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Agonist High- and Low-Affinity States of the D_2-Dopamine Receptor in Calf Brain

Partial Conversion by Guanine Nucleotide

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SUMMARY

In order to determine whether D_2-dopaminergic receptors in brain exist in different affinity states for agonists and whether these receptors could be completely converted from their agonist high-affinity state to their agonist low-affinity state, we examined the effect of a guanine nucleotide on the competition between [^{3}H]spiperone and dopamine agonists for binding to homogenates of calf caudate nucleus. [^{3}H]spiperone labeled sites having different affinities for agonists as well as antagonists. Agonists recognized three components of [^{3}H]spiperone binding. Two of these components were related to the D_2-dopaminergic receptor. These two sites appeared to represent interconvertible states, each having different affinities for agonists. This was supported by the observation of an apparent guanine nucleotide-induced "conversion" of sites with high affinity to those having low affinity for the agonist. This effect of the guanine nucleotide was incomplete, such that a significant proportion of the high-affinity sites (21%) remained in the presence of an excess of the nucleotide. These high-affinity, guanine nucleotide-insensitive sites may represent a distinct class of binding sites having high affinity for both agonists and antagonists or may be the result at equilibrium of an agonist-independent interaction of the receptor and the guanine nucleotide.

INTRODUCTION

Several dopaminergic binding sites have been identified in the central nervous system, where much of the work has been directed toward their qualitative characterization. As a first approach, these sites can be classified according to their relative affinities for dopaminergic agonists and antagonists (1, 2).

The D_1 site has micromolar affinity for dopaminergic agonists and either nanomolar or micromolar affinity for butyrophenone antagonists, but is not sensitive to *S*-sulpiride and domperidone (3). The D_1 site can be studied using [^{3}H]*cis*-flupenthixol and [^{3}H]*cis*-piflutixol (4, 5). This site is associated with the stimulation of adenylate cyclase (6), but at present it has no known physiological relevance.

The D_2 site can be characterized as having nanomolar affinities for antagonists (including *S*-sulpiride and domperidone) and micromolar affinities for agonists, in general. This site can be labeled with ^{3}H-neuroleptics, ^{3}H-ergots and, under appropriate conditions, [^{3}H]NPA[2] (ref. 1). This D_2 site is proposed to be the physiological receptor in brain, since its affinities for dopaminergic congeners correlates very well with the doses required to elicit dopaminergic behavior (1). The D_2 site may be associated with the attenuation of adenylate cyclase (7, 8).

The D_3 site has nanomolar affinities for agonists and micromolar affinities for antagonists. Under appropriate conditions, the D_3 site is labeled by [^{3}H]dopamine, [^{3}H]apomorphine, [^{3}H]ADTN, and [^{3}H]NPA (ref. 1). The D_3 sites may be related to dopamine autoreceptors (9) or to dopamine-stimulated adenylate cyclase (10).

A D_4 site has also been suggested. This site is characterized as having nanomolar affinity for both agonists and antagonists. Binding sites having these characteristics have been identified in rat striatum and calf caudate nucleus (11, 12).

Studies of the D_2-dopaminergic receptor in brain, using [^{3}H]spiperone, have shown that, generally, both agonist and antagonist competition curves are "shallow" (Hill coefficient, $n_H < 1$) (13). Guanine nucleotides specifically decrease the affinity of agonists for D_2-dopaminergic

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[2] The abbreviations used are: NPA, (±)-*N*-*n*-propylnorapomorphine; ADTN, (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; Gpp(NH)p, 5′-guanylyl imidodiphosphate.

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receptors in brain (13, 14). Early reports qualitatively described this effect as a 3- to 4-fold increase in the IC_{50} for agonists against [^{3}H]spiperone with only a slight increase in their Hill coefficients from 0.5 to 0.6 (13).

Recent reports have demonstrated the existence of high- and low-affinity states of the D_2-dopaminergic receptor in the anterior pituitary (15, 16) with the existence of the low-affinity form predominating at equilibrium in the presence of guanine nucleotides.

In light of these observations, it has been suggested that the D_2-dopaminergic receptor in brain may also exist in different affinity states for agonists, and that the D_4 site is actually the high-affinity state of the brain D_2-dopaminergic receptor (14, 15). To test this hypothesis, we have examined the interaction of selected dopaminergic congeners with the binding sites labeled by [^{3}H]spiperone to membranes prepared from calf caudate nucleus. The possible relation between these sites, as differentiated by the ligands, was considered by studying the effect of a guanine nucleotide. Quantitative analysis of the data was conducted using computer-assisted curve fitting. Preliminary reports of these findings have been published (17, 18).

MATERIALS AND METHODS

Drugs. [*phenyl*-^{3}H]Spiperone (29.9–34.9 Ci/mmole in ethanol) was purchased from New England Nuclear Corporation (Boston, Mass.). Stock solutions were prepared in ethanol/0.1% ascorbic acid at a concentration of 200 nM and stored for up to 1 month at −20° in foil-wrapped, glass scintillation vials.

Generous donations of drugs were made by Ayerst Research Laboratories (Montreal, Que.), (+)-butaclamol; Eli Lilly & Company (Toronto, Ont.), (±)-LY156258; Janssen Pharmaceuticals (Beerse, Belgium), R43448, spiperone; Merck Sharp & Dohme Canada, Ltd. (Dorval, Que.), (−)-apomorphine; Professor John Neumeyer, Northeastern University, NPA; Ravizza (Milan, Italy), (−)-sulpiride; Sandoz Products Ltd. (Montreal, Que.), bromocriptine; Research Biochemicals Inc. (Wayland, Md.), ADTN.

All other drugs were purchased from Sigma Chemical Company (St. Louis, Mo.).

Reagents. All materials used in the buffer were purchased from either Fisher Scientific Company (Pittsburgh, Pa.) or Sigma Chemical Company. Gpp(NH)p, as the sodium salt, was purchased from Sigma Chemical Company.

Membrane preparation. Whole calf brains were obtained from a local slaughterhouse (Hunnisett Plant, Canada Packers Company, Toronto). The caudate nucleus was dissected over ice using a glass Petri dish as a supporting platform and kept on ice until the dissection of all brains was completed (2–3 hr postmortem). The tissue, essentially free of the lateral wall of the lateral ventricle and much of the internal capsule, was placed in 10 volumes of a buffer consisting of 50 mM Trizma base (pH 7.4, 21°) and 1 mM EDTA. After briefly dispersing the tissue with a metal spatula, the suspension was homogenized in a glass vessel with a Teflon piston (clearance = 0.18 mm) rotating at 500 rpm, using 20 up-and-down strokes. No attempt was made to keep the suspension ice-cold during this short procedure. Aliquots of either 5 ml or 3 ml of the crude homogenate were then stored in glass scintillation vials at −20° for up to 3 months.

[^{3}H]Spiperone binding assays. To obtain accurate estimates of the binding parameters in an equilibrium binding assay, it was necessary to utilize very low concentrations of both the tissue and [^{3}H]spiperone. These limitations necessitated the use of 20-ml incubation volumes in order to detect sufficient bound radioactivity.

Quadruplicate (for agonists) and triplicate (for antagonists) determinations were conducted in flint glass test tubes (25 × 150 mm), each receiving the following aliquots in the order listed, and delivered, unless otherwise specified, using Brinkmann Eppendorf polypropylene pipette tips: 16.0 ml of basic incubation buffer [using a 10-ml repipettor (Labindustries, Berkeley, Calif.)] containing 50 mM Trizma base (pH 7.4, 22°), 1 mM EDTA, 5 mM KCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.01% (0.6 mM) ascorbic acid; 1 ml of incubation buffer (with or without experimental reagent); and 1 ml of [^{3}H]spiperone (final concentrations of 20–60 pM). The reaction was initiated by the addition of 1 ml of a membrane preparation that had been prepared as follows. Upon thawing, the original crude homogenate was diluted with 9 volumes of preincubation buffer (essentially the same as the incubation buffer except that it lacked ascorbic acid; pH 7.4, 37°). The tissue was incubated in a water bath at 37° for 30 min (shaken at 100 rpm), further homogenized (Brinkmann Polytron; PT-10; 10 sec; setting at 7), and cooled on ice to 2–4°, at which time it was added to the assay tube and then briefly vortexed. This preincubation procedure consistently revealed a large component of high-affinity agonist competition. The final protein concentration, measured by the method of Miller (18a), was 40–60 μg/ml of incubate.

The incubate was left to equilibrate at room temperature for 3 hr. Preliminary experiments showed that steady state was attained within this time under these assay conditions (data not shown). The reaction was terminated by rapid vacuum filtration of the entire 20 ml through a Whatman glass-fiber filter (GF/B; 2.4-cm diameter) supported by a stainless steel mesh in a glass funnel. This filtration was facilitated by decanting the suspension along a disposable glass rod (2 × 50 mm). The filter was then washed with 20 ml of ice-cold incubation buffer, 10 ml first being used to rinse the tube (and decanted using the same glass rod) and the rest being delivered directly onto the filter. The filter was placed directly into a polypropylene scintillation vial to which were added 8 ml of either ACS (Amersham; Arlington Heights, Ill.) or Scint-A (Packard Instrument Company, Inc., Downer Grove, Ill.) and then left at 4° for at least 6 hr. Bound radioactivity was determined by liquid scintillation spectrometry using either a Packard B2450 (19–22% efficiency) or a Packard 450CD (35–40% efficiency).

Data analysis. Equations based on the classical law of mass action were fitted to the experimental data using an iterative procedure based on the nonlinear, least-squares algorithm of Marquardt (19) with a Digital LSI 11/23 processor (20). Total competable binding was determined in two ways, both of which gave similar results; either the minimal binding of [^{3}H]spiperone was measured in the presence of 10 μM (+)-butaclamol or, if suitable data were obtainable, the minimal binding of [^{3}H]spiperone was determined by iteration. The data were weighted by allowing the variance to be a constant percentage of the mean of replicate determinations. This weighting system was chosen as preliminary studies had shown that there was a good correlation of the values for the mean and the standard error. In most cases, obtained estimates of the binding parameters were comparable when any weighting system was used. The "goodness of fit" to the model was evaluated by considering the value of n (the number of binding sites) at which the correlation of neighboring residuals was no longer significant (21). Simultaneous analysis of some of the data was conducted using the curve-fitting program developed by De Lean *et al.* (22). All other statistical applications were conducted using Student's two-tailed t-test.

RESULTS

Competition for [^{3}H]spiperone binding by antagonists. Preliminary saturation curves with [^{3}H]spiperone were first conducted to determine the dissociation constant of the radioligand. The obtained value of 50 pM, determined by Scatchard analysis (Fig. 1), was used in all further analyses. Spiperone/[^{3}H]spiperone curves were "shallow," with Hill coefficients of 0.84 ± 0.01 (Table 1). *S-*

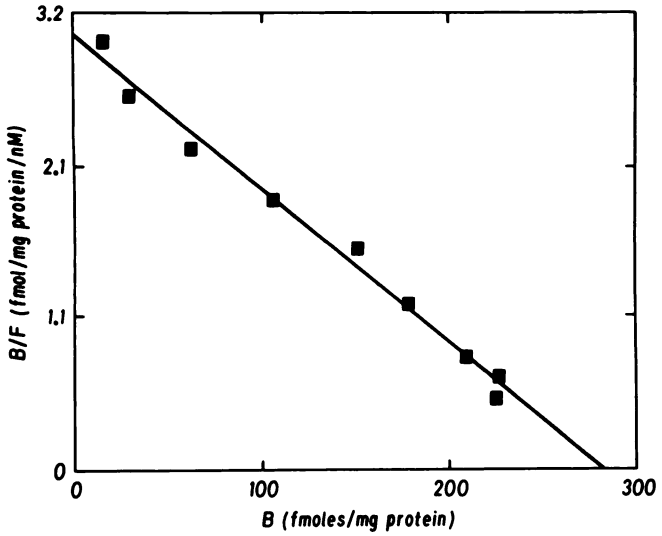

FIG. 1. *Saturation of [^{3}H]spiperone in calf caudate membranes*
Saturation experiments were conducted typically using [^{3}H]spiperone concentrations ranging from 0.01 to 1.0 nM. Scatchard analysis indicated a single binding component with a pK_D of 10.3 ± 0.2 (K_D = 50 ± 30 nM) and a capacity of 280 fmoles/mg of protein.

Sulpiride/[^{3}H]spiperone curves were also shallow, with Hill coefficients of 0.76 ± 0.01 (Table 1). Binding of [^{3}H]spiperone in the presence of an excess of the competing drug was the same for both antagonists and identical with binding measured in the presence of 10 μM (+)-butaclamol. In each case, the data were best described assuming two populations of binding sites. The relative proportions of the two sites were the same for both antagonists, with the low-affinity site contributing to 17% of the total displaceable binding (Fig. 2). Discrimination of these two sites was enhanced by examining *S*-sulpiride competition in the presence of NaCl. With 10 mM NaCl, the binding characteristics of *S*-sulpiride were dramatically altered: Na^+ produced almost a 60-fold increase in the affinity of *S*-sulpiride at the high-affinity site without significantly affecting interaction at the low-affinity site (Fig. 2B). There was no observable effect of 10 mM NaCl on the binding of [^{3}H]spiperone alone. This effect on the binding of *S*-sulpiride was not significantly greater at 100 mM NaCl, although a marked inhibitory effect on the total binding of [^{3}H]spiperone was observed at this high concentration of NaCl (data not shown).

Competition for [^{3}H]spiperone binding by agonists.

TABLE 1

Competition for [^{3}H]spiperone binding by dopaminergic antagonists in homogenates of calf caudate nucleus

Parametric values are those obtained by iteration, with the value of $\%R_L$ fixed at 18%. Dissociation constants and IC_{50} values are expressed as the negative log of the mean; numbers in parentheses are the means expressed in molar units. Values are means ± standard error of the mean for three experiments.

Antagonist	n_H	pIC_{50}	pK_1	pK_2
Spiperone	0.84 ± 0.02	9.76 ± 0.04 (0.17 nM)	9.92 ± 0.04 (0.12 nM)	8.86 ± 0.01 (1.4 nM)
S-Sulpiride	0.76 ± 0.01	4.44 ± 0.01 (36 μM)	4.64 ± 0.03 (23 μM)	3.23 ± 0.07 (600 μM)
S-Sulpiride (with 10 mM NaCl)	0.60 ± 0.05	6.18 ± 0.08 (0.66 μM)	6.40 ± 0.04 (0.44 μM)	3.9 ± 0.1 (130 μM)

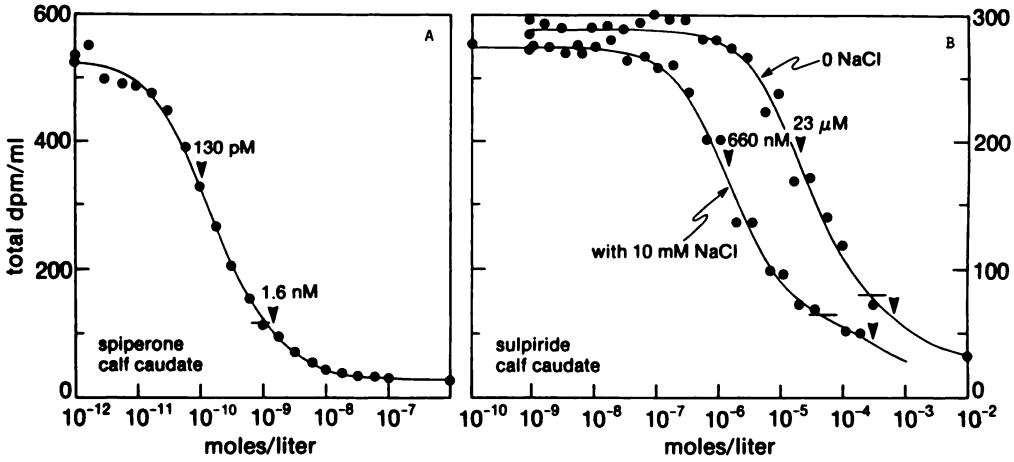

FIG. 2. *Binding of 50 pM [^{3}H]spiperone was measured at various concentrations of antagonist, each point determined in triplicate*
All experiments were performed with a membrane preparation at a final concentration of 40–60 μg of protein per milliliter in a buffer (pH 7.4, 22°) containing 50 mM Tris, 5 mM KCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM EDTA, and 0.01% ascorbic acid. The tubes were incubated at room temperature for 3 hr. With these representative experiments, fitted independently to the model, the curves delineate the fit to the experimental data and are best described as comprising two populations of binding sites. The *horizontal lines* intersecting the curves indicate the relative fraction of binding sites present, with R_L being fixed at 18%. Values of pK_D, obtained by iteration, are indicated by the *arrows*, and the averages of two repetitive experiments are listed in Table 1. A, Spiperone; B, *S*-sulpiride in the presence and absence of 10 mM NaCl.

TABLE 2

Competition for [^{3}H]spiperone binding by dopaminergic agonists in homogenates of calf caudate nucleus

Parametric values are those obtained by iteration, with the value of $\%R_{VL}$ fixed at 18%. Dissociation constants and IC_{50} values are expressed as the negative log of the mean; numbers in parentheses are the means expressed in molar units. Values are means ± standard error of the mean for three experiments. APO, (−)-apomorphine; BROMO, bromocriptine; LY, LY156258.

Agonist	n_H	pIC_{50}	pK_1	pK_2	pK_3	$\%R_L$
ADTN	0.41 ± 0.01	6.6 ± 0.1 (250 nM)	8.0 ± 0.3 (10 nM)	6.1 ± 0.1 (800 nM)	4.7 ± 0.3 (20 μM)	47 ± 4
APO	0.57 ± 0.02	6.92 ± 0.08 (120 nM)	8.6 ± 0.3 (3 nM)	6.74 ± 0.09 (180 nM)	5.9 ± 0.3 (1 μM)	47 ± 4
BROMO	0.99 ± 0.07	8.71 ± 0.04 (2 nM)	8.70 ± 0.03 (2 nM)			
LY	0.53 ± 0.02	5.22 ± 0.03 (6 μM)	6.3 ± 0.1 (500 nM)	4.9 ± 0.1 (12 μM)	3.6 ± 0.1 (250 μM)	44 ± 6
NPA	0.38 ± 0.03	8.35 ± 0.05 (4 nM)	9.1 ± 0.3 (0.8 nM)	7.7 ± 0.3 (20 nM)	5.0 ± 0.4 (10 μM)	36 ± 8

With the exception of bromocriptine, all agonist/[^{3}H] spiperone curves had very shallow slopes, with Hill coefficients ranging from 0.57 ± 0.02 for (−)-apomorphine to 0.38 ± 0.03 for NPA (Table 2). All of the agonists appeared to compete for the same total population of sites competed for by antagonists, since the total binding observed at higher concentrations of all agonists was not different from that observed in the presence of 10 μM (+)-butaclamol or 1 μM spiperone. The standard error of each measurement rarely exceeded 2%.

In all cases, the experimental data for individual agonists were best described as comprising three independent populations of binding sites (Fig. 3). Experiments repeated in different tissue batches or in membrane preparations that had been stored for extended periods of time did not differ significantly in their binding parameters, although the amount of total binding did vary considerably. This accounts for the varying levels of total binding shown in Fig. 3. Binding parameters for the three components of agonist competition are shown in Table 2, where R_H represents the high-affinity site, R_L represents the low-affinity site, and R_{VL} represents the very-low-affinity site, each with its respective dissociation constant. The particular selectivity of NPA for R_{VL} provided an accurate estimate of its contribution to the total competable binding of [^{3}H]spiperone in the calf caudate nucleus. The average value of 18% for R_{VL} was in close agreement with the average value of 17% for the low-affinity component of *S*-sulpiride binding in the presence of NaCl. Therefore, all competition curves were reanalyzed, fixing this component of binding at 18% (this procedure did not worsen the fit of the model to the experimental data); all dissociation constants presented in the tables are those obtained with this constraint.

Individual agonist competition curves, when analyzed separately, suggested that the proportion of R_H might differ between agonists: R_H contributed to 56% of the total competable binding for NPA and 28% for (−)-apomorphine, with intermediate values for ADTN and LY156258. However, simultaneous analysis of data for both NPA and (−)-apomorphine indicated that this difference was not significant, nor was the shared value of $\%R_H$ for both agonists significantly different from 50%.

Effects of Gpp(NH)p on antagonist and agonist binding. Antagonist binding, as determined using competition experiments, did not appear to be affected by 0.1 mM Gpp(NH)p (Fig. 4). Experimental data for either spiperone or *S*-sulpiride (in the presence of Na^+) in the presence or absence of Gpp(NH)p were superimposable. Preliminary experiments could not demonstrate any effect of 0.1 mM Gpp(NH)p on the total binding of 60 pM [^{3}H]spiperone over a Mg^{2+} concentration range of from 0–50 mM, in the presence or absence of 1 mM EDTA (data not shown).

The effect of 0.1 mM Gpp(NH)p on agonist binding was not remarkable; only slight increases in the Hill coefficients were observed, if at all, whereas IC_{50} values were either unchanged or increased by at most a factor of 2 (Fig. 5). Preliminary experiments demonstrated no further effect at 1 mM Gpp(NH)p, indicating that the concentration used was in excess. With some experiments, binding in the presence of 0.1 mM Gpp(NH)p and low (10–100 pM) concentrations of agonist was observed to be about 15% greater in control experiments or binding of [^{3}H]spiperone in the presence of Gpp(NH)p alone; this effect was not seen consistently.

In all competition experiments, the best description of the data for individual agonists was obtained with three independent populations of binding sites (Table 3). Estimates of all dissociation constants were comparable to those obtained in the absence of guanine nucleotide. In the presence of the guanine nucleotide, the relative proportions of the three binding sites were not significantly different for all agonists studied ($R_H = 21 \pm 2\%$, $R_L = 58 \pm 1\%$, $R_{VL} = 24 \pm 3\%$), as one would expect for three independent classes of binding sites. In all cases, the relative proportion of R_H was decreased, and there appeared to be a concomitant increase in the relative proportion of R_L ($p < 0.05$ comparing R_H for ADTN in the presence and absence of guanine nucleotide, $p < 0.01$ for the same comparison with NPA); the contribution of R_{VL} was only slightly increased, by 6% on the average, but this difference was not significant ($p > 0.1$).

Thus, the nucleotide appeared to "convert" sites of high-affinity into those resembling the low-affinity sites observed in the control experiments. This effect was not complete, as a significant proportion of high-affinity sites remained in the presence of an excess of Gpp(NH)p

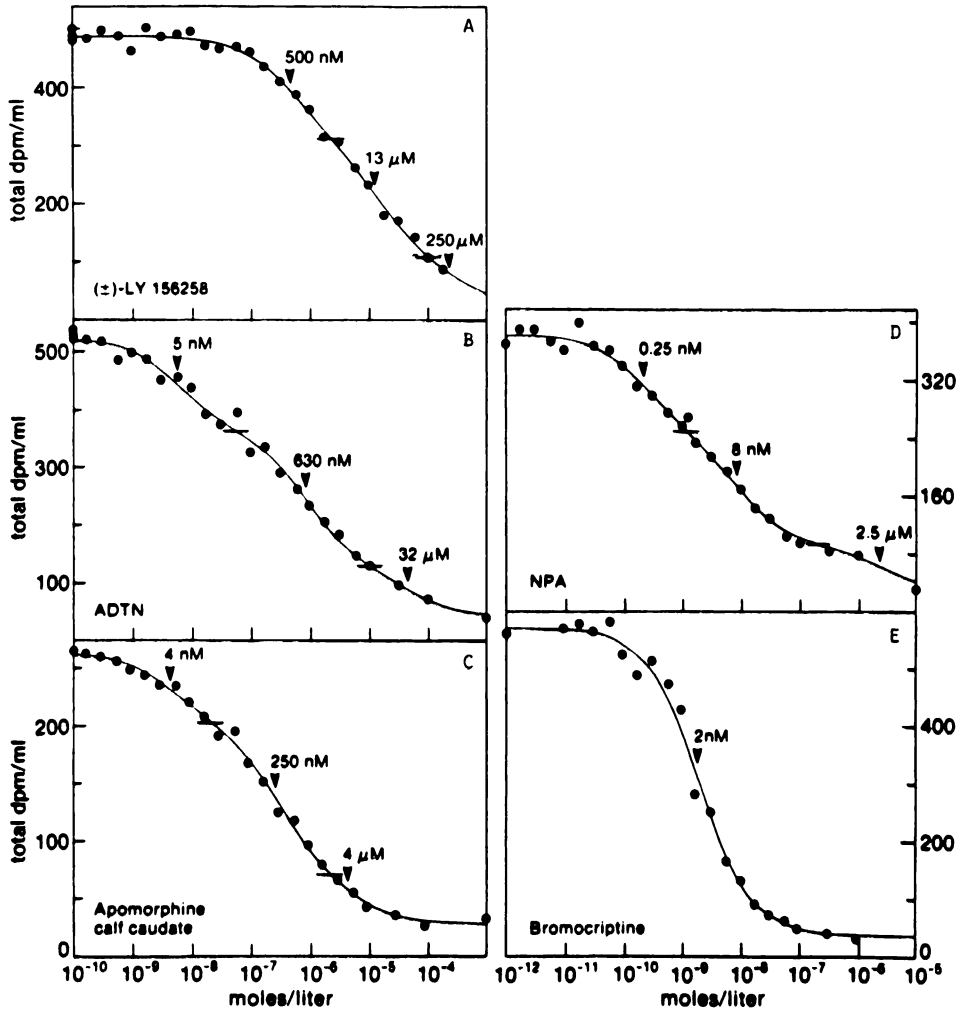

FIG. 3. *Binding of 50 pM [^{3}H]spiperone was measured at various concentrations of agonist, each point determined in quadruplicate*

For further details see the legend to Fig. 2. With these representative experiments, fitted independently to the model, the curves delineate the fit to the experimental data, and competition by each agonist is best described as comprising three populations of binding sites. The *horizontal lines* intersecting the curves indicate the relative fraction of binding sites present, with R_{VL} being fixed at 18%. Values of pK_D, obtained by iteration, are indicated by the *arrows*, and the averages of three repetitive experiments are listed in Table 2. A, LY156258; B, ADTN; C, (−)-apomorphine; D, NPA; E, bromocriptine.

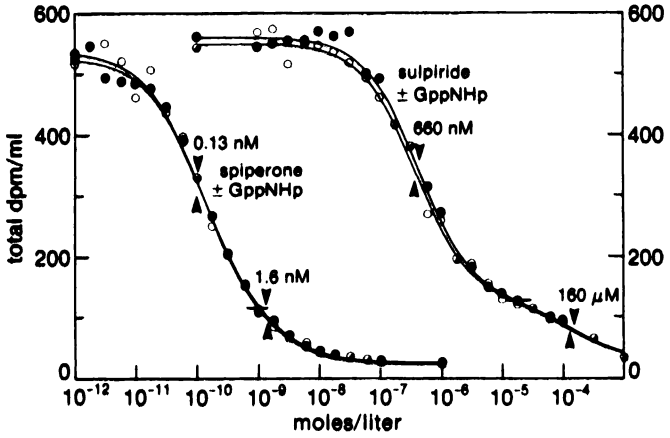

FIG. 4. *Binding of 50–60 pM [^{3}H]spiperone was measured at various concentrations of antagonist in the presence and absence of 0.1 mM Gpp(NH)p*

For further details see the legend to Fig. 2. *Left curve*, Spiperone; *right curve*, *S*-sulpiride in the presence of 10 mM NaCl.

(21%) that may have been identical with those existing in the absence of the nucleotide.

Preliminary experiments were also conducted to determine whether the lack of complete conversion by the guanine nucleotide was due to a temperature-dependent effect. Figure 6 demonstrates that even at 37° there still remains about 20% of the total competable binding in a high-affinity form, in the presence of an excess of Gpp(NH)p.

DISCUSSION

The present results provide the first evidence to suggest that the D_2-dopaminergic receptor in the calf caudate nucleus exists in interconvertible high- (D_2^{high}) and low-affinity (D_2^{low}) states. This conclusion is based on the observation that the relative proportions of high-affinity and low-affinity sites recognized by agonists differed in the presence of a guanine nucleotide such that there appeared to be a "conversion" of sites of high-affinity (R_H) into those resembling the low-affinity (R_L)

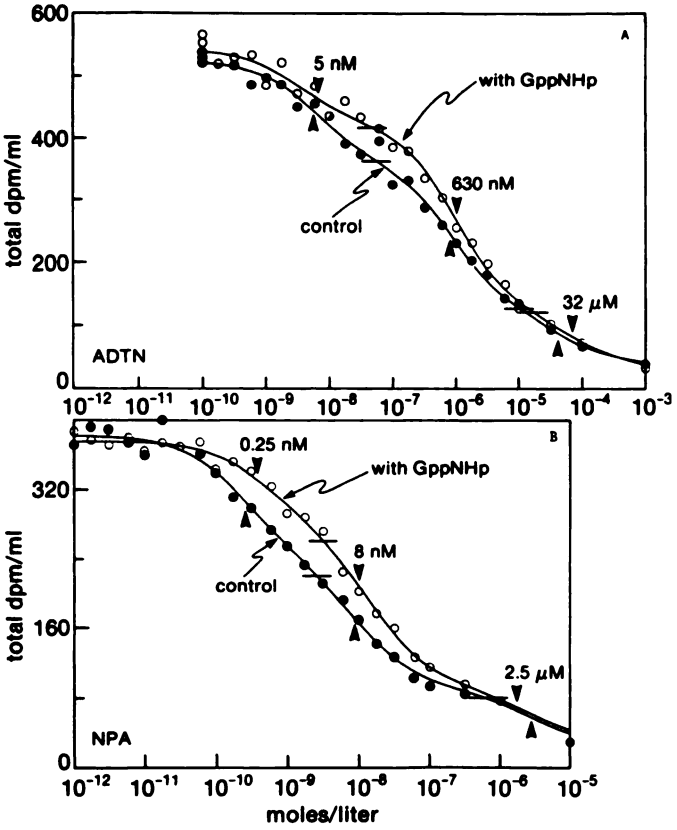

FIG. 5. *Binding of 50–60 pM [³H]spiperone was measured at various concentrations of agonist in the presence and absence of 0.1 mM Gpp(NH)p*

For further details see the legend to Fig. 2. Averages of the pK_D values from three repetitive experiments are given in Table 3. A, ADTN; B, NPA.

sites of the control experiments. Antagonists did not distinguish between these two forms of the receptor, nor were they affected by a guanine nucleotide. The effect of the guanine nucleotide on agonist binding was not complete, such that a significant proportion of high-affinity sites remained in the presence of an excess of Gpp(NH)p.

In this tissue, [³H]spiperone also appeared to label a site that existed in the same proportion of total competable binding (18%) for both agonists and antagonists, as would be expected for an independent class of sites. It is unlikely that this site represents the previously described "spirodecanone" site (23) since the site described here was recognized by compounds other than spiperone. Furthermore, competition by R-43448, a relatively specific serotonergic antagonist, was markedly biphasic, with the high-affinity component comprising about 20% of the total displaceable binding (data not shown). Preliminary work has indicated that this lowest-affinity component may be the S_2-serotonergic binding site that [³H]spiperone has been shown to label also (ref. 1). Although the identity of this component is not yet conclusive, its full characterization was not considered essential for the present study.

It is noted that in light of the biphasic competition curve for spiperone, the radioligand itself must be able to discriminate between these two sites. However, the difference between the two apparent dissociation constants was not great enough to be resolved by Scatchard analysis. By assuming only a single dissociation constant for [³H]spiperone, consistent with the high-affinity component (see Fig. 2), at worst the proportion of the "S_2" component may have been slightly underestimated. There would be no serious alteration in the relative proportions of the other two components, nor in the relative value of K_L/K_H for agonists.

The only model currently available to account for the observation of an agonist-specific effect of guanine nucleotides on the proportions of high and low affinity recognized by agonists involves a reversible, bimolecular interaction between the receptor and an additional membrane component that specifically binds guanine nucleotides. This ternary complex model [based on models first described by Boeynaems and Dumont (24) and by Jacobs and Cuatrecasas (25)] was proposed by De Lean *et al.* (26) to account for the interaction of ligands with the *beta*-adrenergic receptor system of frog erythrocytes. In their discussion of the data, the authors described the positive correlation of the intrinsic activity of an agonist with the proportion of R_H and also with the value of the ratio of the apparent dissociation constants for its interaction with the two forms of the receptor, K_L/K_H. Upon inspection of their data (27) it is suggested that the proportion of R_H is also correlated with the value of K_L/K_H such that an agonist which promoted a higher proportion of R_H would also be the most discriminating between the two forms of the receptor (i.e., have the highest value of K_L/K_H). This correlation between R_H and K_L/K_H is predicted by the ternary complex model: this was also described by Wells *et al.* (28) in a study of the cardiac muscarinic cholinergic receptor.

Several similarities exist between the present results

TABLE 3

Competition for [³H]spiperone binding by dopaminergic agonists in the presence of 0.1 mM Gpp(NH)p in homogenates of calf caudate nucleus

Parametric values are those obtained by iteration, with the value of $\%R_{VL}$ fixed at 18%. Dissociation constants and IC_{50} values are expressed as the negative log of the mean; numbers in parentheses are the means expressed in molar units. Values are means ± standard error of the mean for three experiments. APO, (−)-apomorphine.

Agonist	n_H	pIC_{50}	pK_1	pK_2	pK_3	$\%R_L$
ADTN	0.49 ± 0.02	6.60 ± 0.05 (250 nM)	8.0 ± 0.3 (10 nM)	5.94 ± 0.03 (1 μM)	4.4 ± 0.3 (40 μM)	60 ± 1
APO	0.60 ± 0.03	6.77 ± 0.04 (170 nM)	8.4 ± 0.4 (4 nM)	6.7 ± 0.1 (200 nM)	5.6 ± 0.1 (3 μM)	62 ± 2
NPA	0.38 ± 0.08	8.16 ± 0.03 (7 nM)	9.6 ± 0.1 (0.3 nM)	7.85 ± 0.05 (14 nM)	4.7 ± 0.4 (20 μM)	59 ± 4

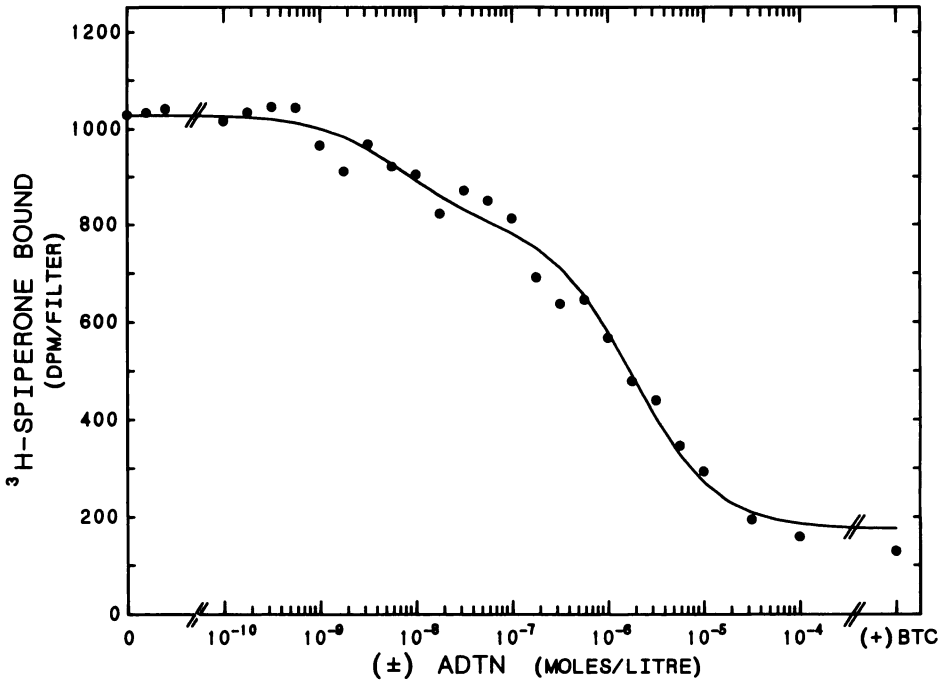

FIG. 6. *Binding of 50–60 pM [^{3}H]spiperone was measured in triplicate at various concentrations of ADTN, in the presence of 0.1 mM Gpp(NH)p* Assay tubes were incubated for 45 min at 37°. Under these conditions, about 20% of the total competable binding remains in a high-affinity form, with a pK_D of 8.1 (K_D = 8 nM).

for the D_2-dopaminergic receptor in brain and those reported for the *beta*-adrenergic receptor in frog erythrocytes (27). In both systems, agonists differentiate two states of the receptor whereas antagonists do not. Guanine nucleotides appear to mediate a "conversion" of receptors in the high-affinity state to a low-affinity state. The present data indicated that the same proportion of R_H was seen for all of the agonists studied. This is consistent with the observation of no difference between K_L/K_H for different agonists (ΔpK; see Table 4), as would be expected if the ternary complex model, as described for the *beta*-adrenergic receptor, does apply to the D_2-dopaminergic receptor in the brain.

Sibley and his collaborators (14, 16) have chosen to interpret their data, obtained in the study of the D_2-dopaminergic receptor in the bovine anterior pituitary, as being adequately described by the ternary complex model. It should be pointed out that, with their observation of the same proportion of the high-affinity state of the receptor for all agonists, the values of K_L/K_H should also be invariant if their model applies. Indeed, this is not the case, as K_L/K_H ranged from 56 for apomorphine to 96 for NPA (16). De Lean *et al.* (15), in a study of the D_2-dopaminergic receptor in porcine anterior pituitary, also reported an invariant proportion of R_H with all agonists, whereas the values of K_L/K_H ranged

TABLE 4

Summary of the binding parameters for dopaminergic agonists for the D_2-dopaminergic receptor in calf caudate nucleus

Dissociation constants are expressed as the negative log of the mean; numbers in parentheses are the means expressed in molar units. The value of ΔpK is the difference between the values of pK^{high} and pK^{low}. Values for $\%D_2^{high}$ were calculated as $\%D_2^{high} = (R_H/R_H + R_L) \times 100$; values for R_H and R_L are taken from Tables 2 and 3. APO, (−)-apomorphine; LY, LY156258.

	ADTN	APO	NPA	LY
Control				
pK^{high}	8.0 ± 0.3 (10 nM)	8.6 ± 0.3 (3 nM)	9.1 ± 0.3 (0.8 nM)	6.3 ± 0.1 (500 nM)
$\%D_2^{high}$	43 ± 6	34 ± 3	60 ± 10	46 ± 1
pK^{low}	6.1 ± 0.1 (790 nM)	6.7 ± 0.1 (200 nM)	7.7 ± 0.2 (20 nM)	4.9 ± 0.1 (13 μM)
ΔpK	1.9 ± 0.1	1.9 ± 0.3	1.3 ± 0.1	1.5 ± 0.3
+ 0.1 mM Gpp(NH)p				
pK^{high}	8.1 ± 0.2 (8 nM)	8.4 ± 0.4 (4 nM)	9.6 ± 0.1 (0.3 nM)	
$\%D_2^{high}$	27 ± 1	24 ± 3	28 ± 1	
pK^{low}	5.9 ± 0.1 (1 μM)	6.7 ± 0.1 (200 nM)	7.9 ± 0.1 (14 nM)	
ΔpK	2.0 ± 0.2	1.7 ± 0.4	1.7 ± 0.1	

from 34 for apomorphine to 222 for NPA. It is noted that the proportion of R_H for agonists in the anterior pituitary (50%) is in excellent agreement with that observed for agonists in the present study.

As well, Sibley and collaborators (14, 16) observed a compete loss of the high-affinity state in the presence of guanine nucleotides while a significant proportion of high-affinity, guanine nucleotide-insensitive sites was observed in very similar systems (15, 29, 30). All of the above points are important discrepancies, and the reasons for them are not clear at this time.

The significance of the incomplete conversion by guanine nucleotide is not well understood. The high-affinity, guanine nucleotide-insensitive sites are not an artifact of temperature, since these sites have been observed at both 25° and 37° in the calf caudate nucleus (this study) and rat striatum (30). Since the relative proportion of the high-affinity, guanine nucleotide-insensitive sites was independent of the agonist studied, they may represent a distinct class of binding sites having high affinity for both agonists and antagonists, like the proposed D_4 site. It is equally possible that these sites may be the result of an agonist-independent, equilibrium interaction of the D_2-dopaminergic receptor with a guanine nucleotide; we are unable to exclude either interpretation with the present results.

The observations of incomplete conversion by guanine nucleotide and an invariant 50% R_H with all agonists are in distinct contrast to those reported for the *beta*-adrenergic system. Also quite atypical are the data for the very potent ergot agonist, bromocriptine. These differences may provide some insight into the mechanisms governing the transduction of these two, physiologically contrasting, receptor systems.

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