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Interactions of Dopamine Agonists with Brain D_1 Receptors Labeled by ^{3}H-Antagonists

Evidence for the Presence of High and Low Affinity Agonist-Binding States

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SUMMARY

The interactions of dopaminergic agonists and antagonists with ^{3}H-antagonist labeled D_1 dopamine receptors of rat striatum have been characterized. [^{3}H]Flupentixol has been found to selectively label D_1 dopamine receptors when its binding to D_2 dopamine receptors is blocked by the inclusion of D_2 selective concentrations of unlabeled spiroperidol or domperidone. Antagonist/^{3}H-antagonist competition curves are of uniformly steep slope ($n_H = 1.0$) suggesting the presence of a single D_1 dopamine receptor. Agonist/^{3}H-antagonist competition curves are extremely shallow ($n_H \leq 0.5$) for agonists of high relative efficacy, suggesting the presence of heterogeneous populations of agonist-binding states of the D_1 dopamine receptor. Computer-modeling techniques were used to estimate affinities and relative site densities for these heterogeneous binding states. This analysis indicates that the ratio of agonist affinities for low and high affinity agonist-binding states is correlated with agonist relative efficacies in activating adenylate cyclase in membrane homogenates. Under the assay conditions employed, the addition of saturating concentrations of guanine nucleotides reduced, but did not abolish, the relative density of high affinity agonist-binding sites. These binding data can, at least in part, be explained by postulating two states of the D_1 dopamine receptor, inducible by agonists but not by antagonists and modulated by guanine nucleotides.

INTRODUCTION

Pharmacological and biochemical evidence indicates that dopaminergic receptors can be divided into two major categories: D_1 and D_2 (1, 2). D_1 dopamine receptors mediate dopamine agonist-induced stimulation of adenylate cyclase activity, whereas agonist occupation of D_2 dopamine receptors has been observed to attenuate this enzymatic activity (1, 2). Extensive characterization of both agonist and antagonist interaction with mammalian D_2 dopamine receptors has been carried out using radioligand-binding techniques (for review, see Ref. 2), coupled more recently with computer-assisted data analyses (3–8). However, except for a preliminary study of some novel dopaminergic agonists (4), no such detailed analyses have been reported describing the interaction of dopaminergic agonists with the D_1 dopamine receptor labeled with ^{3}H-antagonist ligands.

Earlier studies indicate that D_1 dopamine receptors in calf and rat striatum can be labeled by the thioxanthene antagonists [^{3}H]flupentixol and [^{3}H]piflutixol (9–11). The regional distributions in brain of dopamine-stimulated adenylate cyclase activity and specific, high affinity [^{3}H]flupentixol binding are similar, and dopaminergic antagonists block both activities with a highly correlated rank-order of potency (9, 10, 12). However, this laboratory (4) and others (13, 14) have recently found that [^{3}H]flupentixol labels D_2 as well as D_1 dopamine receptors in striatum. As is described in this study, [^{3}H]flupentixol can be used to label selectively D_1 receptors by including in the assays concentrations of unlabeled spiroperidol and domperidone which block [^{3}H]flupentixol binding to D_2 but not to D_1 dopamine receptors.

Most dopamine agonists stimulate adenylate cyclase activity through the D_1 dopamine receptor *in vitro* with micromolar affinity, and ergot alkaloids have been described as partial agonists or antagonists at this receptor where they exhibit submicromolar to micromolar potencies (2). Early radioligand-binding studies have reported

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that the affinities of dopaminergic agonists to inhibit [^{3}H]flupentixol binding are roughly micromolar (12). However, as is described below for the D_2 dopamine receptor, detailed studies of agonist binding to ^{3}H-antagonist-binding sites for some catecholamine receptors have revealed a heterogeneity of agonist-binding states.

Recently, this laboratory has extensively characterized the ligand-binding properties of pituitary (3, 15) and brain (6) D_2 dopamine receptors. The binding of antagonists to D_2 receptors exhibited mass action characteristics while agonist binding displayed properties indicative of two separate classes of agonist-binding sites. This heterogeneity was demonstrated by shallow two-component curves representing agonist competition for radiolabeled antagonist binding. Thus, in the striatum and anterior pituitary, agonists were observed to bind to high and low affinity sites that were not discriminated by antagonists (Refs. 3 and 6; however, see Refs. 5 and 7). In the presence of high concentrations of guanine nucleotides, the high affinity agonist-receptor complex was decreased or abolished, suggesting that guanine nucleotides promote a conversion from high affinity to low affinity agonist-binding states of the receptor. Similar phenomena have been observed for a large number of neurotransmitter and hormone receptors; and in the cases of β- (16, 17) and α_2 (18)-adrenergic and D_2 (3, 5) dopaminergic receptors, a generalized ternary complex model has been proposed to explain these phenomena.

The observation of these properties of agonist binding to some catecholaminergic receptors has recently been extended to include D_1 dopamine receptors of rat striatum (4). In this study, the putatively D_1 receptor-selective agonist SKF 38393 and its derivative SKF 82526 were found to compete for [^{3}H]flupentixol binding biphasically. These curves became monophasic exhibiting only low affinity agonist binding in the presence of guanine nucleotides. Thus, a ternary complex model was suggested for the D_1 dopamine receptor as well. The present study investigates this possibility in detail and provides the first demonstration of heterogeneous agonist-binding states of the D_1 dopamine receptor using a variety of agonists having varying intrinsic activities. The accompanying article (19) describes agonist-binding properties using ^{3}H-labeled agonists and draws correlations between direct agonist labeling and agonist interactions with ^{3}H-antagonist labeling of the D_1 dopamine receptor of rat striatum.

MATERIALS AND METHODS

Preparation of rat striatal membranes. Tissue preparations were similar to those described previously by Leff and Creese (20). Freshly dissected striata from decapitated Sprague-Dawley rats (200–350 g) were weighed and homogenized (Ultra-Turrax, setting 7, 15 sec) in 100 volumes (w/v) of ice-cold Tris-HCl, pH 7.7 (25°). The homogenate was centrifuged at 48,000 × *g* × 10 min (0–4°), and the supernatant was discarded. The pellet was resuspended in 50 volumes (original wet w/v) of ice-cold 50 mM Tris-HCl (pH 7.7 at 25°) containing 2 mM $MgSO_4$, incubated at 37° for 15 min, chilled by the addition of 1–2 times this volume of ice-cold Tris-HCl without ions, and centrifuged as before. This pellet was washed one additional time in Tris-HCl buffer, again with no additional ions, before resuspension of the final pellet in assay buffer. The assay buffer was comprised of (final assay concentrations after the addition of other reagents to the assay) 20 mM 4-morpholinepropanesulfonic acid, 1 mM EDTA free acid, 0.1% ascorbic acid, 4 mM $MgSO_4$, 10 μM pargyline HCl, and 19 mM Tris base to yield a final assay pH of 7.2 at 22°. This tissue preparation, in particular the 15-min 37° preincubation in 2 mM $MgSO_4$, has been previously determined to potentiate high affinity binding of dopamine agonists to rat striatum membranes (20, 21). Ascorbic acid was included in the assay to retard degradation of catechol agonists (22, 23), and preliminary experiments as well as studies by others (24) indicated that ascorbic acid does not affect ^{3}H-antagonist binding in the buffer system used in these studies.

Radioligand-binding assays. Binding was initiated by the addition of the membrane suspension to duplicate or triplicate glass test tubes (12 × 75 mm) containing 250–400 μl of radioligand, unlabeled competitor, and nucleotides before transferring tubes to a 22° water bath. Final assay volume was 2 ml, and tissue concentration was 0.8–0.85 mg/ml (original wet w/v). Specific binding increases in linear proportion to increases in tissue up to this concentration. However, deviation from linearity was observed at higher tissue concentrations, presumably due to the depletion of added ligand to both specific and nonspecific binding sites. Incubations were terminated after 90 min by rapid vacuum filtration (20–22 mm Hg) over GF/C filters using a modified automated cell harvester (Brandel M-48R, Gaithersburg, MD). The filters were then rinsed rapidly with 18 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°). The entire filtration procedure required less than 20 sec, and separate experiments determined that this filtration procedure was optimal. Rinsing filters with a 0.1% solution of BSA[3] in 50 mM Tris-HCl (pH 7.7) before filtration reduced nonspecific binding to the filter without affecting specific binding of [^{3}H]flupentixol. Radioactivity trapped on the filter was counted by standard liquid scintillation spectroscopy at an efficiency of 50% (19). Nonspecific binding was determined in the presence of 1 μM (+)butaclamol or in some experiments 1 μM SCH 23390. At this concentration (−)butaclamol is without effect in displacing [^{3}H]flupentixol. [^{3}H]Flupentixol was used to selectively label D_1 dopamine receptors (see below) by including 10–30 nM spiroperidol (spiperone) or 30 nM domperidone in the assays to prevent [^{3}H]flupentixol from binding to D_2 dopamine receptor sites. When filters were prewetted with 0.1% BSA, [^{3}H]flupentixol-specific binding comprised 30–80% of the total binding in saturation experiments and 68% of total binding for typical competition experiments conducted at 0.4–0.65 nM. For initial experiments conducted using non-BSA-treated filters [^{3}H]flupentixol-specific binding was only 15–60% of total binding in saturation experiments and approximately 55% for competition experiments. At 0.5 nM, specific binding of [^{3}H]flupentixol (New England Nuclear Corp.) was approximately 1600 cpm/filter.

Data analysis. The computer analyses employed are a weighted, nonlinear least squares curve-fitting program, LIGAND (25), using a general model for complex ligand-receptor-binding systems according to mass action law. Analyses were conducted using interactive programs written in BASIC on a VAX11 computer at this university. The exact treatment of experimental data has previously been described in detail (3, 16). Briefly, competition curves were first analyzed according to a four-parameter logistic equation (26) and a pseudo-Hill coefficient (n_H) was derived. Curves were then analyzed according to models for the competition of radioligand and competitor to one, two, or three binding sites. Deviations of observed data points from their predicted values were weighted according to the reciprocal of their predicted variance (27). The statistical difference between models was tested by comparing the residual variances which resulted from the different fits of the data (28). A model for two binding sites was retained only when it fitted the data significantly better than a model for one site ($p < 0.05$; partial F test). The same criterion was applied to the comparison of fits to apparent three-site versus two-site models.

In some experiments, we tested whether respective computer-derived parameter estimates from different curves were significantly different

[3] The abbreviations used are: BSA, bovine serum albumin; NPA, *N*-propylnorapomorphine; Gpp(NH)p, guanosine 5′-(β-γ-imido)triphosphate; ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

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from each other. The curves were first analyzed simultaneously, but independently, allowing the parameter estimates for each curve to assume their optimal values. Subsequently, the curves were analyzed simultaneously, but the parameters under question were constrained to be estimated as a common value. The effect of this constraint on the goodness of fit was tested and the shared parameters were considered indistinguishable if constraining the values did not significantly worsen the simultaneous fit (i.e., if $p > 0.05$). Except as otherwise described in the text, competition curves for which inhibition at the highest competitor concentration was incomplete were modeled constraining the parameter which describes nonspecific binding of the radioligand (N_R) to values determined by the 1 μM (+)butaclamol (or in some cases 1 μM SCH 23390) "blank." This same procedure was applied to curves in which competitor displaced binding in excess of that displaced by 1 μM (+)butaclamol except that data points which dropped below nonspecific binding values were omitted from the analysis. Unless otherwise stated, results from multiple experiments are given as means and standard errors of the mean.

Materials. *cis*-[³H]Flupentixol (10 Ci/mmol) was initially obtained from Institut National des Radioelements (I. R. E., Fleurus, Belgium). Subsequently, *cis*-[³H]flupentixol (12.0 Ci/mmol) was obtained from New England Nuclear Corp. The following drugs were generous gifts from the respective pharmaceutical companies: apomorphine, Merck Co. (Rahway, NJ); butaclamol and propranolol, Ayerst Laboratories (Rouse's Point, NY); teflutixol, *cis* and *trans* isomers of flupentixol, piflutixol, chlorprothixene, and clopentixol, H. Lundbeck and Co. (Denmark); spiroperidol, domperidone, and ketanserin, Janssen Pharmaceutica (Beerse, Belgium); fluphenazine, E. R. Squibb and Sons (New York); chlorpromazine, SKF 83742, SKF 38393, and SKF 82526, Smith, Kline and French (Philadelphia, PA); promethazine, Wyeth Laboratories (Philadelphia, PA); prazosin, Pfizer (New York); sulpiride, Rauizza (Italy); pergolide and lergotrile, Lilly (Indianapolis, IN); lisuride, Schering (Berlin, F. D. R.); SCH 23390, Schering-Plough (Bloomfield, NJ); NPA, Sterling-Winthrop (Rensselaer, NY); ADTN, Burroughs-Wellcome (Research Triangle Park, NC); dopamine, epinine, and scopolamine, Sigma (St. Louis, MO); all other ergot alkaloids were generous gifts of Sandoz Pharmaceuticals (Hanover, NJ). Other reagents were from standard commerical sources.

RESULTS

Characteristics of [³H]flupentixol binding to membranes of rat striatum. In order to determine under our assay conditions and with computer analyses the correct concentration of masking D_2 selective antagonists to use, spiroperidol and domperidone competitions for [³H]flupentixol binding were conducted (Fig. 1). Computer analysis indicates the presence of two binding sites whereby the site having high affinity (0.2–3 nM) for these unlabeled D_2 selective antagonists comprises ~20% of all specific [³H]flupentixol binding. Experiments subsequently described were conducted in the presence of 10–30 nM spiroperidol or 30 nM domperidone to mask binding of [³H]flupentixol to D_2 receptors, thus limiting the specific binding of this ligand to D_1 dopamine receptor sites.

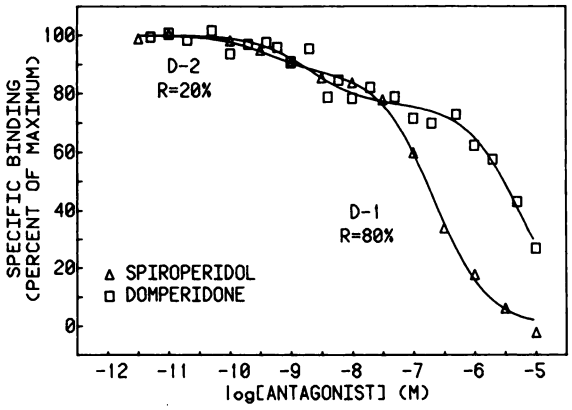

FIG. 1. *Spiroperidol and domperidone discriminate D_1 and D_2 components of [³H]flupentixol binding*

Inhibition of [³H]flupentixol binding. Computer-derived estimates of inhibition constants for spiroperidol and domperidone are 0.3 and 0.7 nM, respectively, for D_2 sites and 152 and 1460 nM for putative D_1 sites, respectively.

D_1 receptor-specific binding of [³H]flupentixol to rat striatal membranes was of high affinity and saturable with Scatchard analysis, indicating the presence of a homogeneous binding site over the ranges of concentrations used. Pooled data from a number of experiments yielded average dissociation constant (K_D) and a maximum binding capacity (B_{max}) of 0.41 ± 0.08 nM and 118.8 ± 8.2 fmol/mg of tissue ($n = 8$).

The kinetics of [³H]flupentixol binding are shown in Fig. 2. At 22° in the absence of sodium [³H]flupentixol, binding reached equilibrium within 90 min (Fig. 2A). The association reaction data were replotted in the inset and a pseudo-first order rate constant, k_{obs}, was determined from the slope of the line. The mean k_{obs} from four independent experiments was 0.068 ± 0.011 min^{-1}. The first order dissociation rate constant (k_{-1}) was determined by allowing [³H]flupentixol to reach equilibrium and then monitoring the dissociation of labeled ligand after the addition of an excess of cold competitor. As shown in Fig. 2B, [³H]flupentixol binding was readily dissociable in the presence of excess (+)butaclamol (10 μM). However, a small percentage of apparent "specific binding" remained undissociated at the longest time point taken (3 hr). This residual "specific binding" was considered undissociable and artifactual when semilogarithmic plots were made (Fig. 2B, inset). Identical data were observed when 1 mM dopamine was used as the dissociation agent. After making a correction for this residual undissociable binding, the dissociation rate constant k_{-1} was determined from the slope of the line and an average value ($n = 5$) of 0.028 ± 0.002 min^{-1} was found. From these pseudo-first order association and dissociation rate data, the association rate constant k_1 was estimated as 0.040 nM^{-1} min^{-1} using the equation:

$$k_{obs} - k_{-1} = k_1 \quad (1)$$

The ratio of the rate constants (k_{-1}/k_1) provides an estimate of the equilibrium dissociation constant (K_D) for [³H]flupentixol binding. Using the averaged data cited above, the kinetically determined K_D is 0.70 nM.

Antagonist competition for radiolabeled antagonist binding. The ability of dopaminergic antagonists to compete for [³H]flupentixol binding to rat striatal membranes was investigated (Fig. 3; Table 1). Representative competition experiments between some typical dopaminergic antagonists and [³H]flupentixol are shown in Fig. 3A. The computer-modeled curves for the antagonists fluphenazine and chlorpromazine exhibit pseudo-Hill slope factors close to 1, and curves model best to a single homogeneous binding site (Fig. 3A).

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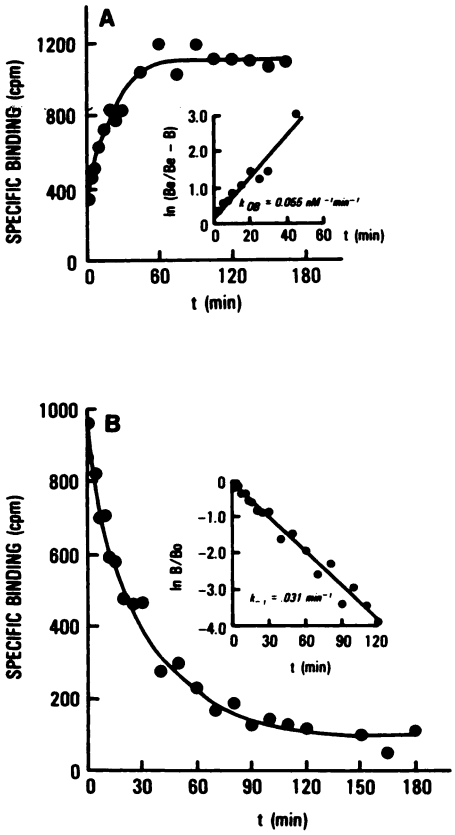

FIG. 2. *Association and dissociation of [³H]flupentixol to D_1 sites on membranes of rat striatum*

A, time course of association. [³H]Flupentixol (0.37 nM) was added to tissue equilibrated at 22°. Specific binding of [³H]flupentixol in the presence of 10 nM spiroperidol was then sampled at the indicated time points. The inset is a pseudo-first order kinetic plot of this data where B represents the amount bound at time t and B_e represents the amount bound at equilibrium. The slope of the linear regression line describes a K_{ob} of 0.055 nM^{-1} min^{-1}. B, dissociation kinetics. [³H]Flupentixol (0.89 nM) was allowed to reach equilibrium in the presence of 10 nM spiroperidol and the amount of specific binding was determined. (+)Butaclamol (10 μM) was then added (t = 0) without significant dilution of the samples and specific binding was sampled at the times indicated. Inset, first order rate plot of the data where B represents binding at time t and B_0 represents binding at time zero. All binding was adjusted by 100 cpm to account for the apparently nondissociable amount of (+)butaclamol-inhibitable binding. The first order dissociation rate constant k_{-1} is determined from the slope of the regression line. Identical rate constants were seen when 1 mM dopamine was used as the dissociating agent.

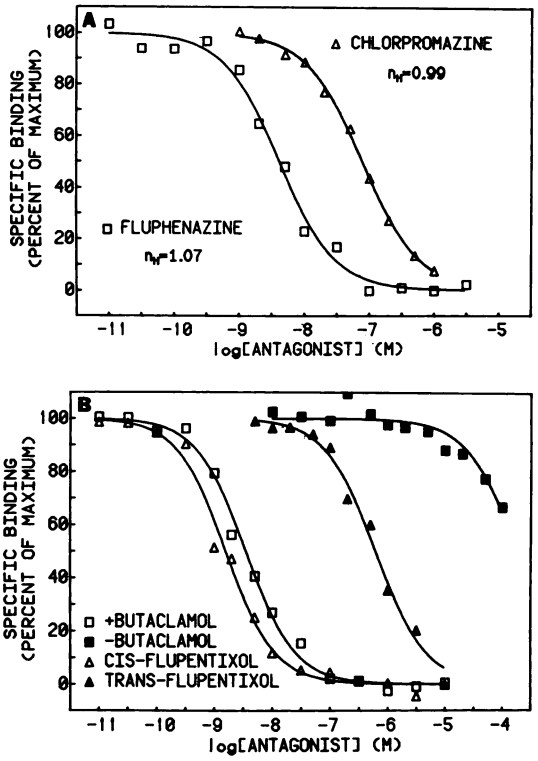

FIG. 3. *Computer-fitted curves for antagonists inhibiting D_1 receptor-specific [³H]flupentixol binding in rat striatum*

The experimentally determined points are from representative experiments using 0.64–0.8 nM [³H]flupentixol. In all cases, computer-drawn curves represent the best fit to the data assuming a single site model. Assumption of a two-site model does not improve the fits. A, computer-fitted curves for phenothiazines inhibiting D_1 specific [³H]flupentixol binding in rat striatum. B, demonstration of stereospecificity of D_1 specific [³H]flupentixol binding in rat striatum. For (−)butaclamol and *trans*-flupentixol, the data were fit while constraining the nonspecific binding parameter equivalent to that seen using 1 μM (+)butaclamol as a blank.

The binding of [³H]flupentixol to rat striatal membranes in the presence of a D_2 masking drug is stereospecific as shown in Fig. 3B and Table 1. The more active *cis* isomer of the thioxanthene antagonist was 425 times more potent than its respective *trans* isomer as an inhibitor of [³H]flupentixol binding. A similar stereoisomeric preference was seen for all other thioxanthene antagonists tested (Table 1) as well as for the (+) versus (−)enantiomer of the antagonist butaclamol (Fig. 3B).

The atypical neuroleptics (−)sulpiride and metoclopramide have been reported to be particularly weak antagonists of dopamine-stimulated adenylate cyclase activity in striatum (29, 30), in contrast to their potent antagonism at brain and pituitary D_2 dopamine receptors. These substituted benzamide antagonists are very weak inhibitors of [³H]flupentixol binding (Table 1).

Table 1 shows that dopaminergic and nondopaminergic antagonists compete for [³H]flupentixol binding (conducted in the presence of 10–30 nM spiroperidol as a D_2 masking drug) with a rank order of potency and stereospecificity expected for a dopamine receptor. Among phenothiazines, fluphenazine is more potent than chlorpromazine which is more potent than promethazine. The active *cis* isomers of thioxanthenes are more potent than the less active *trans* isomers, and the (+)isomer of butaclamol is several orders of magnitude more potent than (−)butaclamol. As has been discussed above, different from D_2 dopaminergic pharmacological profiles, the butyrophenones haloperidol and spiroperidol and antagonists domperidone, pimozide, (−)sulpiride, and metoclopramide are relatively weak inhibitors of [³H]flupentixol binding. In fact, the observed pharmacological profile highly suggests an interaction with D_1 dopamine receptors. This suggestion is supported by data in Table 1 and Fig. 4 where potencies of dopaminergic antagonists to inhibit "non-D_2" [³H]flupentixol binding and dopamine-stimulated adenylate cyclase activity (values derived

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TABLE 1

Antagonist affinities for [³H]flupentixol binding to rat striatal membrane homogenates in the presence of a D_2 masking drug compared to literature-derived values for inhibition of dopamine-stimulated adenylate cyclase activity: characteristics of a D_1 dopamine receptor

Competition curves were conducted using duplicate or triplicate tubes per dose and 10–14 doses/curve. Competitions were conducted in the presence of 10–30 nM spiroperidol to selectively inhibit [³H]flupentixol binding to D_2 dopamine receptor-binding sites. [³H]Flupentixol concentrations ranged from 0.5–1.2 nM. Dissociation/inhibition constants were determined using computer analysis as described in Materials and Methods. Each value represents means ± SE from 2–7 independent experiments. Literature values for inhibition of adenylate cyclase activity are given as means of K_i values when more than one group has investigated the drug. In such cases, ranges are given along with the references. When necessary, K_i values were estimated from IC_{50} values using the equation $K_i = IC_{50}/(1 + [DA]/K_{act})$ where $[DA]$ represents the concentration of dopamine used to stimulate cyclase activity and K_{act} was estimated as 4.5 μM unless studies specified otherwise. Nondopaminergic agents prazosin, yohimbine, and propranolol had $K_i >> 10$ μM).

Antagonist	[³H]Flupentixol binding	D_1 Adenylate cyclase		
		K_i	Range	Refs.
	nM	nM		
cis-Piflutixol	0.4 ± 0.1	3.1		9
trans-Piflutixol	254± 30			
cis-Flupentixol	0.7 ± 0.04	1.3	1.0–1.6	9,31
trans-Flupentixol	292 ± 44	2,258	15–4500	9,32
(+)Butaclamol	1.9 ± 0.14	18.5	9–28	9,31
(−)Butaclamol	53,600 ± 7400	>1,000		9,33
Fluphenazine	2.7 ± 0.2	6.9	4–10	31,34–36
cis-Clopentixol	5.7 ± 2.8	9.1	2.2–16	31,34
trans-Clopentixol	117 ± 34	1,370	40–2700	9,32
cis-Chlorprothixene	6.5 ± 2.7	19.5	2.0–37	31,34
trans-Chlorprothixene	223 ± 33	482	15–950	9,31
Teflutixol	10 ± 0.9	7.0		9
SCH 23390	0.28 ± 0.03	0.66		37
SKF 83742	14.6 ± 2.2	22		38
Chlorpromazine	49 ± 17	62	33–94	9,31,35,36,39
Haloperidol	74 ± 16	82	19–220	33–36,34,40
Spiroperidol	252 ± 777	155	95–270	31,34,41
Ketanserin	191 ± 25			
Pimozide	459 ± 73	550	140–1220	31,34,35,40
Promethazine	2,460 ± 170	1,300	1000–1670	9,35
Domperidone	2,562 ± 745	9,000		42
Molindone	14,000 ± 2,000	3,300		9
Metoclopramide	175,000			
Metoclopramide + NaCl	87,745	>9,000		9
(−)Sulpiride	1,051,000 ± 153,000			
(−)Sulpiride + NaCl	351,000 ± 11,000	(±)30,500	14,000–47,000	34–39
(+)Sulpiride	212,000 ± 34,000			
(+)Sulpiride + NaCl	105,000 ± 10,000			

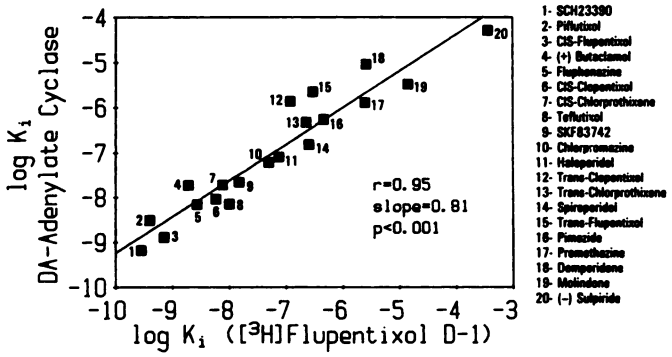

FIG. 4. *Correlation between antagonist inhibition of [³H]flupentixol binding and inhibition of dopamine (DA)-stimulated adenylate cyclase activity in homogenates of rat striatum*

Log K_i values are derived from data in Table 1.

from the literature) are compared. Fig. 4 shows an impressive correlation of antagonist potencies to inhibit these two activities ($r = 0.95$; $p < 0.001$).

Among nondopaminergic antagonists, the α_1-, α_2-, and β-adrenergic antagonists prazosin, yohimbine, and propranolol, respectively, were ineffective inhibitors of D_1-specific [³H]flupentixol binding (K_i values > 10 μM). While *cis*-flupentixol may exhibit some relatively potent anti-α-adrenergic activity (43), prazosin was inactive as an inhibitor of [³H]flupentixol binding (K_i > 100 μM). Thus, [³H]flupentixol did not label these nondopaminergic receptors at the concentrations of labeled ligand used. This finding was supported by detailed competition curves. In contrast, the serotonergic (5-HT_2 receptor selective) antagonist ketanserin (44) had moderately high affinity for [³H]flupentixol binding (K_i = 200 nM). However, competition experiments conducted in the absence of spiroperidol, itself a 5-HT_2 receptor antagonist (45), confirmed that [³H]flupentixol at the concentrations used does not appear to label 5-HT_2 receptors.

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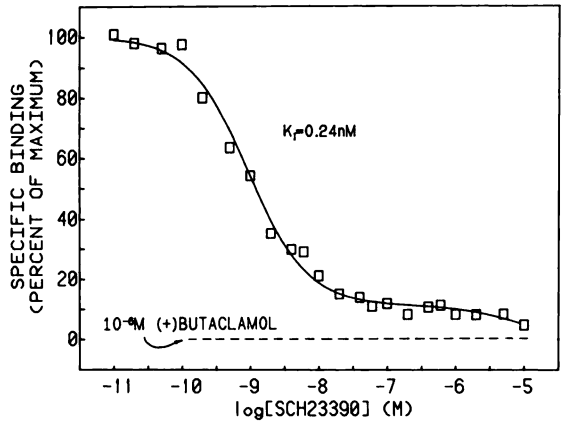

FIG. 5. *Computer-fitted curve for the D_1 dopamine receptor specific antagonist SCH 23390 inhibiting [^{3}H]flupentixol binding*

When the nonspecific binding parameters N is fit unconstrained, the data fit best to a single site model. When specific binding is defined as the difference between binding in the absence and presence of 1 μM (+)butaclamol, the curve is fit best by assuming a two-site model. The K_i for the lower affinity site (comprising 7–15% of the total sites) is greater than 5 μM and probably represents a saturable but non-D_1 dopamine receptor-specific binding site. Thus, nonspecific [^{3}H]flupentixol binding is determined differently by 1 μM (+)butaclamol and 1 μM SCH 23390. The [^{3}H]flupentixol concentration was 0.40 nM.

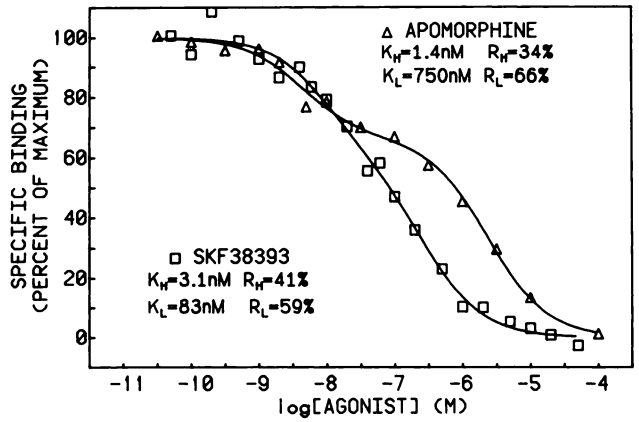

FIG. 6. *Computer-modeled curves for apomorphine and SKF 38393/[^{3}H]flupentixol competition experiments*

Single representative curves are shown. The data are fitted best by a two-site model. The affinity of [^{3}H]flupentixol was constrained to be equal (K_D = 0.4 nM) at both high (R_H) and low (R_L) affinity sites. K_H and K_L denote the estimates of dissociation constants for high and low affinity agonist-binding sites, respectively.

Fig. 5 shows the competition of the newly characterized putatively D_1 dopamine receptor-selective antagonist SCH 23390 (34, 37, 46) and [^{3}H]flupentixol. These data are expressed as inhibition where specific binding is defined using 1 μM (+)butaclamol as a blank drug. These data indicate that (+)butaclamol inhibits a 10% proportion of [^{3}H]flupentixol binding which is not inhibited by SCH 23390.

Agonist competition for radiolabeled antagonist binding. In contrast to antagonist/[^{3}H]flupentixol curves, agonist/[^{3}H]flupentixol curves exhibit heterogeneous characteristics with pseudo-Hill coefficients much less than 1. Fig. 6 shows competitions by the agonists (−)apomorphine and SKF 38393, two partial agonists at the D_1 dopamine receptor which maximally elicit only 50–60% of dopamine's maximal response in stimulating adenylate cyclase (see Table 3). Data for these two agonists were best explained by a two-site model. The full agonist NPA showed competition curves much like those for apomorphine and SKF 38393. Data for this drug were also best fit by a two-site model. However, in these experiments, up to 100 μM (−)NPA could inhibit only 85–95% of the binding which could be blocked by 1 μM (+)butaclamol but all of the SCH 23390-sensitive sites. These aporphine agonists thus appeared to discriminate two agonist-binding sites having high (nanomolar) and low (micromolar) affinity, respectively.

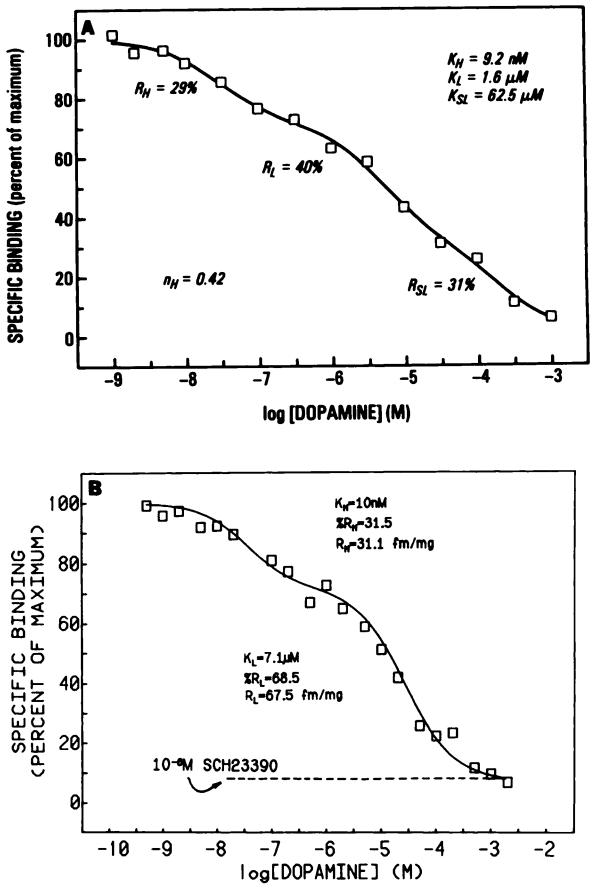

FIG. 7. *Computer-modeled curves for dopamine/[^{3}H]flupentixol competition experiment*

A, a single representative experiment is shown using 1.1 nM [^{3}H] flupentixol in the presence of 30 nM spiroperidol. When nonspecific binding is constrained to a value determined by 1 μM (+)butaclamol, the data are fitted best by a three-site model. The affinity of [^{3}H] flupentixol was constrained to be equal (K_D = 0.45 nM) at all three sites. Sites from these fits were designated high (H), low (L), and super-low (SL). B, a similar representative experiment is shown except that nonspecific binding is constrained to a value determined by 1 μM SCH 23390. The data are fitted best by a two-site model.

Fig. 7 shows a dopamine/[^{3}H]flupentixol competition curve which is very shallow (pseudo-Hill coefficient = 0.42). Computer analysis using 1 μM (+)butaclamol to define nonspecific binding (Fig. 7A) indicates that the data are best explained by a three-site model. Such three-site computer fits were observed frequently (in 15 of 20 experiments) for a number of catechol-containing full or

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nearly full agonists such as epinine and (±)ADTN. While in some experiments involving these agonists assuming a three-site model did not significantly improve the fits derived from a two-site model, in a majority of the experiments, assuming a two-site model did not adequately explain the data. However when nonspecific binding is constrained to that determined with 1 μM SCH 23390, dopamine/[³H]flupentixol curves are best fit to a two-site model (Fig. 7B).

The ability of a third group of potential agonists, ergot alkaloids, to inhibit [³H]flupentixol binding to D_1 striatal dopamine receptors was examined. These compounds are particularly interesting since *in vitro* assays show that they can act as partial agonists of the D_1 dopamine receptor possessing no activity to moderate intrinsic activities (0–40% of the maximal response elicited by dopamine; see Tables 3 and 4). Computer analysis of the competition curves for pergolide and lergotrile produced results which were best explained by a two-site model. None of the ergot compounds examined produced data which consistently were best explained by three-site models. Experiments with other ergots produced data which could be explained by a one-site model. For ergots such as ergotamine, lisuride, and dihydroergocriptine, computer-derived parameters for two-site models could be obtained, but these curves did not consistently fit the data significantly better than those obtained assuming a one-site model (two-site fits were significantly better in 5 of 11 experiments). The affinity estimates for these ergot compounds in these one-site fits matched those shown for their K_L values seen in two-site fits. However, all experiments using pergolide and lergotrile produced data which were best fit by curves assuming a two-site model.

TABLE 2

Computer-modeled parameters for agonists competing for D_1 specific [³H]flupentixol binding to rat striatum

Competition curves were constructed and analyzed as described in Figs. 6 and 7A. [³H]Flupentixol concentrations ranged between 0.5 and 1.1 nM. Data are expressed as means ± SE. For $\%R_H$, values of zero scored in individual experiments when data did not fit better to a two-site model.

Drug	n	K_H	K_L	K_{SL}	$\%R_H$
		nM	nM	μM	
CM 29-712	3	120 ± 31	3700 ± 500		20 ± 4.3
CQ 32-084	3	141 ± 70	6500 ± 2400		24.8 ± 4.1
CF 25-397	4	5.2 ± 4.0	180 ± 25		24.7 ± 11.6
Pergolide	3	44 ± 25	2200 ± 700		34.2 ± 8.0
SKF 38393	3	3.1 ± 0.9	158 ± 52		40 ± 2.0
(−)Apomorphine	9	7.8 ± 2.8	830 ± 120		28.4 ± 3.2
(−)NPA	5	7.9 ± 3.5	1100 ± 700		25.4 ± 2.1
Epinine	6	25.0 ± 4.6	3210 ± 690	90.6 ± 34.4	22.1 ± 1.9
(±)ADTN	8	28.2 ± 7.9	3900 ± 1000	65.5 ± 16.7	25.5 ± 1.2
Dopamine	6	23.2 ± 11.6	3300 ± 1100	262 ± 107	25.5 ± 2.9

TABLE 3

Comparison of the ratio of computer-modeled affinity estimates (K_L/K_H) derived from agonist/[³H]flupentixol competition curves to relative intrinsic activities of agonists to stimulate adenylate cyclase activity in homogenates of rat striatum

Computer-modeled parameters K_H and K_L are taken from Table 2. Per cent maximal response is the maximal stimulation of adenylate cyclase activity elicited by the drug expressed as a percentage of the maximal response elicited by dopamine. These values are the means of values taken from the references listed.

Drug	K_L/K_H	% Maximal response	Range	Refs.
			%	
CM 29-712	31	22	10–34	47,51
CF 25-397	35	26	21–34	47,52,53
CQ 32-084	46	32	32	47
Pergolide	50	33.5	25–46	47–50
SKF 38393	51	65	62–68	54–56
(−)Apomorphine	106	55	32–69	41,47,53,57–62
Epinine	128	100	100	31,51
(±)ADTN	138	93	82–115	31,59,63,64
(−)NPA	139	100	100	65
Dopamine	144	100	100	31,54,57,58,62,66,67

Table 2 summarizes the computer-derived parameters for agonist/[³H]flupentixol curves described above. For drugs fitting to three sites when (+)butaclamol was used to define nonspecific binding, dissociation constants to high affinity (R_H), low affinity (R_L), and super-low affinity (R_{SL}) have been designated K_H, K_L, and K_{SL}, respectively. It is significant that the ratio of K_L/K_H values varies with different compounds (Table 3). The catechol-containing agonists, many of them full or nearly full agonists, have the greatest ratios of K_L/K_H. This relationship, K_L/K_H versus intrinsic agonistic activity, for all of the drugs listed is explored more extensively in Table 3. Values for intrinsic activity were obtained from literature sources and represent the maximal stimulation of adenylate cyclase (cell-free homogenates in the absence of added guanine nucleotides) exhibited by each compound expressed as a percentage of the maximal response elicited by dopamine. Both measures, relative intrinsic activity and K_L/K_H, are tabulated in Table 3, and we examined the correlation between these values for agonists and ergot alkaloids with well documented agonist activity. A significant correlation was found between our experimentally obtained and computer-derived K_L/K_H ratio and relative intrinsic activity ($r = 0.93$; $p < 0.001$). In contrast, no significant correlation was found between either K_H or K_L values and relative intrinsic activity [($r = -0.53$, $p > 0.2$; $r = 0.04$, $p > 0.5$) for K_H and K_L, respectively]. Furthermore no significant relationship between the percentage of total R which is present in the high affinity state for each agonist ($\%R_H$) and that agonist's relative intrinsic activity was demonstrated ($r = -0.04$, slope $= -0.24$, $p > 0.5$). Lisuride, ergotamine, lergotrile, and dihydroergocriptine were omitted from all correlation analyses since they are reported by many to be pure antagonists. Nevertheless, including their values in these analyses did not affect the presence or absence of significant correlation (Table 4).

In Table 5, the relationships between computed parameters K_L and K_H and literature-derived estimates of activation constants (K_{act}) for the agonists listed to stimulate adenylate cyclase were investigated. Fig. 8 shows that a significant correlation between the logs of K_L and K_{act} was observed ($r = 0.93$, slope $= 0.96$, $p < 0.001$). Visual inspection of these data shows that the absolute values for these two constants are also comparable. In contrast, although a significant correlation was found between the logs of K_H and K_{act} values ($r = 0.78$, slope =

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TABLE 4

Computer-modeled parameters for ergot alkaloids of questionable intrinsic activity competing for D_1 specific [^{3}H]flupentixol binding to rat striatal membranes

Competition curves were analyzed as described in Figs. 6 and 7. Data are expressed as means ± SE. Data are tabulated as described in Tables 2 and 3. Data fit better to two-site models in 9 of the 15 experiments.

Drug	*n*	K_H	K_L	K_L/K_H	$\%R_H$	% Maximal response	Refs.
		nM	*nM*				
Lisuride	4	4.0 ± 3.2	109 ± 14	27	4.3 ± 4.3	1.3	47,53,68,69
Ergotamine	4	12.9 ± 5.9	220 ± 20	17	19.2 ± 7.1	4.0	41,57
Lergotrile	4	8.9 ± 7.4	260 ± 30	29	22.0 ± 1.4	7.2	41,47,51–53
Dihydroergocriptine	3	68 ± 18	2260 ± 990	33	14.6 ± 14.6	13	52,53,57,58,70,71

TABLE 5

Comparison of computer-modeled affinity estimates K_H and K_L derived from agonist/[^{3}H]flupentixol competition curves to activation constants (K_{act}) of agonists to stimulate adenylate cyclase activity in homogenates of rat striatum

Computer-modeled parameters K_H and K_L are taken from Table 2. K_{act} refers to activation constant to stimulate adenylate cyclase *in vitro* given as mean and range from references listed.

Drug	K_H	K_L	K_{act}	Range	Refs.
	nM	*nM*	*nM*		
Pergolide	44	2200	2000	600–4500	47–50
CM 29-712	120	3700	4000		47
CF 25-397	5.2	180	150	50–250	47,52
CQ 32-084	141	6500	1600		47
SKF 38393	3.1	158	75	71–80	54–56
(−)Apomorphine	7.8	830	1310	300–2000	47,54,57,60,62
(±)ADTN	28.2	3900	3670	3000–4000	59,63,64
(−)NPA	7.9	1100	1000		65
Epinine	25	3210	4250	1500–7000	31,51
Dopamine	23.2	3300	2600	300–4000	31,54,57,58,62, 66,67

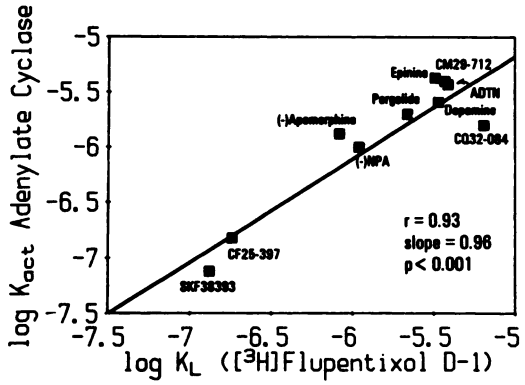

FIG. 8. *Correlation between computer estimates of K_L values derived from agonist/[^{3}H]flupentixol competition curves versus agonist activation constants (K_{act}) in stimulating adenylate cyclase activity in homogenates of rat striatum*

Values are taken from Table 5.

0.85, $p < 0.01$), the absolute values for these two constants differed by 1 to 2 orders of magnitude (not shown). This correlation probably reflects the weak correlation which was found between the logs of K_H and K_L values for these agonists ($r = 0.89$, slope = 0.90, $p < 0.001$; not shown).

Regulation of agonist competition for [^{3}H]flupentixol binding by guanine nucleotides. Guanine nucleotides have been demonstrated to regulate agonist-receptor interactions in a variety of hormone and neurotransmitter systems (72), particularly those which regulate adenylate cyclase activity. In these systems, guanine nucleotides act to reduce agonist affinity for the receptor. Guanine nucleotides may exert no effect on antagonist binding, while in some systems they exhibit positive heterotropic effects on antagonist-receptor interactions (5, 73–75). In the presence of 0.3 mM GTP, dopamine/[^{3}H]flupentixol curves are shifted to the right and slightly steepened. Computer analysis of the curve in the presence of GTP shows that the major effect was to reduce the $\%R_H$. Table 6 shows computer-derived parameters for agonist/[^{3}H] flupentixol curves in the presence and absence of added GTP. Independent experiments with several agonists indicated that no significant difference in results was observed when the hydrolysis-resistant GTP analogue Gpp(NH)p (0.1 mM) was used as the guanine nucleotide. To determine whether guanine nucleotides might affect antagonist affinity for D_1 dopamine receptors, [^{3}H]flupentixol saturation experiments and antagonist/[^{3}H]flupentixol competition experiments were conducted in the presence and absence of 0.3 mM GTP. GTP appeared to have no effect on [^{3}H]flupentixol affinity, B_{max}, or the affinities of (+)butaclamol or fluphenazine for [^{3}H]flupentixol D_1 receptor-specific binding sites (data not shown). Furthermore, examination of a large number of competition experiments ($n = 40$) indicated that GTP did not affect the D_1 receptor-specific binding of [^{3}H] flupentixol.

DISCUSSION

Tritiated thioxanthene and phenothiazine antagonists have been demonstrated to label heterogeneous populations of specific dopaminergic receptors in rat striatum membranes. The binding of [^{3}H]flupentixol to putative D_1 and D_2 dopamine receptors was discriminated by the D_2 selective antagonists domperidone and spiroperidol as shown in Fig. 1. The specific binding of [^{3}H]flupentixol remaining in the presence of a D_2 receptor masking concentration of spiroperidol (10–30 nM) was verified as being dopaminergic on the basis of stereoselectivity and the pharmacological specificity suggestive of a D_1 dopamine receptor (Table 1; Fig. 4). These data corroborate the earlier findings of Hyttel (9–12) and the more recent observations of Cross and Owen (13) and Hyttel (34).

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TABLE 6

Computer-modeled parameters for agonist inhibition of [^{3}H]flupentixol binding in rat striatum: effects of GTP

Competition curves were constructed and analyzed as described in Figs. 6 and 7. Data are tabulated as in Table 2.

Drug	Control			+0.3 mM GTP		
	K_H	K_L	$\%R_H$	K_H	K_L	$\%R_H$
SKF 38393	3.1 ± 0.9	158 ± 52	40 ± 2	6.1 ± 5.9	137 ± 49	20 ± 12
(−)Apomorphine	2.3 ± 0.9	940 ± 190	25 ± 3	1.8 ± 1.1	1660 ± 360	17 ± 2
(−)NPA	5.2 ± 1.9	11130 ± 90	22 ± 2	4.9 ± 2.2	1860 ± 340	13 ± 6
Dopamine	18 ± 11	1900 ± 400	26 ± 1	13 ± 9	4200 ± 1400	10 ± 4

The early studies by Hyttel identified [^{3}H]flupentixol binding to a D_1 dopamine receptor in the absence of a D_2 masking drug. Since the D_2 specific component of [^{3}H]flupentixol binding is relatively minor (this study, 13, 34), a significant correlation between antagonist inhibition of dopamine-stimulated adenylate cyclase and inhibition of [^{3}H]flupentixol binding in rat striatum was observed in those studies. The present study is the first to demonstate selective and detailed agonist and antagonist competition data for D_1 dopamine receptors in the presence of a D_2 masking drug.

The binding of [^{3}H]flupentixol to D_1 dopamine receptors was saturable with respect to time and concentration, exhibited high, apparently homogeneous affinity, and was reversible. The apparent site density in striatum, 100–120 fmol/mg of tissue wet weight, suggests that D_1 dopamine receptors are about 4–5 times more abundant in striatum than D_2 dopamine receptor-binding sites. However, this density is probably an overestimation since it is defined using (+)butaclamol as a blank drug.

Curves describing antagonist competition for D_1 receptor-specific [^{3}H]flupentixol binding were steep (pseudo-Hill slopes = 1) and of uniform affinity. Several thioxanthene antagonists appeared to displace [^{3}H]flupentixol binding in excess of that displaced by 1 μM (+)butaclamol. This was true of both the active, *cis*, and less active *trans* isomers, suggesting an interaction with a nonreceptor-specific, but thioxanthene-specific, binding site. As expected for a D_1 dopamine receptor, domperidone and the benzamide antagonists (−)sulpiride and metoclopramide were weak or virtually inactive. The relatively high affinity inhibition of [^{3}H]flupentixol binding by serotonergic antagonists is not surprising as some of these agents appear to inhibit dopamine-stimulated adenylate cyclase activity more potently than they inhibit serotonin-stimulated activity (76, 77).

In contrast with antagonists, agonist interactions with D_1 specific [^{3}H]flupentixol binding were complex. Competition curves for catechol agonists were very shallow (pseudo-Hill coefficients as low as 0.4), suggesting the presence of either a heterogeneous population of binding sites or a negatively cooperative effect of agonist binding. Using the LIGAND program, competition curves for the full or nearly full agonists dopamine, epinine, and ADTN were often best modeled to three sites. These curves were composed of a high affinity site (R_H) comprising 20–30% of the total number of specific binding sites. An intermediate affinity site (R_L) exhibited roughly micromolar affinity for these drugs and comprised about 45–60% of the specific binding sites. A third very low affinity binding component (R_{SL}) was observed for these fits and comprised 15–30% of the specific binding. The estimated affinities of these drugs for this site was rather variable, ranging from 20–400 μM. Very high concentrations of these cold agonists were required to inhibit [^{3}H]flupentixol binding to the baseline defined by 1 μM (+)butaclamol. However, when the D_1 selective antagonist SCH 23390 (0.1–1μM) was used to define nonspecific binding, dopamine/[^{3}H]flupentixol competition curves were fit best by assuming a two-site model. Parameters from two-site fits corresponded to the high and low affinity sites discriminated by three site fits from other experiments that used (+)butaclamol to determine nonspecific binding.

With respect to the above analyses of agonist/[^{3}H]flupentixol competition data, it is difficult to be certain which drugs provide the most appropriate blank. On the one hand, (+)butaclamol is a well characterized dopaminergic antagonist, and the (+)butaclamol/[^{3}H]flupentixol competition curve in Fig. 3B is sigmoidal and monophasic and shows a long plateau of 100% competition between 0.1 and 10 μM. Furthermore, SCH 23390 is, to date, a relatively uncharacterized D_1 dopamine receptor-selective antagonist. On the other hand, the level of nonspecific binding defined by SCH 23390 is consistent with that seen using other drugs such as SKF 83742, SKF 38393, apomorphine, and (−)NPA. Consequently, our data have been reported as analyzed by using (+)butaclamol as the blank drug (Tables 2–6) with comparisons made to data analyzed using SCH 23390 to define nonspecific binding (Fig. 7).

It is not yet possible to give a precise explanation for the discrepancies we found in antagonist blank determinations or for the appearance of a third super-low affinity agonist-binding site for some agonists. Several possibilities may be raised. First, a super-low affinity portion of these curves may represent interactions between agonists and [^{3}H]flupentixol for a non-dopamine-receptor-specific binding site which, however, is apparently saturable and can be blocked by 1 μM (+)butaclamol. The observation that a minor portion of "(+)butaclamol-displaceable" [^{3}H]flupentixol binding appeared irreversible in dissociation experiments is consistent with this hypothesis. Furthermore, the discrepancies in levels of nonspecific binding defined by either (+)butaclamol or SCH 23390 further support this hypothesis. An alternate hypothesis is that ^{3}H-antagonists such as flupentixol and fluphenazine possess extra hydrophobic portions, in common with many other antagonists, which are involved in binding to the receptor at an accessory

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site. Antagonists such as SCH 23390 and catechol-containing agonists which lack this moiety would not interact with this accessory site. For these agonists, the apparent super-low affinity portion of the competition curve might therefore result from this kind of agonist-antagonist interaction at the receptor-accessory site if receptor-saturating concentrations of agonist still allowed low affinity binding of the ^{3}H-antagonist to the accessory site. In support of this hypothesis is the observation that a number of *trans*-thioxanthenes inhibit [^{3}H]flupentixol binding considerably more potently than they block dopamine stimulation of adenylate cyclase activity. Thus, thioxanthene antagonists appear to utilize accessory binding sites outside the dopamine pharmacophore to enhance their affinity as antagonists (for a discussion of this principle, see Ref. 78) especially as inhibitors of [^{3}H]thioxanthene binding. Whether this phenomenon indeed participates in the appearance of a super-low component of agonist/[^{3}H]flupentixol competition curves cannot be presently evaluated. A third possible explanation for the relative inability of some catechol agonists to completely displace [^{3}H]flupentixol "specific" binding is that agonists might inhibit at least a subclass of [^{3}H]flupentixol-binding sites noncompetitively. However, this possibility is unlikely since identical [^{3}H]flupentixol dissociation rates were observed when an agonist *or* an antagonist was used as the "dissociating" agent.

For agonists such as (−)NPA, (−)apomorphine, and SKF 38393, curves fit best to a two-site model. However, as described in the results, the maximum inhibition produced by the aporphine drugs was only 80–95% of that produced by 1 μM (+)butaclamol. Curves describing the inhibition of [^{3}H]flupentixol binding by (−)NPA at concentrations greater than 0.1 mM leveled out and even reversed at concentrations greater than 0.3 mM (−)NPA. This phenomenon has been previously reported (22, 79) for agonist or catechol competition for [^{3}H]apomorphine or [^{3}H]NPA binding to rat striatum membranes conducted in the absence of reducing agents. Consequently, for these agonists, only the portion of these competition curves which displayed mass action behavior was analyzed. In these analyses, the parameter estimate for the nonspecific binding of [^{3}H]flupentixol was allowed to remain unconstrained, being defined by the agonist [^{3}H]flupentixol competition curve itself.

In contrast to these agonists, competition curves for ergot alkaloids with moderate intrinsic activity were more simply modeled. These compounds, such as pergolide, produced curves that were generally best fit by assuming a two-site model. In no case did assuming a three-site model significantly improve fits of these competition curves. A striking correlation was observed between the per cent maximal response (relative intrinsic activity) found for these compounds in the literature versus the ratio of K_L/K_H values derived from the present study. This correlation extended to all agonists tested.

No significant correlation was observed between relative intrinsic activities of agonists and their average $\%R_H$ derived from competition experiments. Since $\%R_H$ values ranged from 20 to 40% and the error in R_H estimates appeared high, the lack of correlation is difficult to evaluate.

Taken together, the data in this study suggest that the high and low affinity agonist-binding components found in competition experiments might represent partially interconvertible states of a single D_1 dopamine receptor. This hypothesis is partly supported by the observation that saturating concentrations of guanine nucleotides promote an apparent decrease in R_H site densities (Table 6) whereby in some cases agonist/[^{3}H]flupentixol curves model best to a single affinity site in which the affinity is indistinguishable from the K_L value observed in the control curve. Since [^{3}H]flupentixol binding is not affected by guanine nucleotide addition, R_H and R_L appear to be at least partly interconvertible.

Interconversion of agonist-binding states has now been proposed for several other monoaminergic receptors (for review, see Ref. 80), including the D_2 dopamine receptor of brain and pituitary (3–6). These studies have proposed a generalized ternary complex model modified from the original models of Boeynaems and Dumont (81, 82) and Jacobs and Cuatrecasas (83). This model involves a two-step process and three components as shown below:

$$L + R \overset{K_1}{\rightleftharpoons} LR \qquad (2)$$

$$LR + X \overset{K_2}{\rightleftharpoons} LRX \qquad (3)$$

where X is a third membrane component, a guanine nucleotide-binding protein, and K_n are the dissociation constants for the respective reactions. In this model, both agonists and antagonists participate in the first step but only agonists induce a conformational change in the receptor such that the second reaction takes place. Agonists have higher affinity for the ternary complex than they do for the binary complex. Thus, the complex of agonist, receptor, and guanine nucleotide-binding protein represent the R_H state seen in the membranes. The proportion of total receptor represented as R_H may be limited by equilibrium constraints or X/R stoichiometry or both factors. The addition of guanine nucleotides in the model promotes instability of the ternary complex and a concomitant reduction or dissipation of R_H.

An alternative variation of the ternary complex model proposed by Jacobs and Cuatrecasas (83) and Boeynaems and Dumont (84) is shown below: where K_n represents the dissociation constants for either ligand-receptor (L-R, K_1; L-RX, K_3) or receptor-component (X-R, K_4; X-R_L, K_2) interactions.

$$\begin{array}{ccc} X & & X \\ + & & + \\ L + R & \overset{K_1}{\rightleftharpoons} & LR \\ K_4 \downarrow & & \downarrow K_2 \\ L + RX & \underset{K_3}{\rightleftharpoons} & LRX \end{array} \qquad (4)$$

This variation of the ternary complex model has recently been used in computer simulation of ligand interactions with the β-adrenergic receptor of frog erythrocyte (16) and D_2 dopamine receptor of bovine anterior pituitary (85). This model differs from the two-step model in that it considers the possibility of precoupling of R and X in the absence of ligand. Several features of the agonist/[^{3}H]flupentixol data obtained in this study can be ex-

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plained by these two models. However, our data alone do not clearly favor one model over the other. Predicted by these models is the finding that the ratio of K_L/K_H values for various agonist/[^{3}H]flupentixol competition curves correlates with agonist relative intrinsic activity (16, 86). This prediction is based on the hypothesis that a function of agonist action is to promote coupling of R and X. In the first model, agonist intrinsic activity would be inversely related to K_2 (Eq. 3). That is, the equilibrium dissociation constant K_2 is small for agonists of high efficacy; thus, the equilibrium is driven to the right. For the model in reaction sequence 4, agonists possessing high intrinsic activity would be expected to have relatively low values for K_2 and presumably also for K_3. However, due to the principle of detailed balance, K_3 is partially dependent upon K_4 for the system (the equilibrium constraint to precoupling or R-X) since $K_1K_2 = K_3K_4$. A first approximation using this model to analyze the agonist/[^{3}H]flupentixol competition curves in this study might state that K_3 approximates K_H and K_1 approximates K_L where K_H and K_L are derived from the mass action based program LIGAND. If the two-step model is used, K_1 might approximate K_L and K_H would be an emergent value which is dependent upon the actual value of K_2. However, it should be considered that, in some of the experiments described, parameter estimates for agonist-receptor dissociation constants and relative site densities derived from mass action-based computer analyses may not represent *real* dissociation constants or binding site densities. Rather, these parameter estimates may relate *approximations* of real dissociation constants and binding site densities present in the ternary complex model (16).

The observation that some ergot alkaloids such as lergotrile compete for [^{3}H]flupentixol binding with heterogeneous affinity states suggests that these compounds could exert partial agonist activities at the D_1 dopamine receptor, perhaps with the addition of GTP to the assay. A careful inspection of the available literature on these compounds suggests that they can exhibit weak stimulation under certain circumstances (maximal responses are <13% of that produced by 100 μM dopamine) of adenylate cyclase activity in cell-free homogenates of rat striatum (see Table 4). It is also interesting to note that the majority of literature values for the K_{act} and relative intrinsic activities of these ergots to stimulate adenylate cyclase activity in rat striatum homogenates were taken from studies conducted in the absence of added guanine nucleotides. A degree of variability in these values could thus be expected to arise from variability in the levels of endogenous guanine nucleotides that were present in different preparations. In fact, recent studies indicate that ergot alkaloids which are moderately active (pergolide) stimulators of adenylate cyclase activity in control homogenates are markedly more efficacious activators of adenylate cyclase activity in the presence of added guanine nucleotides (49, 50, 87). Such observations may prove important in elucidating the biochemical and pharmacological mechanisms by which such controversial ergot alkaloids with very low intrinsic activity act upon dopaminergic systems in the brain.

In summary, the interactions of agonists and ^{3}H-antagonists with the D_1 dopamine receptor of rat striatum have been characterized. The complex interactions of agonists with this receptor have been explained by a generalized ternary complex model similar to those previously described for a number of other monoaminergic receptors although this model may not explain all of the behavior observed for radioligand binding to the D_1 receptor. Nevertheless, estimates of relative intrinsic activities of agonists to stimulate adenylate cyclase *in vitro* were correlated with the ratio of dissociation constants K_L versus K_H derived from computer-assisted analyses of agonist/^{3}H-antagonist competition curves. Literature estimates of K_{act} for agonists in the adenylate cyclase assay correlated with estimates of K_L from binding assays. Thus, the concentrations of agonists which can promote the formation of primarily high affinity ternary complexes at equilibrium do not significantly activate adenylate cyclase *in vitro*. Current and future studies of the kinetics of agonist stimulation of adenylate cyclase in intact systems (88–90) should better define the significance of these data to the functional receptor. Implicit in the data presented is the suggestion that some agonists compete for a portion of the ^{3}H-antagonist-labeled D_1 dopamine receptor with sufficiently high affinity to be used to label directly a portion of these receptors. This possibility is evaluated in the following article which clarifies the identity of a previously anomolous class of high affinity ^{3}H-agonist-binding sites.

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