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Interactions of Dopaminergic Agonists and Antagonists with Dopaminergic D_3 Binding Sites in Rat Striatum

Evidence That [^{3}H]Dopamine Can Label a High Affinity Agonist-Binding State of the D_1 Dopamine Receptor

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SUMMARY

The interactions of dopaminergic agonists and antagonists with ^{3}H-agonist labeled D_3 dopaminergic binding sites of rat striatum have been characterized by radioligand-binding techniques. When the binding of [^{3}H]dopamine and [^{3}H]apomorphine to D_2 dopamine receptors is blocked by the inclusion of D_2 selective concentrations of unlabeled spiroperidol or domperidone, these ligands appear to label selectively the previously termed "D_3" binding site. Antagonist/[^{3}H]dopamine competition curves are of uniformly steep slope ($n_H = 1.0$), suggesting the presence of a single D_3 binding site. The relative potencies of antagonists to inhibit D_3 specific [^{3}H]dopamine binding are significantly correlated with their potencies to block D_1 dopamine receptors as measured by the inhibition of both dopamine-stimulated adenylate cyclase and [^{3}H]flupentixol-binding activities. The affinities of agonists to inhibit D_3 specific [^{3}H]dopamine binding are also correlated with estimates of these agonists' affinities for the high affinity binding component of agonist/[^{3}H]flupentixol competition curves. Both D_3 specific [^{3}H] dopamine binding and the high affinity agonist-binding component of dopamine/[^{3}H]flupentixol competition curves show a similar sensitivity to guanine nucleotides. Taken together, these data strongly suggest that the D_3 binding site is related to a high affinity agonist-binding state of the D_1 dopamine receptor.

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 stimulation of adenylate cyclase activity, whereas agonist occupation of D_2 dopamine receptors has been observed to attenuate this enzymatic activity (1, 2). The previous article (3) and earlier studies (4, 5) have demonstrated that the [^{3}H]thioxanthene antagonist *cis*-[^{3}H]flupentixol can be used to selectively label D_1 dopamine receptors in rat striatum provided that [^{3}H]flupentixol binding to D_2 receptors is blocked. In the previous article (3), it was demonstrated that agonist competitions of ^{3}H-antagonist binding to the D_1 dopamine receptor discriminated high and low affinity agonist-binding states that can be modulated by guanine nucleotides. In addition to D_1 dopamine receptors, studies by many laboratories (for review, see Ref. 2) have extensively characterized both agonist and antagonist interactions with mammalian D_2 dopamine receptors labeled with ^{3}H-antagonist ligands. Several of these studies also identified high affinity agonist-binding states of the D_2 receptor in the brain (6–11) and pituitary (12, 13).

In addition to studies of agonist interaction with dopamine receptors labeled by ^{3}H-antagonists, ^{3}H-agonists have been used to directly label dopaminergic receptors/recognition sites in the brain (reviewed in Ref. 2) and pituitary (12). Recently, we (6, 14) and others (15–18) have identified that ^{3}H-agonist ligands label both the high affinity agonist binding state of the D_2 dopamine receptor and an additional site, termed D_3 (19), in membrane preparations of rat striatum. Competition by the potent D_2 dopamine receptor antagonists spiroperidol and domperidone for radiolabeled agonist high affinity binding sites demonstrated markedly biphasic curves. The portion of ^{3}H-agonist binding for which these antagonists exhibited subnanomolar affinity identified high affinity agonist binding to the D_2 dopamine receptor,

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while the portion of ^{3}H-agonist binding inhibited by submicromolar concentrations of these antagonists defined the D_3 binding site. Early studies by Nagy *et al.* (20) and Sokoloff *et al.* (17) suggested that the D_3 binding site represents in part high affinity agonist binding to presynaptic dopamine autoreceptors since removal of nigrostriatal terminals by 6-hydroxydopamine lesions of the medial forebrain bundle produced decreased levels of D_3 specific binding. Recently, however, we (14) and others (18) have demonstrated that such decreased D_3 specific binding was an artifact of depleting endogenous dopamine and that D_3 binding sites are entirely postsynaptically located in striatum.

Since earlier studies have indicated that D_1 dopamine receptors are localized, like D_3 dopaminergic binding sites, primarily to intrinsic neurons of the striatum (21–23), and since the previous article (3) suggested that D_1 dopamine receptors can exist in a high affinity agonist-binding state, the present study determines whether D_3 dopamine-binding sites represent such a high affinity agonist-binding state of the D_1 dopamine receptor of the rat neostriatum. Our ensuing characterization of agonist- and antagonist-binding properties of ^{3}H-agonist-labeled D_3 dopaminergic binding sites in comparison to such properties of ^{3}H-antagonist-labeled D_1 dopamine receptors strongly supports such a hypothesis.

MATERIALS AND METHODS

Preparation of rat striatal membranes. Tissue preparations were virtually identical to those described previously (3). Briefly, freshly dissected rat striatum was homogenized in 100 volumes (w/v) of ice-cold Tris-HCl, pH 7.7 (25°). The homogenate was centrifuged (48,000 × *g* × 10 min) and the resulting pellet was resuspended in 50 volumes of ice-cold Tris-HCl containing 2 mM $MgSO_4$. This homogenate was incubated at 37° for 15 min, chilled with Tris-HCl buffer, and centrifuged as before. This pellet was washed one additional time in Tris-HCl buffer before resuspension of the final pellet in assay buffer (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°. Ascorbic acid was included in the assay to retard degradation of catechol agonists (24, 25) and preliminary experiments as well as previous studies (6, 26) indicated that ascorbic acid does not affect [^{3}H]dopamine specific binding while nonspecific binding was reduced in the buffer system used here.

Radioligand-binding assays. Binding was initiated by the addition of the membrane suspension to duplicate or triplicate glass test tubes containing 200–250 μl of radioligand, unlabeled competitor, and nucleotides (if any) before transferring tubes to a 22° water bath. Final assay volumes were 1 ml and tissue concentrations were 2.4–3.2 mg/ml (original wet w/v). Incubations were terminated after 90 min by rapid vacuum filtration as described previously (3) over GF/C filters except that filters were washed with only 10 ml of cold Tris-HCl buffer and were prewetted with Tris-HCl buffer only. Damp filters were immediately transferred to polypropylene minivials (Research Products International, Mt. Prospect, IL) which were subsequently filled with 4.0 ml of scintillation cocktail (Cytoscint, Westchem Products, San Diego, CA). Filters were shaken for 30 min, until translucent, and radioactivity trapped on the filter was determined by standard liquid scintillation spectroscopy at an efficiency of 50%. Separate experiments determined that incomplete extraction of trapped [^{3}H]dopamine from the filter occurred if filters were allowed to dry before the addition of scintillation cocktail. In some cases, apparent recoveries as low as 30% of the trapped radioactivity occurred. However, if filters were rewetted with 100–250 μl of water, apparent recoveries of radioactivity were 80% of values found on comparable filters which had not been allowed to dry. Nonspecific binding of radiolabeled agonists was determined in the presence of 10 μM *cis*-flupentixol. [^{3}H]Dopamine and [^{3}H]apomorphine were used to label selectively D_3 dopaminergic binding sites (see below) by including 10–50 nM spiroperidol or 30–100 nM domperidone in the assays to prevent ^{3}H-agonist from binding to D_2 dopamine receptor sites. For competition studies the D_2 "mask" concentrations used were 10 nM spiperone or 50 nM domperidone. For D_3 binding site-specific [^{3}H]dopamine saturation experiments, specific binding comprised 35–70% of total binding. For typical competition experiments conducted at 2.0–2.5 nM [^{3}H]dopamine, specific binding comprised 50–60% of total binding. Specific binding of [^{3}H]apomorphine to D_3 binding sites in saturation experiments comprised 30–70% of total binding. At a concentration of 2.0 mM, specific binding of [^{3}H]dopamine to D_3 binding sites was approximately 675 cpm/filter and [^{3}H]apomorphine binding to these sites was approximately 750 cpm/filter.

Data analysis. The computer analyses employed have been described in detail elsewhere (3, 12, 27). For saturation analyses, data were first analyzed by the method of Scatchard (28) in which only specific binding was considered. Data were subsequently analyzed by weighted nonlinear regression analysis (29) as better estimates of affinity and site density may be achieved by such methods especially for data of high scatter (30). For ease of graphic comparison, such data are presented in the form of Scatchard plots. Analyses of competition curves were conducted as described in the preceding article (3). Unless otherwise stated, results from multiple experiments are given as the mean and standard error of the mean.

Materials. [*ring*-2,5,6-^{3}H]Dopamine (41.6–41.8 Ci/mmol) and [8,9-^{3}H]apomorphine (23.6 Ci/mmol) were obtained from New England Nuclear Corp. Drugs were purchased or were generous gifts from the sources described in the preceding article (3). Other reagents were obtained from standard commercial sources.

RESULTS

^{3}H-Agonists label D_2 dopamine receptors and D_3 dopaminergic binding sites. Specific binding of agonists [^{3}H]dopamine and [^{3}H]apomorphine was saturable and of high affinity (Fig. 1). For most experiments, saturation data could be explained by assuming a single site model. In occasional experiments saturation data were significantly better explained by a two-site model. However, the inconsistency of this observation made its significance unclear. Thus, it appears that [^{3}H]dopamine and [^{3}H]apomorphine label either a single site of high affinity or a mixed population of binding sites having similar affinity.

Indeed, we (6, 14) and others (16, 17, 31) have previously reported that radiolabeled dopaminergic agonists

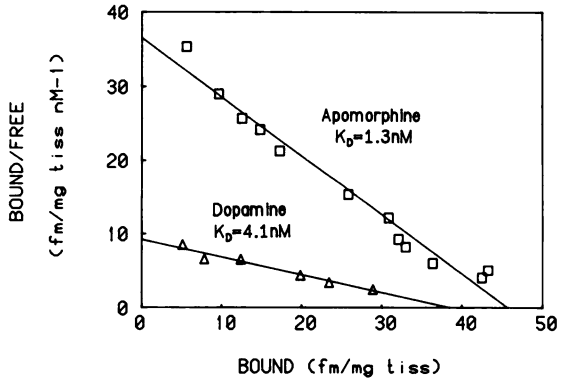

FIG. 1. *Scatchard plots of specific ^{3}H-agonist binding in rat striatum* Nonspecific binding was determined in the presence of 10 μM *cis*-flupentixol. Concentrations ranged from 0.5–15 and 0.1–20 nM for [^{3}H]dopamine and [^{3}H]apomorphine, respectively.

label heterogeneous populations of binding sites in striatum. Fig. 2 shows competition curves between the butyrophenone antagonist spiroperidol or the butyrophenone-like domperidone and agonists [^{3}H]dopamine and [^{3}H] apomorphine. These competition curves are clearly biphasic, suggesting that these antagonists discriminate two or more binding sites labeled by ^{3}H-agonists. Indeed computer-assisted analyses indicated the presence of two binding sites, one site having high and one site having low affinity for these antagonists. As has been suggested previously (6, 14, 16, 17, 31), the site having high affinity for these antagonists represents the D_2 dopamine receptor, while the remaining binding site has been called the D_3 dopamine-binding site (6, 14, 17, 19).

In Fig. 2, it can be seen that ^{3}H-agonist binding to D_3 dopaminergic sites could be studied selectively by including appropriate concentrations of spiroperidol or domperidone in the binding assays. Thus, 10–30 nM spiroperidol or 30–50 nM domperidone could be used to block ^{3}H-agonist binding to D_2 dopamine receptors without significantly inhibiting ^{3}H-agonist binding to D_3 binding sites. Employing this strategy, saturation studies of [^{3}H] dopamine and [^{3}H]apomorphine binding to D_3 binding sites were conducted (Fig. 3). Separate competition experiments were conducted at high and low concentrations of each radiolabeled agonist to determine the appropriate concentration(s) of spiroperidol or domperidone for these saturation studies. Thus, within the concentration ranges of ^{3}H-agonists used, 10–30 nM spiroperidol and 30–100 nM domperidone were used to selectively block [^{3}H]dopamine and [^{3}H]apomorphine binding, respectively, to D_2 dopamine receptors allowing saturation studies of D_3 binding sites to be conducted.

Pharmacological characteristics of antagonist interaction with D_3 sites labeled by [^{3}H]dopamine. For D_3 specific [^{3}H]dopamine binding in the presence of 10 nM spiroperidol, it can be shown that unlabeled antagonist competition curves are monophasic, with pseudo-Hill coefficients equal to 1. This observation is consistent with a simple bimolecular reaction of antagonists for this site. For example, Fig. 4 shows the experimental data and resulting computer-modeled competition curves for the

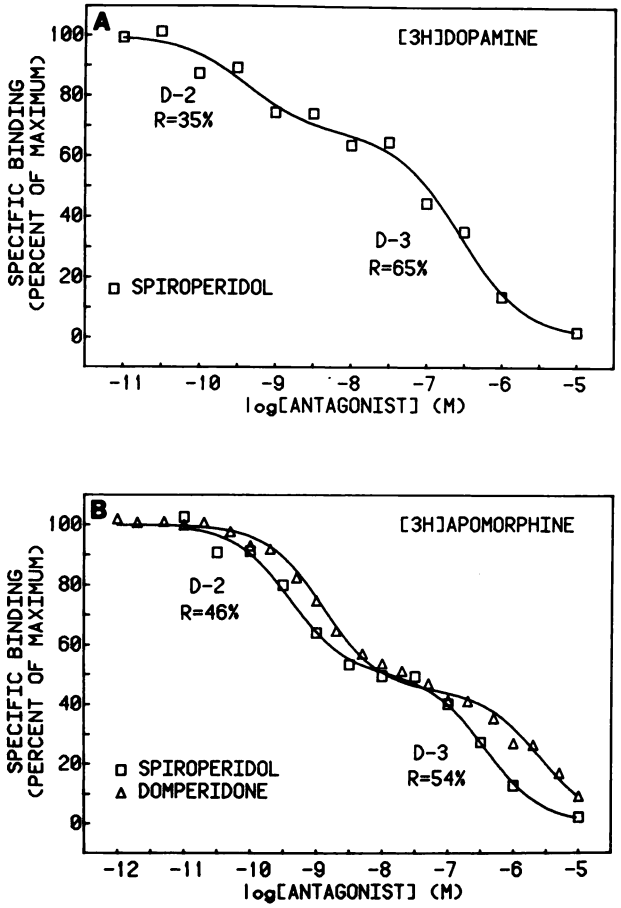

FIG. 2. *Spiroperidol and domperidone discriminate D_2 and D_3 components of [^{3}H]dopamine and [^{3}H]apomorphine binding*

A, inhibition of [^{3}H]dopamine binding. Inhibition constants for spiroperidol are 0.19 and 134 nM for D_2 and D_3 sites, respectively. [^{3}H] Dopamine concentration was 1.3 nM. B, inhibition of [^{3}H]apomorphine binding. Inhibition constants for spiroperidol and domperidone are 0.14 and 0.3 nM, respectively for D_2 sites, and 193 and 983 nM for D_3 sites, respectively.

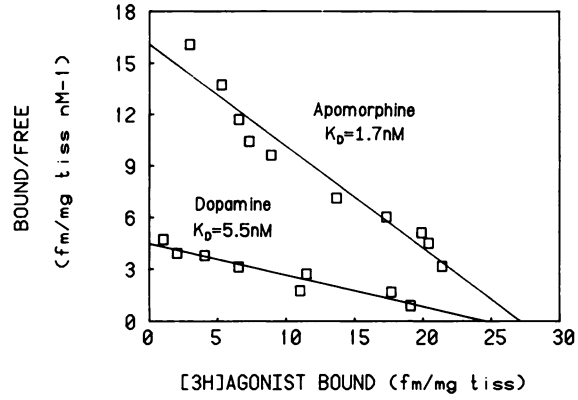

FIG. 3. *Scatchard plots of D_3 specific ^{3}H-agonist binding to membranes of rat striatum*

[^{3}H]Dopamine (0.2–16 nM) binding were conducted in the presence of 10 nM spiroperidol. [^{3}H]Apomorphine (0.1–16 nM) binding was conducted in the presence of 50 nM domperidone. *cis*-Flupentixol (10 μM) was used to determine nonspecific binding for both ligands.

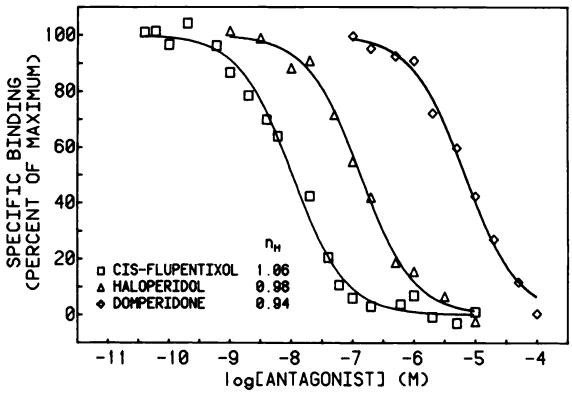

FIG. 4. *Computer-fitted curves for antagonist inhibition of D_3 specific [^{3}H]dopamine binding in rat striatum*

The experimentally determined points are from representative experiments using 2.3–2.8 nM [^{3}H]dopamine. The computer-drawn curves represent the best fit to the data assuming a single site model. Assumption of a two-site model did not improve the fit. Estimated inhibition constants are 6.4, 83, and 3960 nM for *cis*-flupentixol, haloperidol, and domperidone, respectively.

antagonists *cis*-flupentixol, haloperidol, and domperidone. These curves model best to a single homogeneous receptor population having apparent dissociation constants of 6.4, 83, and 3960 nM, respectively. The D_3 dopamine-binding site shows clear stereoselectivity. For the antagonist butaclamol, the active enantiomer at both D_1 and D_2 dopamine receptors (+)butaclamol is several orders of magnitude more potent than the inactive (−)butaclamol as an inhibitor of D_3 specific [³H]dopamine binding. A similar retention of stereospecificity is observed for all thioxanthenes (Table 1).

Antagonist inhibition of [³H]dopamine binding to the D_3 site appears to be competitive as indicated in Fig. 5. Performing D_3 specific [³H]dopamine saturation analyses in the presence of the antagonist fluphenazine decreases the apparent affinity of the radioligand but not its binding capacity. Table 1 shows the dissociation constants for a large number of dopaminergic antagonists and nondopaminergic drugs computed from competition experiments with [³H]dopamine. The rank order potency of these drugs is markedly similar to that observed for both the inhibition of [³H]flupentixol binding and dopamine-stimulated adenylate cyclase activity observed in the preceding article (3). This observation is explored in more detail in Fig. 6 (see Ref. 3; Fig. 5 and Table 1). These figures show that an impressive correlation exists between the K_i values for antagonists to inhibit D_3 binding and dopamine-stimulated adenylate cyclase activity ($r = 0.94, p < 0.001$). Also a strong correlation ($r = 0.945$, $p < 0.001$) is found between K_i values for antagonist

TABLE 1

Antagonist affinities for D_3 specific [³H]dopamine binding to membrane homogenates of rat striatum

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. [³H]Dopamine concentrations ranged from 2.1–2.8 nM. Dissociation/inhibition constants were determined using computer analysis as described in Materials and Methods. Each value represents means ± SE from 3–8 independent experiments. Other nondopaminergic agents tested, prazosin, propranolol, and scopolamine, had $K_i >> 10$ μM.

Antagonist	K_i
	nM
SCH 23390	0.72 ± 0.06
Piflutixol	6.7 ± 1.5
trans-Piflutixol	1,839 ± 403
cis-Flupentixol	12.4 ± 1.9
trans-Flupentixol	3,685 ± 528
(+)Butaclamol	31.6 ± 6.2
(−)Butaclamol	79,972 ± 10,452
Fluphenazine	28.9 ± 10.9
cis-Clopentixol	19.2 ± 3.4
trans-Clopentixol	881 ± 33
cis-Chlorprothixene	6.8 ± 0.6
trans-Chlorprothixene	443 ± 104
Teflutixol	120 ± 6.2
SKF 83742	13.6 ± 2.9
Chlorpromazine	68.3 ± 3.3
Haloperidol	67.7 ± 8.0
Spiroperidol	278 ± 55
Ketanserin	366 ± 51
Pimozide	969 ± 45
Domperidone	5,782 ± 1,433
Promethazine	7,293 ± 1,814
Molindone	8,039 ± 990
Yohimbine	3,492 ± 14
Cinnanserin	1,531 ± 143
Mianserin	371 ± 63
(−)Sulpiride	>10,000
(+)Sulpiride	>10,000
Metoclopramide	>10,000

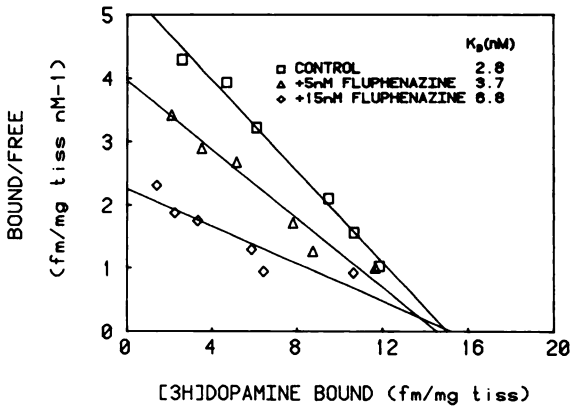

FIG. 5. *Scatchard plots from D_3 specific [³H]dopamine saturation experiments in the presence of increasing concentrations of fluphenazine*

Concentrations of [³H]dopamine ranged from 0.6 to 12 nM. Fluphenazine exhibits apparent competitive inhibition at D_3 binding sites.

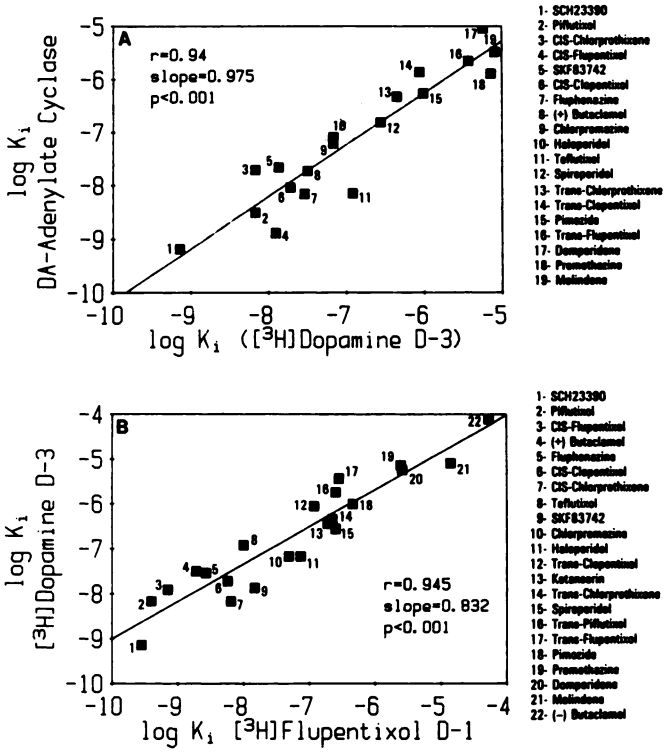

FIG. 6. *Correlation between antagonist inhibition of D_3 specific [³H] dopamine binding and inhibition of (A) dopamine (DA)-stimulated adenylate cyclase activity in homogenates of rat striatum or (B) D_1 specific [³H]flupentixol binding to membranes of rat striatum*

Log K_i values for inhibition of D_3 binding sites are derived from data in Table 1. Log K_i values for inhibition of dopamine-stimulated adenylate cyclase activity or D-1 receptor-specific [³H]flupentixol binding are derived from data in Table 1 of the preceding article (3).

inhibition of D_3 specific [^{3}H]dopamine binding and D_1 specific [^{3}H]flupentixol binding to rat striatum (Fig. 6B).

Interaction of agonists and ergot alkaloids with D_3 specific [^{3}H]dopamine binding. The ability of dopaminergic agonists and ergot alkaloids to compete for D_3 site specific [^{3}H]dopamine binding to rat striatum membranes was examined (Table 2). (−)*N*-Propylnorapomorphine and epinine curves exhibit a pseudo-Hill coefficient (n_H) of 0.91 and 1.02, respectively, and they model best to single homogeneous binding sites with K_D values of 2.8 and 7.4 nM, respectively. Ergot/[^{3}H]dopamine competition data and computer-modeled curves for ergot alkaloids exhibit pseudo-Hill coefficients (n_H) of around 1.0 and they model best to a single homogeneous site.

Table 2 shows the mean values for dissociation constants of dopamine agonists computed from competition experiments with D_3 specific [^{3}H]dopamine binding. Apparent dissociation constants were computed from fits modeled to a single binding site. To test the hypothesis that the D_3 dopaminergic binding site is related to the D_1 dopamine receptor, these K_D values are shown in comparison with K_H estimates derived from agonist and ergot versus [^{3}H]flupentixol competition experiments described in the preceding article (3). Fig. 7 shows that a significant correlation exists ($r = 0.875$, $p < 0.001$) between these two measures of agonist/ergot interactions with D_3 binding sites and D_1 dopamine receptors. In contrast, a much weaker or no significant correlation is found between dissociation constants of agonist/ergots at D_3 binding sites versus either their K_{act} for stimulating adenylate cyclase activity ($r = 0.51$, $p < 0.1$) or their K_L values derived from [^{3}H]flupentixol competition experiments ($r = 0.64$, $p < 0.02$).

In Table 3, the relationship between agonist interactions at the D_3 binding site and the D_1 dopamine receptor is examined further. The dissociation constants and B_{max} for [^{3}H]dopamine and [^{3}H]apomorphine binding to D_3 sites is compared to K_H and R_H values from [^{3}H]flupentixol competition experiments using these two agonists. Though comparable, these agonists apparently bind with higher affinity and to somewhat fewer numbers of D_3 sites when compared to their K_H and R_H estimates derived from competition experiments with [^{3}H]flupentixol. Also shown in Table 3 are apparent dissociation constants for dopamine and (−)apomorphine binding to D_3 sites derived from competition experiments using [^{3}H]dopamine. These affinity estimates (14 and 3.4 nM) are more similar to K_H estimates (23.2 and 7.8 nM for dopamine and (−)apomorphine, respectively) for agonist interactions with [^{3}H]flupentixol-labeled D_1 dopamine receptors.

Regulation of D_3 specific [^{3}H]dopamine binding by guanine nucleotides. Guanine nucleotides have been demonstrated to regulate agonist-receptor interactions in a variety of hormone and neurotransmitter systems (32), particularly those which regulate adenylate cyclase activity. In these systems, guanine nucleotides generally reduce the apparent agonist affinity for the receptor. Fig.

TABLE 2

Affinities of dopaminergic agonists and ergot alkaloids for D_3 specific [^{3}H]dopamine binding to membranes of rat striatum

Competition curves were constructed and analyzed as described in Fig. 4. [^{3}H]Dopamine concentrations ranged between 2.0 and 2.9 nM. Data are expressed as mean ± standard error for 3–5 independent experiments. NPA, *N*-propylnorapomorphine; ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

Drug	K_i
	nM
(−)Apomorphine	3.4 ± 1.4
(−)NPA	6.3 ± 3.5
(±)ADTN	5.9 ± 0.8
Dopamine	13.6 ± 4.3
Epinine	6.4 ± 0.5
SKF 38393	2.2 ± 0.7
Lisuride	6.6 ± 1.4
CF 25-397	5.6 ± 1.1
Ergotamine	6.8 ± 1.1
Lergotrile	8.4 ± 3.9
Dihydroergotamine	11.4 ± 0.9
Pergolide	12.6 ± 0.3
Dihydroergocriptine	23 ± 6
CQ 32-084	64 ± 14
CM 29-712	91 ± 26
Bromocriptine	75 ± 19

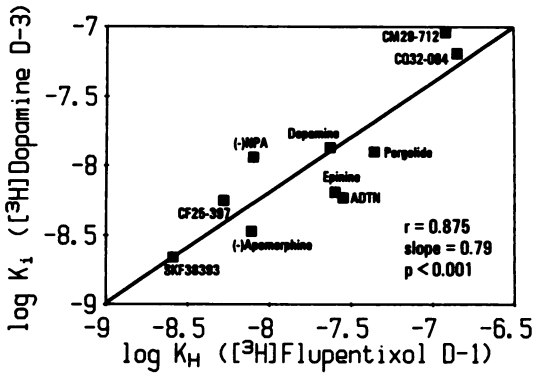

FIG. 7. *Correlation of agonist affinities for D_3 specific [^{3}H]dopamine binding and the high affinity agonist binding component of agonist/[^{3}H]flupentixol competition curves*

Data are from Table 2 of this article (K_i for D_3) and Table 2 of the preceding article (3) (K_H for D_1).

TABLE 3

Computed parameter estimates for agonist interactions at putative D_1 dopaminergic sites labeled by ^{3}H-agonists or ^{3}H-antagonists in rat striatal membrane homogenates

Means of computer-modeled parameter estimates for direct agonist-binding assays were determined for several independent D_3 specific ^{3}H-agonist saturation experiments ($n = 3$ for apomorphine and $n = 13$ for dopamine). Data for agonist/[^{3}H]dopamine competition experiments are derived from Table 2.

Agonist	^{3}HAgonist saturation		Agonist/[^{3}H] dopamine competition	Agonist/[^{3}H] flupentixol competition	
	K_D	R	K_i	K_H	R
	nM	*fmol/mg tissue*	*nM*	*nM*	*fmol/mg tissue*
Dopamine	5.7 ± 0.5	20 ± 1.5	14 ± 4	23 ± 12	30 ± 4
(−)Apomorphine	1.9 ± 0.2	28 ± 4	3.4 ± 1.4	7.8 ± 2.8	34 ± 4

8 shows that D_3 specific [^{3}H]dopamine binding is guanine nucleotide sensitive. However, even at high concentrations of guanine nucleotides [0.3 mM GTP or 0.1 mM Gpp (NH)p[2]] the inhibition of D_3 specific [^{3}H]dopamine binding was incomplete. In Fig. 8, saturation analyses of D_3 specific [^{3}H]dopamine binding shows that the addition of 0.3 mM GTP produces only a 40–50% decline in B_{max}. A similar decrease was observed using 0.1 mM Gpp(NH)p. The addition of these nucleotides did not appear to significantly affect the affinity of [^{3}H]dopamine binding. Separate dose-effect curves (not shown; see Ref. 6) indicate that these concentrations of guanine nucleotides produce maximal or near-maximal effect. Experiments using ATP suggested that at higher concentrations of guanine nucleotides nonspecific inhibition of [^{3}H]dopamine would be likely to occur.

The incomplete inhibition of D_3 specific [^{3}H]dopamine binding by guanine nucleotides resembles the incomplete "conversion" of D_1 agonist R_H sites to R_L sites observed in the preceding article (3). Fig. 9 shows a comparison of the effects of guanine nucleotides on both D_3 specific [^{3}H]dopamine binding and the relative density of R_H derived from D_1 specific dopamine/[^{3}H]flupentixol competition curves. GTP decreased these two putative measures of high affinity agonist binding to the D_1 dopamine receptor by 39 and 48% for D_3 [^{3}H]dopamine binding and R_H (D_1), respectively. The similarity in guanine nucleotide effects on these two measures of high affinity agonist binding is consistent with the hypothesis that the D_3 binding site is related to a high affinity agonist binding state of the D_1 dopamine receptor.

DISCUSSION

These studies as well as others (6, 14, 16, 17, 31) demonstrate that tritiated dopaminergic agonists label both D_2 dopamine receptors and D_3 binding sites on membranes of rat striatum. While much evidence supports the hypothesis that dopaminergic agonists can label a pharmacologically definable D_2 dopamine receptor in brain and pituitary (for review, see Ref. 2), the pharmacological identity of the D_3 dopaminergic binding site in the past has been unclear although we (2, 33) and others (31) have suggested it may represent a high affinity agonist-binding state of the D_1 dopamine receptor. The current studies were designed to test this hypothesis.

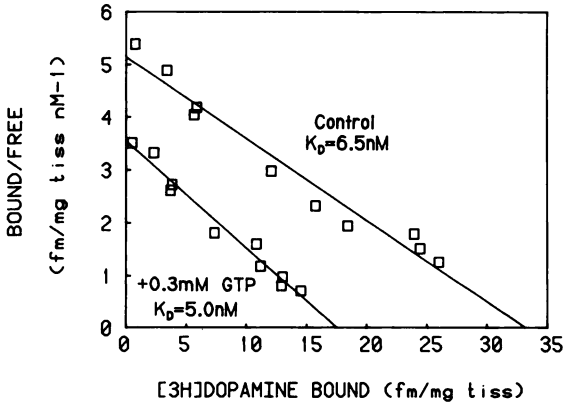

FIG. 8. *Scatchard plots of D_3 specific [^{3}H]dopamine binding in the presence and absence of GTP*

Representative experiments indicate a 40–50% decline in B_{max} in the presence of GTP. A similar decrease was observed using 0.1 mM Gpp(NH)p.

[2] The abbreviation used is: Gpp(NH)p, guanosine 5′-(β,γ-imido)triphosphate.

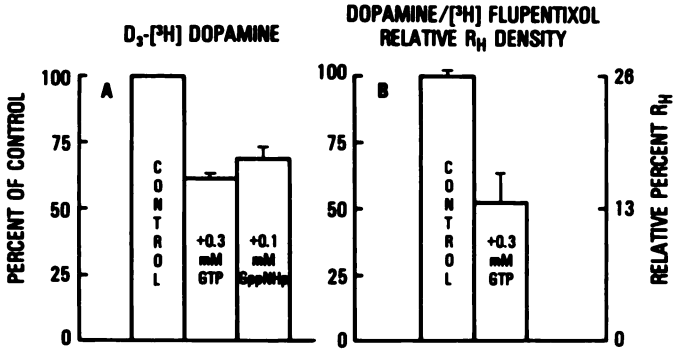

FIG. 9. *Effects of guanine nucleotides on D_3 specific [^{3}H]dopamine binding and %R_H estimates from dopamine/[^{3}H]flupentixol competition curves*

[^{3}H]Dopamine concentrations ranged from 2.3 to 3.2 nM. Results of guanine nucleotide effects on D_3 binding are shown as the mean ± standard error of the per cent control specific binding for seven independent experiments. Values for relative R_H site density from dopamine/[^{3}H]flupentixol competition curves are derived from data in the preceding article (3).

It was first necessary to characterize radiolabeled agonist binding to D_3 binding sites selectively. Competition curves between spiroperidol or domperidone and ^{3}H-agonists (Fig. 2) show that these drugs can be used to selectively block ^{3}H-agonist binding to D_2 dopamine receptors. Consequently, in the presence of low concentrations of spiroperidol (10–30 nM) or domperidone (30–50 nM), ^{3}H-agonist binding to D_3 sites can be characterized. [^{3}H]Dopamine and [^{3}H]apomorphine binding to D_3 sites was of high affinity (K_D values, 1–10 nM), was saturable, and reached equilibrium at 22° within 90 min (not shown).

The D_3 binding sites labeled by [^{3}H]dopamine are most certainly dopaminergic recognition sites. They showed clear stereoselectivity in interacting with dopaminergic antagonists such as butaclamol and thioxanthenes (Table 1). However, unlike the D_2 dopamine receptor, antagonists such as spiroperidol, pimozide, and domperidone are relatively weak (K_i values, 0.1–5 μM) while substituted benzamide neuroleptics such as sulpiride and metoclopramide are virtually inactive. This profile of antagonist rank order potencies is very similar to those seen for the D_1 dopamine receptor identified either by [^{3}H] flupentixol binding or by dopamine activation of adenylate cyclase. Indeed, impressive correlations are seen between antagonist inhibition constants for all three measures (Fig. 6; Fig. 5 in Ref 3). These results strongly support the hypothesis that D_3 binding sites are related to the D_1 dopamine receptor. Of course, the possibility remains that these two binding sites may be independent but share a highly similar pharmacological specificity for antagonists.

While a good correlation is found between antagonist

inhibition constants measured at [^{3}H]dopamine-labeled D_3 sites versus [^{3}H]flupentixol-labeled D_1 sites (Fig. 6B), careful inspection of these data shows that the most potent antagonists are as much as 10–30 times more potent inhibitors of ^{3}H-antagonist binding compared to their affinities at [^{3}H]dopamine-binding sites. Several factors may have contributed to this apparent discrepancy. First, the tissue concentrations used for [^{3}H]flupentixol- and [^{3}H]dopamine-binding assays differed by about 3.5-fold (0.85 vs. 3.0 mg/ml, respectively). At the higher tissue concentrations used in [^{3}H]dopamine-binding assays, depletion of the cold antagonists to specific and nonspecific binding sites at low antagonist concentration may produce apparently higher inhibition constants. Second, as discussed in the preceding article (3), the involvement of an accessory antagonist-binding site adjacent to the D_1 receptor "pharmacophore" may explain why thioxanthenes and phenothiazines inhibit [^{3}H]flupentixol binding more potently than they inhibit dopamine-stimulated adenylate cyclase. Indeed, the absolute potency of thioxanthene and phenothiazine antagonists to inhibit D_3 specific [^{3}H]dopamine binding agrees more closely with their *in vitro* potencies to inhibit dopamine-stimulated adenylate cyclase (Fig. 6A). Finally, ^{3}H-agonists may bind to an agonist-preferring state of the D_1 dopamine receptor which has lower affinity for some antagonists when compared to an "antagonist-preferring" conformation of the receptor.

It is well known that dopamine agonists generally stimulate D_1 dopamine receptors *in vitro* with micromolar potencies. How can the hypothesis that D_3 binding sites (exhibiting nanomolar affinity for these agonists) are related to the D_1 dopamine receptor be reconciled with this observation? The preceding article (3) has presented data suggesting that the D_1 dopamine receptor exhibits heterogeneous binding properties for agonists. Competition curves between full agonists and [^{3}H]flupentixol exhibited extremely shallow slope factors ($n_H <$ 0.5), and computer modeling of these curves suggested either the presence of more than one agonist-binding state of the D_1 receptor or the presence of multiple receptor subtypes having identical affinity for [^{3}H]flupentixol but different affinities for agonists. As shown in Fig. 7, a significant correlation exists between the K_H agonist affinity estimates and agonist affinities for D_3 specific [^{3}H]dopamine-binding sites. These data again suggest that dopaminergic agonists interact with a high affinity form of the D_1 dopamine receptor which appears to be also the D_3 dopaminergic binding site.

On the other hand, agonist affinities at a lower affinity component of agonist/[^{3}H]flupentixol competition curves (K_L) correlate well with agonist activation constants (K_{act}) for stimulating adenylate cyclase activity (see Ref. 3, Fig. 12). Thus, agonists discriminate high and low affinity states of the D_1 dopamine receptor, and affinities for the low affinity agonist-binding state appear to correlate in absolute potency with estimates of K_{act} for adenylate cyclase activation (3). It should be noted that a weaker, although significant, correlation was observed between agonist K_H estimates from agonist/[^{3}H]flupentixol competition experiments and K_{act} estimates from the literature (3). However, K_{act} estimates are shifted by up to 2.5 orders of magnitude weaker than K_H estimates. Indeed the presence of large absolute differences in agonist K_{act} and K_i values for [^{3}H]dopamine binding explains the observation of Seiler and Markstein (34) that the K_{act} for a series of monohydroxyaminotetralins correlates strongly with their IC_{50} values against [^{3}H]dopamine binding.

These data suggest that agonists exhibit heterogeneous affinities for the D_1 dopamine receptor, demonstrable by agonist interactions at both radiolabeled agonist- and antagonist-binding sites. This heterogeneity may represent the presence of interconvertible states of a single D_1 dopamine receptor as has been proposed previously for other catecholaminergic receptors (12, 27, 35, 36). This hypothesis is partly supported by the observation that saturating concentrations of guanine nucleotides promote an apparent decrease in D_3 site specific ^{3}H-agonist binding site density as well as an apparent decrease in agonist R_H densities derived from agonist/[^{3}H]flupentixol competition curves.

As described in the preceding article (3), these data may be explained in part by a generalized ternary complex model modified from the original models of Boeynaems and Dumont (37, 38) and Jacobs and Cuatrecasas (39). The effect of guanine nucleotides on high affinity agonist binding was incomplete. At the highest concentrations of guanine nucleotides used, high affinity agonist binding decreased by only 40–60%. This incomplete effect of guanine nucleotides may be due to equilibrium constraints whereby even saturating concentrations of guanine nucleotides do not decrease affinities of receptor (R) for a third membrane component (X) sufficiently to prevent entirely ternary complex formation. On the other hand, the incomplete conversion may reflect the occurrence of a covalent coupling between receptor and guanine nucleotide-binding protein as has been proposed for the cardiac β-adrenergic receptor (40). Such incomplete effects of guanine nucleotides on high affinity agonist binding have been observed for a number of other central nervous system neurotransmitter receptors (8, 10, 41–43).

In the current study, we observe that ergot alkaloids compete for D_3 specific [^{3}H]dopamine binding with high affinity. Our estimates of ergot affinities for these sites are similar to values published previously by others (44–47). However, data from the present study taken together with ergot/[^{3}H]flupentixol competition data detailed in the preceding article (3) provide the first evidence that ergot alkaloid agonists can discriminate high and low affinity agonist-binding states of the D_1 dopamine receptor. As these compounds are partial agonists at the D_1 dopamine receptor, it appears that partial agonists compete with comparable affinities for both [^{3}H]dopamine binding and R_H derived from ergot/[^{3}H]flupentixol competition experiments. Interestingly, four ergots that are generally considered antagonists at the D_1 dopamine receptor (lisuride, lergotrile, ergotamine, dehydroergocriptine) also competed for D_3 sites with high affinity. As discussed in the preceding article (3), we speculate that these compounds may be predicted to exert some

minimal partial agonist activity under optimal conditions at the D_1 dopamine receptor. Experiments to test this prediction are currently in progress.

Table 3 shows a comparison of estimates of agonist affinities and binding site densities for high affinity sites determined either by direct ^{3}H-agonist labeling or agonist/[^{3}H]flupentixol competition experiments. The apparent discrepancies between mean affinity and B_{max} values for these two putative measures of high affinity agonist binding to the D_1 receptor are difficult to evaluate. Indeed, these estimates depend upon accurate measures of radioligand specific activities and correct estimates of K_D and B_{max} for the radiolabeled antagonist used in competitions. Furthermore, individual affinity and site density values derived from indirect or direct agonist-binding experiments showed some overlap for both dopamine and apomorphine. Thus, given the limitations inherent in our available means of analysis, the significance of the apparent discrepancies in absolute values for these comparative affinity and site density estimates for high affinity agonist binding is unclear.

Regardless of the means by which high affinity agonist-binding sites were measured, the sites comprised only 15–40% of the number of sites which could be labeled by ^{3}H-antagonists. Such limits on the formation of the agonist R_H state may reflect either equilibrium constraints on the formation of LRX or a functional stoichiometric limitation of X relative to R (48). We speculate that limitations on the formation of LRX are due to the stoichiometric limitation of available X for the following reasons. The relatively large K_L/K_H ratio seen for full agonists suggests that these agonists strongly favor RX formation ($K_2 << K_4$ and $K_3 << K_1$ in the ternary complex model). However, agonists of much lower intrinsic activity appear to promote the formation of nearly as many apparent R_H sites even though their accompanying K_L/K_H ratios (and thus K_4/K_2 and K_1/K_3) are much lower. Indeed, a stoichoimetric limitation of X relative to R could produce such behavior (48) whereby a "ceiling" is present on the maximum formation of R_H by agonists. This hypothesis could be tested theoretically by varying the normal ratios of X/R in the membrane. This can be accomplished by decreasing the relative concentration of the total functional R in the membrane using an irreversible blocking agent (49). Under these conditions, the ratio of X to R would be increased and the proportion of total R that would be present as R_H would be predicted to increase. Unfortunately, when R is decreased significantly (>50%) using the irreversible receptor alkylating agent phenoxybenzamine (7, 49), agonist/[^{3}H]flupentixol competition curves are unsatisfactory due to the compromised ratio of specific to total [^{3}H]flupentixol binding under these conditions.[3] Nevertheless, phenoxybenzamine concentrations which inhibit 90% of [^{3}H]flupentixol binding inhibit only 50–60% of D_3 specific [^{3}H]dopamine or [^{3}H] apomorphine binding.[3]

The possibility that the X component, presumably a guanine nucleotide-binding protein, may be limiting in this system poses intriguing possibilities regarding the regulation of the functional sensitivity of the D_1 dopamine receptor. The involvement of a ternary complex as a functional intermediate in adenylate cyclase-stimulatory hormonal receptor systems has been studied extensively by Lefkowitz and colleagues (50). If indeed the guanine nucleotide-binding protein is a limiting factor for the D_1 dopamine receptor function in striatal membranes, and if these *in vitro* measurements reflect the conditions of D_1 receptor-effector coupling *in vivo*, then one might speculate that the functional sensitivity of D_1 dopamine receptors in striatum may be strongly modulated by changes in the relative availability of the guanine nucleotide-binding protein which regulates adenylate cyclase activation by this transmitter.

[3] S. Leff, unpublished observation.

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REFERENCES

1. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature* **277**:93–96 (1979).
2. Creese, I., D. R. Sibley, M. W. Hamblin, and S. E. Leff. The classification of dopamine receptors: relationship to radioligand binding. *Annu. Rev. Neurosci.* **6**:43–71 (1983).
3. Leff, S. E., M. W. Hamblin, and I. Creese. 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. *Mol. Pharmacol.* **27**:171–183 (1984).
4. Hyttel, J. Effects of neuroleptics on [^{3}H]-haloperidol and [^{3}H]-*cis*-(*Z*)-flupentixol binding and on adenylate cyclase activity in vitro. *Life Sci.* **23**:551–556 (1978).
5. Hyttel, J. A comparison of the effect of neuroleptic drugs on the binding of ^{3}H-haloperidol and ^{3}H-*cis*(*Z*)-flupentixol and on adenylate-cyclase activity in rat striatal tissue in vitro. *Prog. Neuro-Psychopharm.* **2**:329–335 (1978).
6. Hamblin, M. W., and I. Creese. ^{3}H-Dopamine binding to rat striatal D-2 and D-3 sites: enhancement by magnesium and inhibition by sodium. *Life Sci.* **30**:1587–1595 (1982).
7. Hamblin, M., and I. Creese. Phenoxybenzamine treatment differentiates dopaminergic ^{3}H-ligand binding sites in bovine caudate membranes. *Mol. Pharmacol.* **21**:44–51 (1982).
8. Hamblin, M. H., S. E. Leff, and I. Creese. Interactions of agonists with D-2 dopamine receptors: evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes. *Biochem. Pharmacol.*, **33**:877–887 (1984).
9. Huff, R. M., and P. B. Molinoff. Quantitative determination of dopamine receptor subtypes not linked to activation of adenylate cyclase in rat striatum. *Proc. Natl. Acad. Sci. U.S.A.* **79**:7561–7565 (1982).
10. Lazareno, S. Effects of GTP on ^{3}H-domperidone and its displacement by dopamine in rat striatal homogenates. *J. Receptor Res.* **3**:163–175 (1983).
11. Sibley, D. R., S. E. Leff, and I. Creese. Interactions of novel dopaminergic ligands with D-1 and D-2 dopamine receptors. *Life Sci.* **31**:637–645 (1982).
12. Sibley, D. R., A. De Lean, and I. Creese. Anterior pituitary dopamine receptors: demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J. Biol. Chem.* **257**:6351–6361 (1982).
13. De Lean, A., B. R. Kilpatrick, and M. G. Caron. Dopamine receptor of the porcine anterior pituitary gland: evidence for two affinity states discriminated by both agonists and antagonists. *Mol. Pharmacol.* **22**:290–297 (1982).
14. Leff, S. E., and I. Creese. Dopaminergic D-3 binding sites are not presynaptic autoreceptors. *Nature* **306**:586–589 (1983).
15. Bacopoulos, N. G. Acute changes in the state of dopamine receptors: in vitro monitoring with [^{3}H]dopamine. *Life Sci.* **29**:2407–2414 (1981).
16. List, S. J., K. A. Wreggett, and P. Seeman. Striatal binding of 2-amino-6,7-[^{3}H]dihydroxy-1,2,3,4-tetrahydronaphthalene to two dopaminergic sites distinguished by their low and high affinity for neuroleptics. *J. Neurosci.* **2**:895–906 (1982).
17. Sokoloff, P., M.-P. Martres, and J.-C Schwartz. [^{3}H]Apomorphine labels both dopamine post-synaptic and autoreceptors. *Nature* **288**:283–286 (1980).
18. Schweitzer, B. I., and N. G. Bacopoulos. Reversible decrease in dopaminergic

^{3}H-agonist binding after 6-hydroxydopamine and irreversible decrease after kainic acid. *Life Sci.* **32**:531–540 (1982).
19. Titeler, M., S. List, and P. Seeman. High affinity dopamine receptors (D_3) in rat brain. *Comm. Psychopharmacol.* **3**:411–420 (1979).
20. Nagy, J. T., T. Lee, P. Seeman, and H. C. Fibiger. Direct evidence for presynaptic and post-synaptic dopamine receptors in brain. *Nature* **278**:577–578 (1978).
21. Schwarcz, R., I. Creese, J. T. Coyle, and S. H. Snyder. Dopamine receptors localized on cerebral cortical afferents to rat corpus striatum. *Nature* **271**:766–768 (1978).
22. Leff, S., L. Adams, J. Hyttel and I. Creese. Kainate dissociates striatal dopamine receptor radioligand binding sites. *Eur. J. Pharmacol.* **70**:71–75 (1981).
23. Cross, A. J., and J. L. Waddington. Kainic acid lesions dissociate [^{3}H] spiperone and [^{3}H]*cis*-flupenthixol binding sites in rat striatum. *Eur. J. Pharmacol.* **71**:327–332 (1981).
24. Leff, S., D. R. Sibley, M. Hamblin, and I. Creese. Ascorbic acid enables reversible dopamine receptor ^{3}H-agonist binding. *Life Sci.* **29**:2081–2090 (1981).
25. Heikkila, R. E., and F. S. Cabbat. Studies on the stability of ^{3}H-dopamine in neostriatal membrane preparations: effects of ascorbic acid. *Life Sci.* **32**:847–853 (1983).
26. Bacopoulos, N. G. Dopamine receptors in rat brain regions. *Biochem. Pharmacol.* **31**:3085–3091 (1982).
27. De Lean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
28. Scatchard, G. The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
29. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
30. Burgisser, E. Model testing in radioligand/receptor interaction by Monte Carlo stimulation. *J. Receptor Res.* **3**:261–281 (1983).
31. Bacopoulos, N. G. [^{3}H]Dopamine binds to D-1 and D-2 receptors in rat striatum. *Eur. J. Pharmacol.* **87**:353–356 (1983).
32. Rodbell, M. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* **284**:17–22 (1980).
33. Creese, I., D. R. Burt, and S. H. Snyder. Biochemical actions of neuroleptic drugs: focus on the dopamine receptor. In *Handbook of Psychopharmacology*, (L. L. Iversen, S. D. Iversen, and S. H. Snyder, eds.), Vol. 10. Plenum Press, New York 37–81 (1978).
34. Seiler, M. P., and R. Markstein. Further characterization of structural requirements for agonists at the striatal dopamine D-1 receptor. *Mol. Pharmacol.* **22**:281–289 (1982).
35. Heidenreich, K. A., G. A. Weiland, and P. B. Molinoff. Effects of magnesium and *N*-ethylmaleimide on the binding of [^{3}H]hydroxybenzylisoproterenol to β-adrenergic receptors. *J. Biol. Chem.* **257**:804–810 (1982).
36. Hoffman, B. B., T. Michel, T. B. Brenneman, and R. J. Lefkowitz. Interactions of agonists with platelet alpha$_2$-adrenergic receptors. *Endocrinology* **110**:926–932 (1982).
37. Boeynaems, J. M., and J. E. Dumont. Quantitative analysis of the binding of ligands to their receptors. *J. Cyclic Nucleotide Res.* **1**:123–142 (1975).
38. Boeynaems, J. M., and J. E. Dumont. The two-step model of ligand-receptor interaction. *Mol. Cell Endocrinol.* **7**:33–47 (1977).
39. Jacobs, S., and P. Cuatrecasas. The mobile receptor hypothesis and "cooperativity" of hormone binding: application to insulin. *Biochim. Biophys. Acta* **433**:482–495 (1976).
40. Venter, J .C., C. M. Fraser, A. I. Soiefer, D. R. Jeffery, W. L. Strauss, R. R. Charlton, and R. Greguski. Autoantibodies and monoclonal antibodies to β-adrenergic receptors: their use in receptor purification and characterization. *Adv. Cyclic Nucleotide Res.* **14**:135–143 (1981).
41. Birdsall, N. J. M., C. P. Berrie, A. S. V. Burgen, and E. C. Hulme. Modulation of the binding properties of muscarinic receptors: evidence for receptor-effector coupling. In *Receptors for Neurotransmitters and Peptide Hormones* (G. Pepeu, M. J. Kuhar, and S. J. Enna, eds.). Raven Press, New York, 107–116 (1980).
42. Ehlert, F. J., W. R. Roeske, and H. I. Yamamura. Regulation of muscarinic receptor binding by guanine nucleotides and *n*-ethylmaleimide. *J Supramol. Struct.* **14**:149–162 (1980).
43. Mallat, M., and M. Hamon. Ca^{2+}-guanine nucleotide interactions in brain membranes. I. Modulation of central 5-hydroxytryptamine receptors in the rat. *J. Neurochem.* **38**:151–161 (1982).
44. List, S. J., and P. Seeman. [^{3}H]Dopamine labeling of D_3 dopaminergic sites in human, rat, and calf brain. *J. Neurochem.* **39**;1363–1373 (1982).
45. Goldstein M., J. Y. Lew, A. Sauter, and A. Lieberman. The affinities of ergot compounds for dopamine agonist and dopamine antagonist receptor sites. In *Ergot Compounds and Brain Function: Neurotransmitter and Neuropsychiatric Aspects* (M. Goldstein *et al.*, eds.). Raven Press, New York, 75–82 (1980).
46. Makman, M. H., H. S. Ahn, L. J. Thal, N. S. Sharpless, B. Dvorkin, S. G. Horowitz and M. Rosenfeld, Makman, M. H., R. K. Mishra, and J. H. Brown. Drug interactions with dopamine-stimulated adenylate cyclases of caudate nucleus and retina: direct agonist effect of a piribedil metabolite. *Adv. Neurol.* **9**:213–222 (1975).
47. Templeton, W. W., and G. N. Woodruff. Binding of [^{3}H]ADTN to rat striatal membranes. *Biochem. Pharmacol.* **31**:1629–1632 (1982).
48. Boeynaems, J. M., and J. E. Dumont. *Outlines of Receptor Theory.* Elsevier/North-Holland (1980).
49. Sibley, D. R., and I. Creese. Regulation of ligand binding to pituitary D-2 dopaminergic receptors: effects of divalent cations and functional group modification. *J. Biol. Chem.* **258**:4957–4965 (1983).
50. Lefkowitz, R. J., M. G. Caron, T. Michel, and J. M. Stadel. Mechanisms of hormone receptor-effector coupling: the β-adrenergic receptor and adenylate cyclase. *Fed. Proc.* **41**:2664–2670 (1982).

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