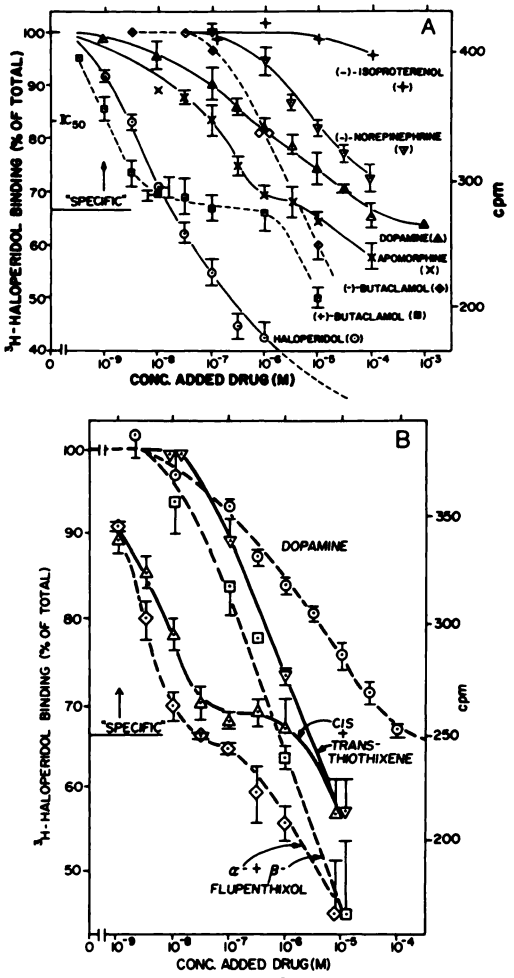

FIG. 1. *Competition for [^{3}H]haloperidol binding*

A. Competition by nonradioactive drugs. Increasing concentrations of each drug were added to tubes containing 2 nM [^{3}H]haloperidol and membranes from 20 mg of calf caudate in 2 ml. Results are expressed as a percentage of the total binding in the absence of added drugs and are the means of multiple experiments. Bars give standard error of the mean, where applicable. The "specific" portion of binding was defined with reference to tubes containing 0.1 μM (+)-butaclamol or 100 μM dopamine, as shown. The "cpm" axis is based on the mean counts per minute observed in many experiments.

B. Competition by geometrical isomers of flupenthixol and thiothixene. Experiments were the same as in Fig. 1A, except that the results were first obtained as mean percentages of the "specific" binding and then re-expressed as a percentage of the total binding.

[^{3}H]haloperidol binding than is dopamine, which in turn is more potent than (−)-norepinephrine. (−)-Isoproterenol, the po-

tent agonist for norepinephrine *beta* receptors, is essentially inactive. The relative potencies of these catecholamines in competing for [^{3}H]haloperidol binding resemble their potencies in competing for [^{3}H]dopamine binding associated with dopamine receptor sites (1, 3) and for the dopamine-sensitive adenylate cyclase (13, 14).

Geometrical isomers of certain neuroleptics differ in their pharmacological capacity to block dopamine receptors. Thus, among the thioxanthenes, dopamine-blocking activity in pharmacological tests is exhibited by α-flupenthixol but not by β-flupenthixol (15), and by *cis*- but not by *trans*-thiothixene (16). α-Flupenthixol competes for [^{3}H]haloperidol binding with distinct high- and low-affinity components. The high-affinity component reduces [^{3}H]haloperidol binding 50% at about 2 nM. Essentially no high-affinity competition by β-flupenthixol can be demonstrated, while in its low-affinity competition β-flupenthixol has about the same potency as α-flupenthixol. Similar differences exist between *cis*- and *trans*-thiothixene. *Cis*-thiothixene has an IC_{50} of about 3 nM for its high-affinity component but does not differ in potency from *trans*-thiothixene in low-affinity inhibition of [^{3}H]haloperidol binding. The extent of the high-affinity inhibition of [^{3}H]haloperidol binding by *cis*-thiothixene and α-flupenthixol is the same as that displayed by (+)-butaclamol and by 100 μM dopamine (Fig. 1B). This further supports the suggestion that the portion of [^{3}H]haloperidol binding competed for by 100 μM dopamine and by the high-affinity components of *cis*-thiothixene, α-flupenthixol, and (+)-butaclamol represents binding of [^{3}H]haloperidol associated with dopamine receptors.

The slopes of inhibition of [^{3}H]haloperidol binding by antagonists such as butaclamol, thiothixene, and haloperidol itself are steeper than those displayed by agonists such as dopamine and apomorphine (Fig. 1A and B). The Hill coefficients for these slopes are 1.1 and 0.9 for (+)-butaclamol and *cis*-thiothixene, respectively, and 0.5 and 0.7 for dopamine and apomorphine (see Fig. 2A for dopamine). Haloperidol inhibits [^{3}H]haloperidol binding with a steeper slope (Hill coefficient = 1.3), presumably because at higher concentrations there is simultaneous competition for nonspecific but saturable sites.

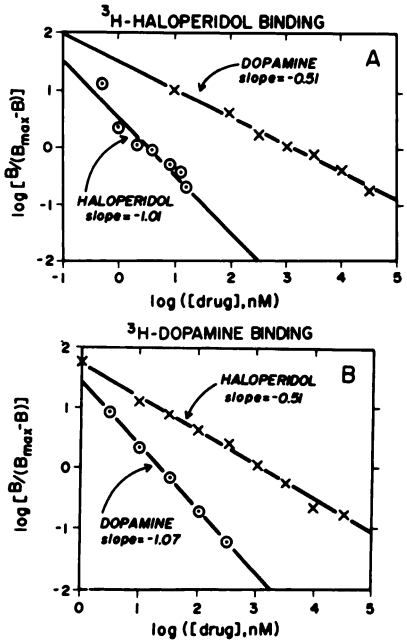

FIG. 2. *Hill plots*

A. Competition of dopamine for specific [^{3}H]haloperidol binding (data from Fig. 1B) and of the saturation of specific [^{3}H]haloperidol binding (data from Fig. 3). The saturation data were first converted to the form of a competition curve by using the *B/F* data from the Scatchard plot of Fig. 3 at each [^{3}H]haloperidol concentration and taking the maximum point as the intercept with the *B/F* axis. For the dopamine competition curve, the maximum point was taken as the observed specific [^{3}H]haloperidol binding in the absence of added drug.

B. Competition of nonradioactive dopamine and haloperidol for specific [^{3}H]dopamine binding (data from Fig. 5). The maximum point was taken as the observed specific [^{3}H]dopamine binding in the absence of added drug.

With increasing concentrations of [^{3}H]haloperidol, specific binding becomes saturated (Fig. 3). Binding appears to be fully saturated at about 10 nM. The Hill coefficient of this saturation curve (plotted in the form of a competition curve in Fig. 2A) is about 1. Scatchard analysis of the saturation data indicates a dissociation constant (K_D) for [^{3}H]haloperidol binding of about 3 nM. The K_D value obtained in these experiments is somewhat greater

than the apparent value from competition of nonradioactive haloperidol for [^{3}H]-haloperidol binding. The density of receptors calculated from these data is about 17 pmoles/g of caudate nucleus tissue, wet weight.

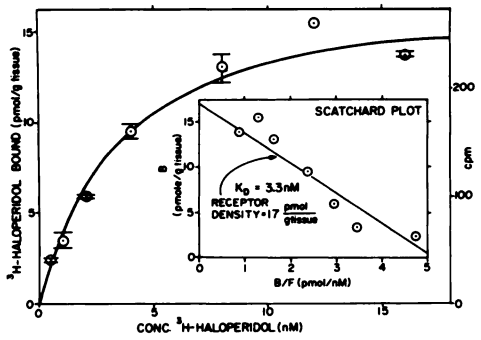

FIG. 3. *Saturation of specific [^{3}H]haloperidol binding*

Increasing concentrations of [^{3}H]haloperidol were incubated with membranes of calf caudate. Binding was measured relative to blanks containing 0.1 μM (+)-butaclamol or 100-μM dopamine, as in Fig. 1. Results are the means and standard errors of four experiments. The inset gives a Scatchard plot of the same data.

[^{3}H]Haloperidol binding takes place rapidly (Fig. 4). Binding appears to reach equilibrium at 37° between 1 and 3 min, with half-maximal binding attained at about 15–20 sec. The rate constant for association of [^{3}H]haloperidol binding, calculated from the initial slope of the association curve and the known concentrations of [^{3}H]haloperidol and receptor density derived from saturation curves, is 0.3 nM^{-1} min^{-1}. The dissociation rate of [^{3}H]-haloperidol binding was evaluated by measuring the amount of [^{3}H]haloperidol bound at various time intervals after adding 100 μM dopamine or 0.1 μM (+)-butaclamol to incubation media in which binding had attained equilibrium (Fig. 4). Employing either (+)-butaclamol or dopamine to prevent rebinding of dissociated [^{3}H]haloperidol, we observed the same rate of dissociation. Specific [^{3}H]haloperidol binding dissociates according to simple first-order kinetics, with a half-life at 37° of 40 sec, corresponding to a rate constant for dissociation of 1 min^{-1}. The equilibrium dissociation constant for [^{3}H]-haloperidol binding can be calculated from

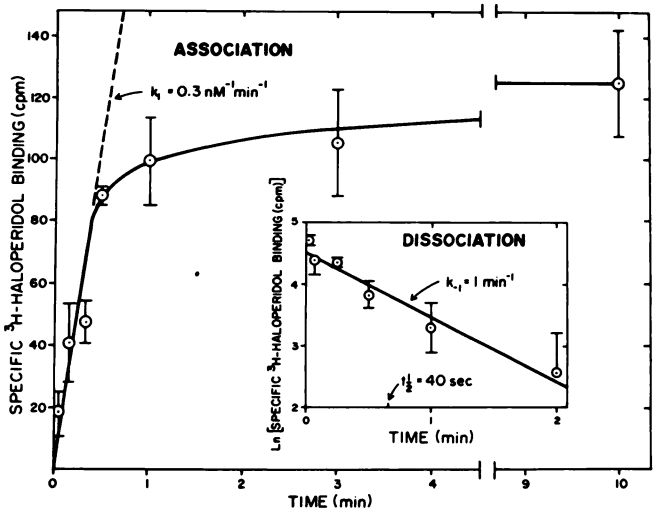

FIG. 4. *Association and dissociation of specific [^{3}H]haloperidol binding*

For the measurement of association, membranes of calf caudate were incubated at 37° with 2 nM [^{3}H]haloperidol for increasing periods before filtration. Binding in four experiments was measured as described in Fig. 3. The rate constant for association (k_1) was determined from the initial rate (0.1 nM/min) by dividing by the known concentration of [^{3}H]haloperidol (2 nM) and of receptors (0.2 nM, determined from the Scatchard plot of Fig. 3). To determine dissociation, specifically bound [^{3}H]haloperidol was measured at increasing periods after addition of 0.1 μM (+)-butaclamol or 100 μM dopamine to tubes already incubated for 10 min at 37° as in the association experiments. The results of four experiments are plotted semilogarithmically; the slope gives the rate constant for dissociation (k_{-1}).

the ratio of the rate constant for dissociation to the rate constant for association. This value is about 3 nM, which is the same as the K_D value calculated from saturation experiments.

Saturability and kinetics of [³H]dopamine binding. Previously we observed that [³H]dopamine binding to calf striatum measured by a centrifugation assay achieves half-maximal saturation at about 7 nM (3). In the present study, using a filtration assay, [³H]dopamine binding exhibits half-maximal saturation at a concentration of 10–15 nM. Scatchard analysis of the saturation curves yields a density of [³H]dopamine binding sites in the range of 20–30 pmoles/g of caudate nucleus tissue. Nonradioactive dopamine reduces binding of 5 nM [³H]dopamine half-maximally at about 22 nM (Fig. 5). Scatchard analysis of the competition curve indicates that the K_D of binding is about 17 nM while the density of binding sites is about 31 pmoles/g of caudate nucleus tissue, wet weight.

Haloperidol is much less potent in competing for [³H]dopamine than for [³H]haloperidol binding sites, with half-maximal inhibition at about 1 μM. In repeated experiments the slope for inhibition of [³H]dopamine binding by dopamine is steeper than for inhibition by haloperidol. The Hill coefficient for dopamine inhibition is 1.07, while for haloperidol inhibition of [³H]dopamine binding the Hill coefficient is 0.51 (Fig. 2B). This pattern is the opposite of that observed for [³H]-haloperidol binding, in which displacement by antagonists exhibited a steeper slope than displacement by agonists. Also, as reported previously (1), the agonist dopamine has much higher affinity for [³H]dopamine than for [³H]haloperidol binding sites, while the opposite is true for haloperidol and other antagonists.

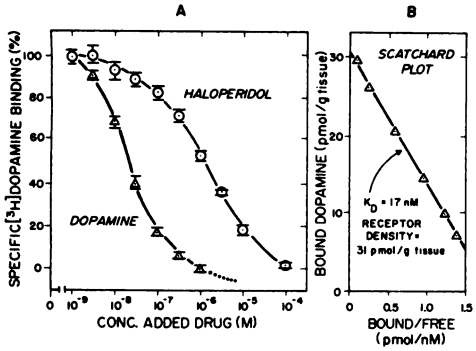

FIG. 5. *Competition by nonradioactive dopamine and haloperidol for [³H]dopamine binding*

A. Increasing concentrations of drug were added to tubes containing 5 nM [³H]dopamine and calf caudate membranes. Results are the means and standard errors of 5–14 experiments. The dotted continuation of the dopamine curve signifies that concentrations of dopamine greater than 1 μM do compete for additional [³H]dopamine binding. However, this low-affinity competition is also present in boiled tissue and on filters alone and clearly does not represent specific binding.

B. The Scatchard plot was drawn from the solid portion of the dopamine competition curve.

[³H]Dopamine binding associates more slowly than [³H]haloperidol binding. For [³H]dopamine, binding attains equilibrium after about 5–10 min, with half-maximal binding at about 1 min (Fig. 6). The rate constant for association (k_1), calculated from the initial rate of [³H]dopamine association, the concentration of [³H]-dopamine, and the receptor density determined in equilibrium saturation experiments, provides a value of 0.02–0.03 nM^{-1} min^{-1}. Unlike specific [³H]dopamine binding, "nonspecific" binding of [³H]-dopamine in the presence of 10 μM (+)-butaclamol changes very little, with most binding completed at the earliest time point measured.

The dissociation of [³H]dopamine binding was evaluated by conducting incubations to equilibrium, whereupon 100 μM nonradioactive dopamine was added to prevent rebinding of [³H]dopamine and samples were filtered at various time points. [³H]Dopamine binding dissociates with simple first-order kinetics, with a half-life at 37° of 1.3 min, corresponding to a rate constant for dissociation (k_{-1}) of 0.5 min^{-1} (Fig. 6). The K_D value for [³H]dopamine binding, calculated from the ratio of the rate constant for dissociation to the rate constant for association, is about 20 nM, similar to the value determined by equilibrium saturation experiments.

Regional variations in [³H]haloperidol and [³H]dopamine binding. In preliminary studies we observed that specific [³H]haloperidol and [³H]dopamine binding is most enriched in areas of the brain that

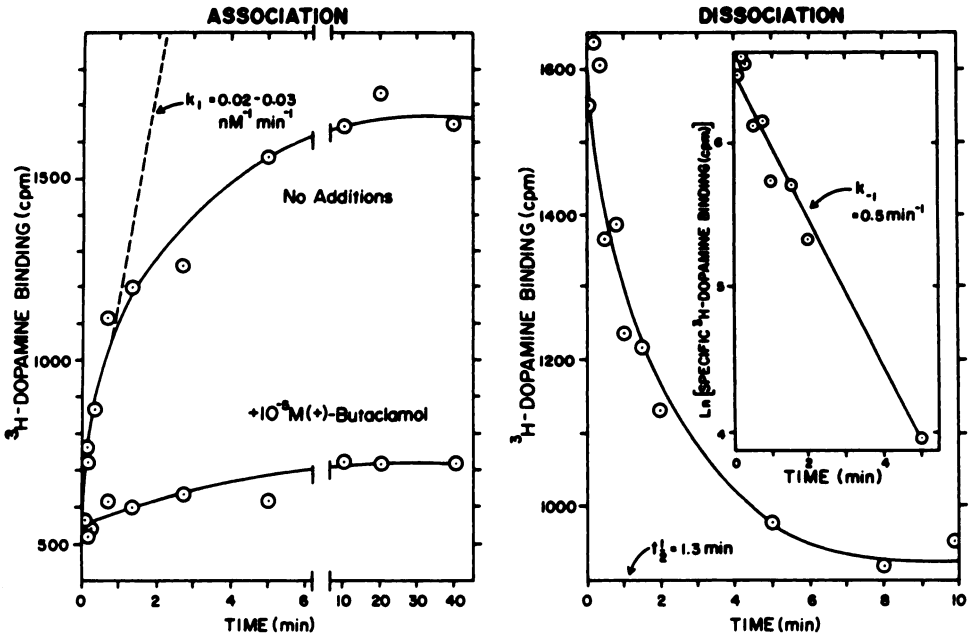

FIG. 6. *Association and dissociation of [^{3}H]dopamine binding*

In association experiments, membranes of calf caudate were incubated with 5 nM [^{3}H]dopamine in the presence and absence of 10 μM (+)-butaclamol for increasing periods before filtration. Results are those of a single experiment, which was replicated once. The rate constant for association (k_1) was determined from the initial rate (0.05 nM/min) by dividing by the known concentration of [^{3}H]dopamine (5 nM) and of receptors (0.3 nM, determined from the Scatchard plot of Fig. 5). In dissociation experiments, [^{3}H]dopamine binding was measured at increasing periods after addition of 100 μM dopamine to tubes already incubated for 10 min at 37° as in the association experiments. Results are those of a single experiment, replicated once.

contain the densest dopamine innervation (1). There appeared to be some differences in the relative amounts of [^{3}H]haloperidol and [^{3}H]dopamine binding in various brain regions, suggesting the possibility that the proportion of "agonist" and "antagonist" states of the dopamine receptor might differ in various brain regions. In the present study we have evaluated regional variations of [^{3}H]haloperidol and [^{3}H]dopamine binding in greater detail (Table 1). As observed before, highest binding occurs in the caudal caudate nucleus for both [^{3}H]haloperidol and [^{3}H]-dopamine, with binding in the rostral caudate being 80–90% of values in the caudal caudate. For both tritiated ligands, binding in the globus pallidus is about 60% of that of the caudal caudate, while binding in the anterior putamen is about 50–55% of the caudal caudate. Similar levels of [^{3}H]haloperidol and [^{3}H]dopamine binding are detected in the olfactory tubercle and nucleus accumbens, being about 40–45% of caudal caudate values. In all other areas examined, binding is not significantly different from zero. Unlike our previous preliminary observations, the present study has revealed no consistent specific binding of [^{3}H]haloperidol in the amygdala and midbrain. Moreover, the relatively greater amount of [^{3}H]haloperidol than [^{3}H]dopamine binding previously observed in the olfactory tubercle and nucleus accumbens has not been confirmed in the present, more extensive study. No specific binding of [^{3}H]haloperidol or [^{3}H]-dopamine can be detected in the spleen, spinal cord, heart, lung, liver, or testis.

The antischizophrenic efficacy and the tendency of neuroleptics to elicit extrapyramidal parkinsonism-like side effects are both thought to be caused by dopamine receptor blockade. The observation that, with comparable antischizophrenic doses, neuroleptics vary in their tendency to pro-

TABLE 1

Regional distribution of [³H]haloperidol and [³H]dopamine binding in calf brain

Calf brain regions, frozen for up to 1 week, were assayed with 2 nM [³H]haloperidol or 5 nM [³H]dopamine. Results are means ± standard errors for three experiments (except hindbrain). Note that data represent the binding actually observed and not the total number of binding sites in each region. Nonspecific binding in all regions was approximately the same and was equivalent to about 300–350 fmoles/mg of protein for [³H]haloperidol binding and about 200–250 fmoles/mg of protein for [³H]dopamine binding. Generally it was impossible to detect specific binding that amounted to less than 10% of these blank values.

Region	[³H]Haloperidol binding	[³H]Dopamine binding
	fmoles/mg protein	
Caudal caudate	244 ± 29	354 ± 27
Rostral caudate	186 ± 7	323 ± 35
Globus pallidus	148 ± 21	229 ± 73
Anterior putamen	118 ± 20	198 ± 67
Olfactory tubercle	111 ± 28	155 ± 27
	111 ± 18	130 ± 33
Areas in which specific binding was not significantly different from zero:		
Amygdala area	41 ± 29	6 ± 5
Thalamus	27 ± 33	21 ± 17
Hypothalamus	40 ± 10	8 ± 5
Frontal cortex	0 ± 8	−4 ± 7
Occipital cortex	17 ± 15	−2 ± 13
Hippocampus	12 ± 13	2 ± 2
Cerebellum	−3 ± 9	3 ± 2
Midbrain	3 ± 24	3 ± 3
Hindbrain[a]	51 ± 12	3 ± 5

[a] Two experiments.

duce extrapyramidal side effects has suggested that dopamine receptors in areas of the brain such as the caudate, which presumably mediate the extrapyramidal side effects, may differ in their sensitivity to neuroleptics from dopamine receptors in other parts of the brain. Accordingly we have compared the potencies of several neuroleptics with widely varying tendencies to elicit extrapyramidal side effects for competition with [³H]haloperidol and [³H]dopamine in membranes prepared from caudate nucleus, olfactory tubercle, or nucleus accumbens (Table 2). The relative potencies of the agonists dopamine and apomorphine, as well as the neuroleptic antagonists fluphenazine, clozapine, haloperidol, pimozide, molindone, and thioridazine, are the same in all three brain regions. This suggests that discrepancies between antischizophrenic and extrapyramidal actions of drugs are not due to variations in drug sensitivity of the dopamine receptors in these regions, although presumed dopamine receptors in the cerebral cortex, not detectable in our studies, may differ. An alternative explanation for the varying pharmacological effects of these drugs relates to their relative anticholinergic effects (17, 18).

Effects of drugs on [³H]haloperidol and [³H]dopamine binding. In preliminary studies we observed that agonists and antagonists of dopamine receptors have substantial affinity for [³H]haloperidol and [³H]dopamine binding sites (1–6). To ensure that these ligands selectively label dopamine receptors, it would be important to show that drugs unrelated to the dopamine system lack affinity for these sites. Accordingly we have evaluated a wide range of agents of varying structure not generally thought to act via direct interactions with dopamine receptors. None of these have substantial affinity for specific [³H]haloperidol or [³H]dopamine binding sites (Table 3E). Phenoxybenzamine, imipramine, and benzotropine, which are not neuroleptics, displace [³H]haloperidol binding with affinity similar to the weak neuroleptic clozapine. Clozapine displacement of [³H]haloperidol binding at 1 μM is not additive with maximal displacement by 0.1 mM dopamine or 0.1 μM (+)-butaclamol. However, there is partial additivity between displacement by 1 μM phenoxybenzamine, imipramine, and benzotropine and maximal displacement by dopamine and (+)-butaclamol, suggesting that these three non-neuroleptics compete for haloperidol sites other than dopamine receptors.

In preliminary studies using a limited number of agonists and antagonists, it appeared that dopamine receptor agonists have much higher affinity for [³H]-dopamine than [³H]haloperidol binding

TABLE 2

Regional variations in drug competition for [³H]haloperidol and [³H]dopamine binding

Fresh or frozen calf brain regions were assayed with three or more concentrations of drug in duplicate or triplicate. The concentrations of drugs required to inhibit specific binding by 50% (IC_{50}) were determined from log probit plots and converted to K_i values according to the equation $K_i = IC_{50}/(1 + c/K_D)$, where c is the concentration of radioactive ligand (1 to 2 nM for [³H]haloperidol and 5 nM for [³H]dopamine) and K_D is its dissociation constant (taken as 2 nM for [³H]haloperidol and 20 nM for [³H]dopamine). Results are means ± standard errors for the number of experiments indicated in parentheses. K_i values for individual drugs were the same whether 1 or 2 nM [³H]haloperidol was employed.

Drug	K_i for [³H]haloperidol binding		
	Striatum	Olfactory tubercle	Nucleus accumbens
	nM	nM	nM
Dopamine	550 ± 100 (6)	700 ± 40 (2)	475 ± 75 (2)
Apomorphine	43 ± 8 (6)	44 ± 10 (2)	40 ± 13 (2)
Haloperidol	1.4 ± 0.1 (5)	0.8 ± 0.3 (3)	0.8 ± 0.1 (2)
Pimozide	0.6 ± 0.2 (2)	0.6 ± 0.04 (2)	0.6 ± 0.1 (2)
Fluphenazine	0.6 ± 0.1 (6)	0.3 ± 0.01 (2)	0.8 ± 0.2 (2)
Thioridazine	14 ± 1.3 (5)	14 ± 2 (3)	16 (1)
Clozapine	100 ± 6 (6)	64 ± 24 (2)	80 ± 0 (2)
Molindone	65 ± 20 (3)	85 ± 15 (2)	90 (1)

Drug	K_i for [³H]dopamine binding	
	Striatum	Olfactory tubercle
	nM	nM
Dopamine	16.8 ± 1.8 (5)	11.7 ± 3.0 (4)
Apomorphine	8.0 ± 0.3 (4)	7.2 ± 1.8 (4)
Haloperidol	1,060 ± 140 (5)	1,100 ± 215 (4)
Pimozide	7,600 ± 1,200 (2)	6,800 ± 1,200 (2)
Fluphenazine	242 ± 46 (5)	340 ± 75 (4)
Thioridazine	1,780 ± 330 (4)	1,810 ± 210 (3)
Clozapine	1,890 ± 340 (5)	2,800 ± 950 (4)
Molindone	22,900 ± 4,600 (4)	18,900 ± 4,000 (3)

sites while the reverse is true for the neuroleptic antagonists (1). This trend is confirmed in the present study with a much more extensive series of drugs. Moreover, in the present study we observe a close correlation between the potencies of drugs in clinical and pharmacological studies *in vivo* and their effects on [³H]haloperidol binding. For instance, spiroperidol *in vivo* is the most potent of the butyrophenones evaluated in this study. Spiroperidol is 5–10 times more potent than haloperidol in most pharmacological and clinical studies (19) and displays 6 times the affinity of haloperidol for [³H]haloperidol binding sites. Benperidol, which in clinical and pharmacological studies has about the same potency as spiroperidol, also has about the same affinity as spiroperidol for [³H]haloperidol binding sites. On the other hand, azaperone and pipamperone, which pharmacologically and clinically are weak butyrophenone neuroleptics, are correspondingly weak in competing for [³H]haloperidol sites. Among the phenothiazines there is also a close correlation between clinical and pharmacological potency and affinity for [³H]haloperidol binding. Fluphenazine is about 10 times as potent as chlorpromazine in most pharmacological screens (19), and has about 12 times the potency of chlorpromazine for [³H]haloperidol binding. Promazine and promethazine, which are weak and ineffective neuroleptics, respectively, are among the least potent agents in competing for [³H]haloperidol binding. Potencies of phenothiazines as inhibitors of [³H]dopamine binding show some correlation with pharmacological and clinical activities. However, the butyrophenones, which are extremely potent

TABLE 3

Inhibition of [³H]haloperidol and [³H]dopamine binding by drugs

Each drug was tested at three or more concentrations in duplicate or triplicate for the indicated number of experiments. K_i values were derived as described in Table 2.

Drugs	K_i							
	[³H]Haloperidol				[³H]Dopamine			
	nM				nM			
A. Neuroleptics and related drugs								
Spiroperidol (spiperone)	0.25	±	0.02	(4)	1,400	±	190	(3)
Benperidol	0.33	±	0.02	(4)	4,100	±	540	(4)
Clofluperol	0.50	±	0.03	(4)	360	±	20	(3)
(+)-Butaclamol	0.54	±	0.08	(8)	80	±	11	(12)
(−)-Butaclamol	700	±	120	(4)	>10,000			(3)
Fluspirilene	0.60	±	0.13	(4)	1,400	±	220	(4)
Pimozide	0.81	±	0.09	(4)	5,300	±	1,100	(6)
Fluphenazine	0.88	±	0.12	(12)	230	±	30	(12)
Piflutixol	0.90	±	0.10	(3)	67	±	10	(3)
Trifluperidol	0.95	±	0.19	(3)	740	±	20	(3)
α-Flupenthixol	0.98	±	0.11	(6)	180	±	30	(8)
β-Flupenthixol	48	±	15	(6)	8,000	±	900	(3)
Droperidol	1.0	±	0.10	(4)	880	±	80	(3)
Bromoperidol	1.4	±	0.15	(4)	600	±	90	(3)
Haloperidol	1.4	±	0.10	(18)	920	±	90	(16)
Methiothepin	1.4	±	0.09	(4)	210	±	30	(7)
cis-Thiothixene	1.5	±	0.10	(6)	540	±	140	(6)
trans-Thiothixene	145	±	41	(3)	15,000	±	2,100	(3)
Moperone	1.9	±	0.26	(4)	1,200	±	160	(4)
Trifluprομazine	2.1	±	0.12	(4)	530	±	80	(5)
Trifluoperazine	2.1	±	0.34	(4)	740	±	80	(5)
Teflutixol	2.6	±	0.28	(3)	1,900	±	180	(3)
cis-Clopenthixol	3.1	±	0.24	(3)	480	±	78	(4)
trans-Clopenthixol	88	±	21	(4)	13,000	±	1,600	(4)
Fluanisone	3.8	±	0.8	(4)	800	±	180	(4)
Chlorprothixene (*cis*)	4.4	±	0.6	(4)	250	±	17	(3)
Penfluridol	5.6	±	1.4	(4)	1,600	±	310	(4)
Azaperone	10.0	±	0.6	(4)	1,700	±	290	(4)
Chlorpromazine	10.2	±	1.6	(5)	900	±	200	(7)
Thioridazine	15	±	1.5	(9)	1,800	±	180	(8)
Pipamperone	31	±	5	(4)	4,900	±	500	(4)
Molindone	68	±	8	(7)	19,000	±	3,000	(8)
Promazine	72	±	3	(4)	7,100	±	1,600	(8)
Clozapine	120	±	11	(10)	2,050	±	260	(10)
Promethazine	240	±	30	(4)	12,000	±	3,600	(7)
Bulbocapnine	930	±	290	(6)	800	±	100	(5)
B. Dopamine agonists								
Apomorphine	51	±	8	(7)	8.6	±	0.5	(11)
ADTN	120	±	16	(4)	11	±	2	(3)
Epinine	530	±	80	(4)	23	±	2	(4)
Dopamine	670	±	80	(17)	17.5	±	0.9	(19)
(−)-Epinephrine	2,600	±	660	(4)	280	±	3	(3)
(+)-Epinephrine	37,000	±	6,800	(4)	1,200	±	30	(3)
(−)-Norepinephrine	5,600	±	530	(8)	200	±	19	(5)
(+)-Norepinephrine	21,000	±	1,300	(5)	820	±	140	(3)
C. LSD and related agents								
2-Bromo-LSD	4.0	±	0.5	(2)	54	±	7	(6)
d-LSD	20	±	1	(4)	30	±	3	(9)
l-LSD	20,000	±	10,000	(2)	56,000	±	14,000	(4)
d-Lysergic acid amide	390	±	90	(3)	180	±	23	(3)
d-Isolysergic acid amide	430	±	80	(2)	190	±	23	(3)

continued

TABLE 3—*Continued*

Drugs	K_i							
	[³H]Haloperidol				[³H]Dopamine			
Ergocornine	0.7	±	0.3	(2)	36	±	4	(5)
α-Ergocryptine	1.3	±	0.2	(2)	69	±	7	(6)
Ergocristine	1.4	±	0.2	(2)	52	±	8	(5)
Ergotamine	1.8	±	0.4	(3)	30	±	7	(3)
2-Bromo-α-ergocryptine	2.5	±	1.0	(3)	106	±	12	(6)
Ergometrine	160	±	16	(3)	61	±	15	(6)
Methysergide	45	±	10	(2)	290	±	20	(5)
Cyproheptadine	65	±	8	(3)	1,200	±	160	(7)
Mianserin	620	±	130	(2)	2,500	±	400	(3)
D. Miscellaneous								
Phenoxybenzamine	140	±	60	(2)	90,000	±	22,000	(2)
Impiramine	180	±	14	(3)	22,000	±	2,000	(2)
Benztropine	230	±	60	(4)	30,000	±	8,000	(3)
Phentolamine	2,000	±	0	(2)	25,000	±	3,000	(2)
Methadone	2,700	±	700	(3)	>100,000			(1)
Propranolol	2,800	±	600	(3)	>100,000			(1)
Pentazocine	3,500	±	1,000	(2)	>100,000			(1)

E. Inactive drugs (IC_{50} = 10,000 nM or greater for both [³H]haloperidol and [³H]dopamine binding): Acetophenetidine, *l*- and *d*-amphetamine, atropine, benactyzine, (+)- and (−)-bicuculline, bufotenine, carbachol, chlordiazepoxide, chlormezanone, dexchlorpheniramine, diazepam, dibenamine, *N,N*-dimethyltryptamine, diphenylhydantoin, *R*(−)- and *S*(+)-2,5-dimethoxy-4-ethylamphetamine, (+)-2,5-dimethoxy-4-methylamphetamine, eserine, ethosuximide, fenfluramine, γ-aminobutyric acid, glutamic acid, glycine, harmaline, homovanillic acid, 6-hydroxydopamine, isoproterenol, lidocaine, mescaline, meprobamate, 5-methoxytryptamine, α-methyldopa, α-methyl-*p*-tyrosine, morphine, naloxone, octopamine, oxotremorine, probenecid, psilocin, psilocybin, pyridoxine, reserpine, methylphenidate, serotonin, strychnine, Δ^9-tetrahydrocannabinol, tranylcypromine, tryptamine, tyramine.

on [³H]haloperidol binding and as neuroleptics, are relatively weak in competing for [³H]dopamine binding, and their relative potencies at [³H]dopamine sites do not correlate well with their pharmacological and clinical activities. Of the dopamine agonists (Table 3B), apomorphine is most potent at both [³H]haloperidol and [³H]dopamine sites. ADTN, whose potency in enhancing the dopamine-sensitive adenylate cyclase resembles that of apomorphine (14), is the second most active inhibitor of [³H]haloperidol and [³H]dopamine binding. The presence of a β-hydroxyl group reduces binding potency, as is evident from the weaker activity of norepinephrine and epinephrine compared with dopamine and epinine, respectively. Stereoselectivity at the β-carbon is manifested by the 4–14-fold greater potencies of (−)-norepinephrine and (−)-epinephrine than of their (+) isomers. These data coincide with our previous results (1–7, 10) and are in reasonable agreement with those of Seeman *et al.* (8, 9). Differences such as the lower K_D for [³H]dopamine reported by Seeman *et al.* (8, 9) may relate to species variations, tissue preparation, or assay technique.

In previous studies we demonstrated that *d*-LSD has a relatively high affinity for both [³H]haloperidol and [³H]dopamine binding (4). We now report that many ergot derivatives also have substantial affinity for both [³H]haloperidol and [³H]dopamine binding sites (Table 3C). This suggests that these drugs may exert some of their behavioral effects through direct interactions with dopaminergic receptors. Interestingly, ergot derivatives block dopamine influences in invertebrates (20) and produce dopamine-like actions in prolactin release (21) and behavior (22–27) in mammals.

DISCUSSION

The major finding of the present study is that [³H]haloperidol and [³H]dopamine label the same or related sites in the brain with characteristics expected of postsyn-

aptic dopamine receptors. The major portion of haloperidol and dopamine binding does not appear to involve presynaptic dopamine neurons, as lesions of the nigroneostriatal dopamine pathway fail to reduce [^{3}H]haloperidol or [^{3}H]dopamine binding (1, 3, 6). The relative activities of drugs in competing for these binding sites resemble their effects on postsynaptic dopamine receptors and not their influence on dopamine uptake sites (1, 3). The finding that regional variations in [^{3}H]haloperidol and [^{3}H]dopamine binding are essentially the same and correlate with the relative distribution of endogenous dopamine also suggests that the binding of these ligands is associated with postsynaptic dopamine receptors.

These binding results differ in several respects from data obtained with the dopamine-sensitive adenylate cyclase (13, 14). Pharmacological and clinical potencies of butyrophenones correlate closely with affinity for [^{3}H]haloperidol binding sites, but much less well with effects on the cyclase (10). Butyrophenones represent the clinically most potent clase of neuroleptics, as reflected in their high affinity for [^{3}H]haloperidol binding sites. However, butyrophenones are relatively weak inhibitors of the dopamine-sensitive adenylate cyclase and of [^{3}H]dopamine binding. Affinities of agonists for [^{3}H]dopamine binding sites are considerably greater than for the dopamine-sensitive adenylate cyclase. These discrepancies indicate that the recognition or binding site of the dopamine receptor is not identical with the dopamine-sensitive adenylate cyclase.

Although for both [^{3}H]haloperidol and [^{3}H]dopamine binding the relative activities of various drugs resemble their relative potencies at dopamine receptors, there are differences between drug effects on binding of the two tritiated ligands. Dopamine antagonists have 20–12,000 times greater affinity for [^{3}H]haloperidol than for [^{3}H]dopamine binding sites. On the other hand, dopamine agonists have 6–38 times more affinity for [^{3}H]dopamine than for [^{3}H]haloperidol binding sites. The concentrations of haloperidol and dopamine binding sites in the brain may be different, with up to twice as many dopamine as haloperidol binding sites. The differences in substrate specificity suggest that dopamine and haloperidol label different portions or states of the dopamine receptor (1). There might be distinct dopamine receptor sites binding agonists and antagonists. Alternatively, one dopamine receptor might interconvert between conformations which have high affinities for agonists or for antagonists. The reduced slope of "mixed" competition curves, corresponding to Hill coefficients less than 1 (Fig. 2), are consistent with both interpretations. If the dopamine receptor can exist in interconvertible "agonist" and "antagonist" states, one might explain the pharmacological properties of agonists and antagonists in terms of their relative affinities for the two states of the receptor (28). According to this model, relatively pure agonists should have much greater affinity for [^{3}H]dopamine than for [^{3}H]haloperidol binding sites, while the reverse situation should hold true for pure antagonists. Mixed agonist-antagonists should have similar affinities for both [^{3}H]haloperidol and [^{3}H]dopamine binding sites. LSD behaves as a mixed agonist-antagonist in stimulating the dopamine-sensitive adenylate cyclase as well as in blocking the effects of dopamine on the cyclase (29–31). Apomorphine also behaves as a mixed agonist-antagonist on the dopamine-sensitive adenylate cyclase (14) and the dog renal artery dopamine receptor (32). As predicted by this model, LSD and apomorphine have similar affinities for both [^{3}H]haloperidol and [^{3}H]dopamine binding (4) (Table 3C). Evidence for a two-state model of neurotransmitter receptor functioning has also been obtained in studies in this laboratory of the opiate receptor (33), the glycine receptor (34), the serotonin receptor (35), and the muscarinic cholinergic receptor (36).

ACKNOWLEDGMENT

We thank Janet Ryan for excellent technical assistance.

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