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Dopamine Receptor of the Porcine Anterior Pituitary Gland

Evidence for Two Affinity States Discriminated by Both Agonists and Antagonists

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SUMMARY

Quantitative analysis of radioligand binding to the porcine anterior pituitary dopaminergic receptor indicates the existence of two affinity states of the same receptor population. Biphasic competition curves of agonists for the binding of the antagonist [^{3}H]spiroperidol suggest that agonists discriminate among the sites labeled by the antagonist, a high- and a low-affinity form of the receptor. The agonist high-affinity form of the receptor, which represents 50% of the total binding sites, can be labeled by direct ligand binding using the agonist [^{3}H]*n*-propylapomorphine. Guanine nucleotides modulate the proportion of the sites with high affinity for agonists as evidenced by their ability to shift the agonist competition curves for ^{3}H-labeled antagonist binding to a lower potency or decrease the high-affinity binding of [^{3}H]*n*-propylapomorphine. Antagonists also appear to discriminate the same two affinity forms of the receptor. Saturation binding isotherms of [^{3}H] spiroperidol document the presence of two different affinity forms of roughly equal proportions which are converted to a single high-affinity form for the antagonist [^{3}H] spiroperidol in the presence of guanine nucleotides. These data suggest that the two forms of this dopamine receptor are reciprocally discriminated by agonists and antagonists. Thus, in porcine anterior pituitary membrane preparations in the absence of guanine nucleotides, 50% of the receptor sites display high affinity for agonists and low affinity for antagonists with the remaining sites showing low affinity for agonists and high affinity for antagonists. In the presence of guanine nucleotides, the agonist high-affinity form (antagonist low-affinity form) is convertible to the agonist low-affinity form (antagonist high-affinity form). The presence of these specific nucleotide effects suggests the involvement of a guanine nucleotide-binding protein in the mechanism of ligand binding and activation of the dopaminergic receptor of porcine anterior pituitary gland.

INTRODUCTION

The original observation of Kebabian *et al.* (1) that a dopamine-responsive adenylate cyclase was present in the central nervous system suggested that specific receptors for that catecholamine might be mediating certain physiological responses. Subsequently, central nervous system dopamine receptors were identified by direct ligand binding using both ^{3}H-labeled agonists, such as dopamine itself, and ^{3}H-labeled antagonists, such as haloperidol (2, 3). Considerable pharmacological differences were observed in the binding of dopaminergic agonists and antagonists to an area of the brain rich in these sites, the corpus striatum. On that basis it was proposed that separate agonist and antagonist receptor sites might exist or that a unique dopamine receptor might interconvert between conformations which have high affinities for agonists or for antagonists (4). More recently, Kebabian and Calne (5), on the basis of pharmacological differences between sites identified by ligand binding and sites mediating dopamine stimulation of adenylate cyclase, proposed that dopaminergic sites existed as two different receptor subtypes, termed D_1 and D_2. The D_1 subtype of receptor would include those receptors coupled to stimulation of adenylate cyclase. However, because of the lack of a readily identifiable biochemical response to occupancy of receptor with D_2 pharmacological specificity, properties of ligand binding to these sites in brain could be correlated only with behavioral data (6).

In the anterior pituitary gland, where dopamine regulates the secretion of prolactin, we identified (using the ergot alkaloid [^{3}H]dihydroergocryptine) dopaminergic binding sites with the specificity of the D_2 subtype of binding sites (7). We were able for the first time to correlate the pharmacological properties of ligand bind-

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ing with a direct physiological effect, the inhibition of prolactin release (7). Very recently it has been shown that, at least in the pituitary gland (intermediate lobe), agonist occupancy of sites which possess the pharmacology of the D_2 receptor subtype are inhibitory to adenylate cyclase activity in broken-cell preparations (8) or inhibit levels of cyclic AMP in whole cells (9).

These dopaminergic binding sites have been identified in both brain and pituitary gland systems by direct ligand binding using potent labeled agonists (10–12) and antagonists (13, 14), but the relationship between the labeling of these sites with agonists or antagonists and their different numbers and characteristics has not been fully elucidated. In order to clarify the mechanism of interaction of dopaminergic ligands with the dopamine receptor of the porcine anterior pituitary gland we have examined in detail and compared binding of the agonist [^{3}H]*n*-propylapomorphine and the antagonist [^{3}H]spiroperidol in this system. We have also examined the effects of guanine nucleotides on the binding of these ligands and used computer-based methods to obtain a quantitative analysis of the data. From these data we propose that the dopaminergic receptor of the porcine anterior pituitary system can exist in two affinity forms that are discriminated reciprocally by agonists and antagonists and modulated by guanine nucleotides.

EXPERIMENTAL PROCEDURES

Materials

[^{3}H]Spiroperidol (33.2–51 Ci/mmole) and [^{3}H]*N-n*-propylapomorphine (~60 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). (±)-ADTN[2] was a gift from Dr. J. D. McDermed, Burroughs Wellcome Company (Research Triangle Park, N. C.). Dopamine, pargyline, and GTP were obtained from Sigma Chemical Company (St. Louis, Mo.). Gpp(NH)p was obtained from Boehringer Mannheim GmbH (Mannheim, West Germany). The following drugs were gifts from the respective pharmaceutical companies: (−)-*N-n*-propylapomorphine HCl, Sterling-Winthrop Research Institute (Rensselaer, N. Y.); apomorphine, Merck & Company (Rahway, N. J.); (+)-butaclamol, Ayerst Laboratories (Rouses Point, N. Y.) All other chemicals were from commercial sources and of the best available quality.

Methods

Membrane preparation. Fresh female porcine pituitary glands were obtained from a local slaughterhouse and were kept in 25 mM Tris-HCl/2 mM $MgCl_2$ (pH 7.4) or in buffered saline containing 250 mM sucrose at 0–4° until preparation of the membranes (3–5 hr after slaughter). The anterior lobes were separated from the posterior and intermediate lobes, minced, and homogenized with 12 strokes of a glass-Teflon homogenizer in 250 mM sucrose/2 mM $MgCl_2$/25 mM Tris-HCl (pH 7.4 at 4°) (Buffer A) (20 ml/g of tissue). All steps were performed at 0–4°. The homogenate was filtered through two layers of cheesecloth and centrifuged at 300 × *g* for 10 min. The supernatant was retained and the pellet was rehomogenized and centrifuged again. The combined supernatants were layered over 50% sucrose (w/v)/2 mM $MgCl_2$/50 mM Tris-HCl (pH 7.4) and centrifuged at 30,000 × *g* for 30 min. The sucrose-buffer interface was collected, diluted with Buffer A, and centrifuged at 30,000 × *g* for 20 min. The pellet was washed by resuspension in Buffer A and centrifugation at 30,000 × *g* for 10 min. The resulting pellet was suspended and homogenized in 50 mM Tris-HCl/6 mM $MgCl_2$/1 mM EDTA/100 mM NaCl/0.1% ascorbate/10 μM pargyline (pH 7.4 at 25°) (Buffer B) at a concentration of 3.6 ± 1.0 mg/ml (2 ml of buffer/g equivalent of tissue). This particulate material, which presumably contained elements of plasma membranes as well as other cellular organelles but was essentially devoid of secretory granules, is referred to as the particulate or membrane preparation. This particulate preparation contained more than 75–80% of the specific measurable [^{3}H]spiroperidol binding activity. These preparations were frozen in liquid nitrogen and stored at −90° until used.

Radioligand binding assay. Porcine anterior pituitary membranes (0.36 ± 0.10 mg) were incubated with 150–200 pM [^{3}H]*n*-propylapomorphine or 120–200 pM [^{3}H] spiroperidol in the presence of the indicated agents in a total assay volume of 1 ml of Buffer B for 60 min at 25°. Binding was initiated by the addition of membranes. The incubation was terminated by the addition of 5 ml of cold 2 mM $MgCl_2$/25 mM Tris-HCl (pH 7.4 at 4°) buffer and rapid vacuum filtration onto Whatman GF/C or GF/B filter discs with four additional 5-ml washings. Bound radioactivity trapped on the filters was counted by liquid scintillation spectrophotometry. Nonspecific binding was determined in the presence of 1 μM (+)-butaclamol.

Data analysis. Saturation and competition binding data were analyzed by a nonlinear least-squares curve-fitting procedure based on a generalized model for complex ligand-receptor systems according to the law of mass action (15, 16). For saturation binding data, we considered a model for a radioligand binding to one or two forms of the receptor (R_H and R_L) with high affinity (K_H) or low affinity (K_L), respectively, for the ligand. In the case of competitive binding data, the curves were initially analyzed according to a four-parameter logistic equation in order to determine the IC_{50} and the steepness factor of the curve as described (16). The competition curves were then analyzed according to a model for one or two forms of the receptor discriminated by both the radioligand and the competitor. These two forms (R_H and R_L) were commonly distinguished according to their high (K_H) or their low (K_L) affinity for agonists. In the case where the radioligand was an antagonist (i.e., [^{3}H] spiroperidol), the model also incorporated the distinct affinities (K_L' and K_H') of the radiolabeled antagonist for the two forms of the receptor simultaneously discriminated by the agonist in the competitive binding experiments. These affinity constants of the radiolabeled antagonist (K_L' and K_H') were separately estimated from a saturation binding experiment with the radioligand. Statistical analysis comparing "goodness of fit" between one- and two-affinity state models was also provided and was

[2] The abbreviations used are: ADTN, 2-amino-6,7-dihydroxytetrahydronaphthalene; Gpp(NH)p, guanyl-5′-yl imidodiphosphate.

used to determine the most appropriate model for the ligand being examined. For ligands whose interaction with the dopamine receptor were best described by two states of binding, K_H and K_L represent the high- and low-affinity dissociation constants whereas R_H and R_L represent the proportion of receptor corresponding to the high- and low-affinity states, respectively. The full model with two states discriminated by agonists and antagonists is schematized in Fig. 7.

RESULTS

Analysis of dopaminergic agonist and antagonist binding to porcine anterior pituitary membranes. In order to study the binding of dopaminergic ligands to the porcine anterior pituitary gland system, we performed studies with both the agonist [^{3}H]*n*-propylapomorphine and the antagonist [^{3}H]spiroperidol. These ligands have been shown previously in several systems to label binding sites with specificity appropriate for dopamine receptors (10–14). To verify the specificity of binding of these ligands in the anterior pituitary gland, competition curves using various agonists were obtained for the binding of both [^{3}H]spiroperidol and [^{3}H]*n*-propylapomorphine. The results suggest that both ligands bind to anterior pituitary sites with a typical dopaminergic specificity: *n*-propylapomorphine > ADTN > apomorphine > dopamine (Fig. 1A and B).

Figure 1B shows the competition curves of these various agonists for [^{3}H]*n*-propylapomorphine binding (ADTN not determined). Computer analysis of the data indicates that the competition curves are monophasic, of normal steepness (slope factor ~ 1) and were significantly better analyzed by a model for the agonists competing for the binding of [^{3}H]*n*-propylapomorphine to a single affinity form of the receptor. On the other hand, competition curves of [^{3}H]spiroperidol by the same agonists are complex, multiphasic, and shallow (slope factor 0.3–0.7) and are significantly better analyzed by a model for two different affinity states of the receptor discriminated by all agonists (Fig. 1A). Quantitative analysis (Table 1) of the agonist competition curves shown in Fig. 1A and B indicates that agonists discriminate between two forms of the receptor sites labeled by [^{3}H]spiroperidol. The high- and the low-affinity forms appear to be in roughly equal proportions (50%/50%) of the total sites labeled by [^{3}H]spiroperidol, with ratios (K_L/K_H) of the lower affinity (K_L) to the higher affinity (K_H) ranging from 30 to 200. However, when the agonist competition curves for [^{3}H]*n*-propylapomorphine are similarly analyzed, a single high-affinity form is observed for each of the agonists, with a unique dissociation constant corresponding closely to that of the high-affinity form evidenced in the [^{3}H] spiroperidol competition curves (Table 1). In contrast to agonists, antagonists compete for both [^{3}H]spiroperidol and [^{3}H]*n*-propylapomorphine with the expected specificity but with competition curves of normal steepness (data not shown).

In order to compare further the binding of agonists and antagonists, saturation binding isotherms for both [^{3}H]*n*-propylapomorphine and [^{3}H]spiroperidol were generated on the same membrane preparation (Fig. 2). [^{3}H]*n*-propylapomorphine labeled with high affinity (K_D

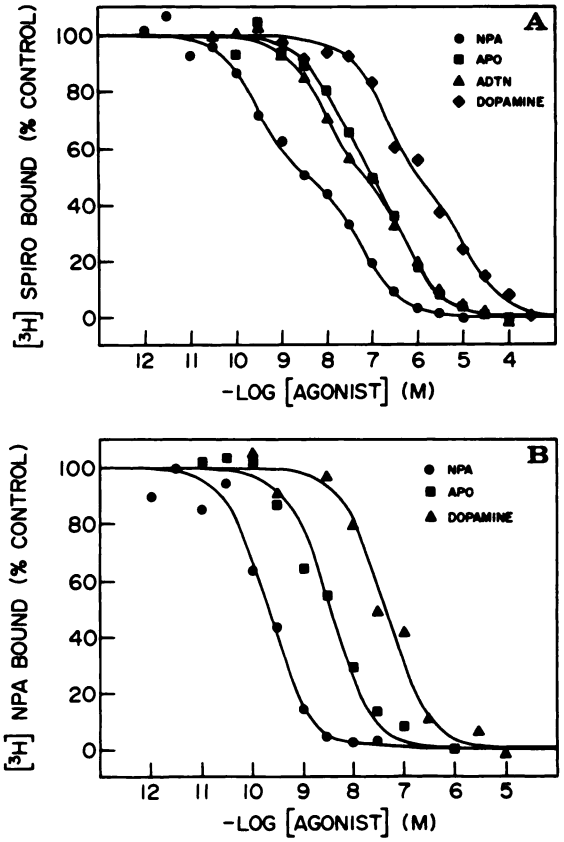

FIG. 1. *Competition curves of a series of dopaminergic agonists for the binding of [^{3}H]spiroperidol (A) and [^{3}H]n-propylapomorphine (B)*

Porcine anterior pituitary membranes prepared as described under Methods were incubated with [^{3}H]spiroperidol ([^{3}*H*]*SPIRO*) (~140 pM) or [^{3}H]*n*-propylapomorphine ([^{3}*H*]*NPA*) (~150 pM) and increasing concentrations of the various agonists as shown. Incubations were performed as described under Methods except that ~0.6 mg of membrane protein per assay was used in [^{3}H]*n*-propylapomorphine experiments. In the experiment shown, 100% [^{3}H]spiroperidol binding corresponded to 32 pM or ~107 fmoles/mg, whereas 100% [^{3}H]*n*-propylapomorphine was 33 pM or 55 fmoles/mg. The points represent experimental data whereas the lines through the points represent the best fit of the data according to a model for one or two binding sites. The experiment shown was performed in duplicate and is representative of three (A) and two (B) such experiments. *APO*, apomorphine.

~ 260 pM) only about 50% as many sites as those labeled by [^{3}H]spiroperidol (16.6 pM versus 29.8 pM). This was to be expected. The estimates of the dissociation constants K_H and K_L of the agonist *n*-propylapomorphine for both forms of the dopaminergic receptor obtained from the competition curve for [^{3}H]spiroperidol binding (Table 1) indicate that K_H is low enough (0.081 nM) to permit direct labeling of the agonist high-affinity form by the radiolabeled agonist. However, the lower dissociation constant K_L (18 nM) would be too high to detect the low-affinity form of the receptor with the usual concentrations used to perform direct [^{3}H]*n*-propylapomorphine binding isotherms (<1000 pM). Binding experiments with higher concentrations of [^{3}H]*n*-propylapomorphine are technically less satisfactory because of high nonspecific binding. Therefore, it would appear that, by direct binding, [^{3}H] *n*-propylapomorphine labels only the agonist high-affinity form of the receptor population labeled by [^{3}H]spiroperidol.

TABLE 1

Quantitative analysis of agonist competition curves for [^{3}H]spiroperidol ([^{3}H]Spiro) and [^{3}H]n-propylapomorphine ([^{3}H]NPA) binding to porcine anterior pituitary membranes

Data shown in Fig. 1A and B were computer-analyzed as described under Methods. K_H represents the dissociation constants of agonist calculated for the high-affinity component of [^{3}H]spiroperidol competition curves or of [^{3}H]n-propylapomorphine binding. K_L is the dissociation constant calculated for the lower-affinity component of [^{3}H]spiroperidol competition curves. K_L/K_H is the ratio of the two dissociation constants, whereas $\%R_H$ represents the percentage of sites in a high-affinity form for the agonist.

Competing agonist	Radioligand				
	[^{3}H]Spiro				[^{3}H]NPA
	K_H[a]	K_L[a]	K_L/K_H	$\%R_H$	K_H[a]
	nM	nM			nM
NPA	0.081 ± 0.022	18.0 ± 3.9	222	51.6	0.075 ± 0.022
Apomorphine	4.7 ± 2.4	160 ± 61	34	46.5	1.4 ± 0.3
ADTN	2.3 ± 0.8	190 ± 59	82	50.6	—
Dopamine	66 ± 37	4300 ± 2700	65	56.3	18 ± 5

[a] Values are means ± standard error of the mean.

Effects of guanine nucleotides on agonist and antagonist binding. Guanine nucleotides have been shown to modulate the binding of agonist agents in several hormone receptor systems (17). Usually, the effects can be domonstrated as a selective decrease of the affinity of the receptor for agonists (18). Similar effects have been observed in several dopaminergic systems (19–24). Therefore, it was of interest to examine the effect of guanine nucleotides on dopaminergic ligand binding to porcine anterior pituitary membranes in an attempt to gain further understanding of the mode of interaction of ligands with this dopaminergic receptor.

The effects of guanine nucleotides were examined on both agonist competition curves for [^{3}H]spiroperidol binding and direct [^{3}H]*n*-propylapomorphine binding. As can be seen from Fig. 3, the competition curve of *n*-propylapomorphine (or other agonists, data not shown) for [^{3}H]spiroperidol binding, which is biphasic, shallow, and best explained by two-agonist affinity forms of the receptor in the absence of guanine nucleotide, is steepened and shifted to the right in the presence of the guanine nucleotide Gpp(NH)p.[3] Analysis of these data (Table 2) indicates that the number of the agonist high-affinity form of the receptor is markedly decreased from roughly 50% of the total [^{3}H]spiroperidol sites to 10–15%, in the presence of Gpp(NH)p, with the remaining sites having the same high affinity for the agonist. Consequent to the decrease in the number of high-affinity form, the number of agonist low-affinity form increases to 80–85% of total receptor sites with no significant change in the K_L value for *n*-propylapomorphine (8,300 ± 2,000 versus 12,000 ± 1,900 pM).

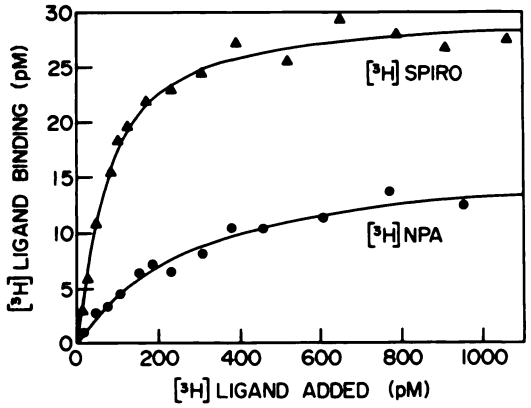

FIG. 2. *Saturation binding isotherms for [^{3}H]spiroperidol and [^{3}H]n-propylapomorphine*

Anterior pituitary membranes were incubated for 1 hr at 25° with concentrations of [^{3}H]spiroperidol ([^{3}H]*SPIRO*) or [^{3}H]*n*-propylapomorphine ([^{3}H]*NPA*) from 10 to 1000 pM. Data points shown represent specific binding for both ligands (total minus nonspecific binding which was determined in the presence of 1 μM (+)-butaclamol). As estimated from computer-assisted analysis, [^{3}H]spiroperidol binding was 29.8 ± 0.5 pM whereas [^{3}H]*n*-propylapomorphine was 16.1 ± 1.1 pM. The K_D for [^{3}H]spiroperidol was 58 ± 4 pM and for [^{3}H]n-propylapomorphine was 261 ± 40 pM.

Since nucleotides seem to affect the agonist high-affinity binding which, as suggested above, can be labeled by [^{3}H]*n*-propylapomorphine, it was of interest to examine the effects of nucleotides on the direct binding of [^{3}H]*n*-propylapomorphine. The saturation isotherms shown in Fig. 4 indicate that in the presence of 1 mM GTP the direct binding of [^{3}H]*n*-propylapomorphine is decreased by more than 75% of the number of sites identified by the ^{3}H-labeled agonist (24 versus 6.2 pM) with no significant change in the affinity of the remaining sites for the ^{3}H-labeled agonist (K_D = 220 versus 210 pM). In absolute value, the small number of high-affinity [^{3}H]*n*-propylapomorphine sites remaining in the presence of guanine nucleotides corresponds closely to the 10–15% proportion of total [^{3}H]spiroperidol sites still possessing high affinity for agonists in the presence of guanine nucleotides (Fig. 3). Lower concentrations of GTP (10 μM–1 mM) produced a gradual decrease in the number of agonist sites labeled with no significant change in the affinity of the residual sites. These results appear entirely consistent with the formulation that the dopaminergic receptor in porcine anterior pituitary gland membranes can exist in two affinity forms that are discriminated by agonists and modulated by guanine nucleotides.

[3] In these studies, we have used interchangably 1 mM GTP or 100 μM Gpp(NH)p to elicit maximal nucleotide effects on both agonist and antagonist binding. The specificity of the nucleotide effects was the same as that reported for other systems (17), Gpp(NH)p > GTP > GDP, with GMP and other nucleotide triphosphates (ITP, CTP, and ATP) being weak or inactive.

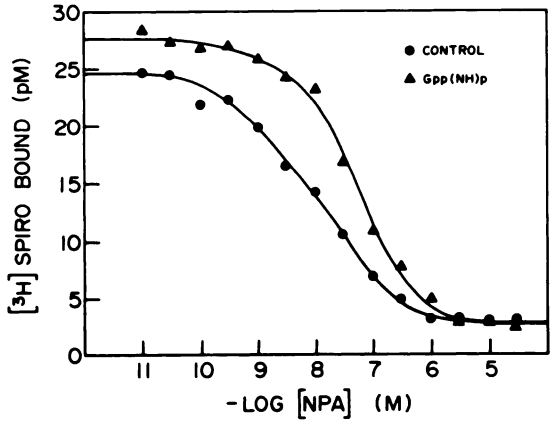

FIG. 3. *Effect of the guanine nucleotide Gpp(NH)p on competition of n-propylapomorphine for [^{3}H]spiroperidol*

Membranes were incubated as described under Methods with [^{3}H] spiroperidol ([^{3}*H*]*SPIRO*) (~160 pM) and increasing concentrations of *n*-propylapomorphine (*NPA*) in the presence and absence of 100 μM Gpp(NH)p. The lines are the best fit obtained with a model for two affinity forms of the receptor. The experiment shown is representative of 10–12 experiments.

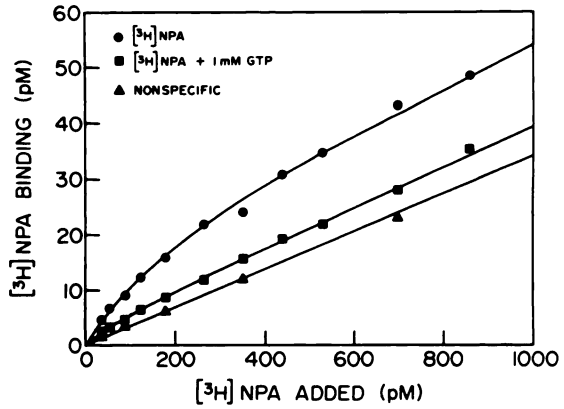

FIG. 4. *Effects of guanine nucleotides on the direct binding of [^{3}H]-n-propylapomorphine*

Porcine anterior pituitary membranes were incubated with increasing concentrations (10–900 pM) of [^{3}H]*n*-propylapomorphine ([^{3}*H*]-*NPA*) in the presence and absence of 1 mM GTP. Nonspecific binding was determined in the presence of 1 μM (+)-butaclamol and was the same with or without the addition of GTP. Data were analyzed as described under Methods. The numbers of sites labeled by [^{3}H]*n*-propylapomorphine were 24 pM (absence) and 6.2 pM (presence of GTP), and the dissociation constants for the ligand were 220 and 210 pM, respectively. The experiment shown was performed in duplicate and is representative of three such experiments.

As shown in Fig. 3, when competition curves for [^{3}H] spiroperidol were obtained in the presence of guanine nucleotides, an apparent increase in the binding of [^{3}H] spiroperidol was observed. This effect is contrary to the usually well-characterized effects of guanine nucleotides on agonist binding in several systems (17). However, similar findings have been obtained for muscarinic cholinergic antagonists (25–26). Therefore, we examined the effect of guanine nucleotides on the direct binding of [^{3}H] spiroperidol. Figure 5 shows detailed saturation isotherms for the binding of [^{3}H]spiroperidol in the presence and absence of a guanine nucleotide (1 mM GTP). An apparent increase in the binding of [^{3}H]spiroperidol was observed. Analysis of these data suggests that the control saturation isotherm can be best described by [^{3}H]spiroperidol binding to two forms of the receptor (Fig. 5, *solid line*), approximately equal in number but different in affinity (R_{total} = 38 pM; K_H' = 45 pM, K_L' = 415 pM). In the presence of 1 mM GTP, the apparent increase in the binding of [^{3}H]spiroperidol can be explained by the ^{3}H-labeled antagonist binding to a single high-affinity form of the receptor with no change in the estimated total number of sites (R_{total} = 38 pM; K_H' = 56 pM). A more

TABLE 2

Quantitative analysis of n-propylapomorphine competition curves for [^{3}H]spiroperidol binding to porcine anterior pituitary dopamine receptors in the absence and presence of guanine nucleotide

Data from Fig. 3 were computer-analyzed as described under Methods. K_H, K_L, and R_H represent the same as that described in the legend to Table 1, whereas R_L and $\%R_L$ represent the fraction of the receptor sites possessing low affinity for the agonist in concentration units and as a percentage of the total binding sites. Because of the effects of nucleotides on [^{3}H]spiroperidol binding (cf. Fig. 5), two dissociation constants for [^{3}H]spiroperidol (30 and 70 pM) were used as estimated from separate saturation binding experiments with the radiolabeled antagonist. The slope factor was obtained as described under Methods.

Parameter	Control[a]	Gpp(NH)p (1×10^{-4} M)
K_H (pM)	290 ± 130	200 ± 350
K_L (pM)	8300 ± 2000	12000 ± 1900
R_H (pM)	14.9 ± 2.2	4.6 ± 2.2
R_L (pM)	16.7 ± 1.7	29.4 ± 1.7
$\%R_L$	52.8	86.0
Slope factor	0.57 ± 0.04	0.76 ± 0.05

[a] Values are means ± standard error of the mean.

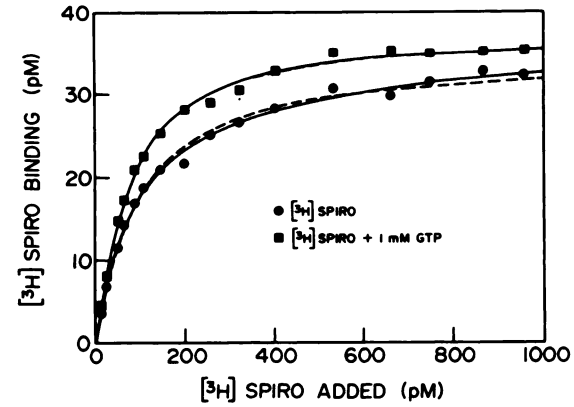

FIG. 5. *Effects of guanine nucleotides on the direct binding of [^{3}H]spiroperidol.*

Saturation binding isotherms for [^{3}H]spiroperidol ([^{3}*H*]*SPIRO*) were generated by incubating membranes with [^{3}H]spiroperidol (5–1000 pM in the presence and absence of 1 mM GTP. Nonspecific binding was determined in the presence of 1 μM (+)-butaclamol and was the same with or without nucleotide. The control saturation curve (●) was analyzed according to a model for one form (*dashed line*) or two forms of the receptor (*solid line*). A statistically significant improvement in the fit was obtained with the analysis according to a two-site model. For the curve in the presence of GTP (■), no improvement in the fit was obtained with analysis assuming a model for two affinity forms of the receptor. The quantitative results of the analysis are given in the text (Results). The experiment shown is representative of three experiments.

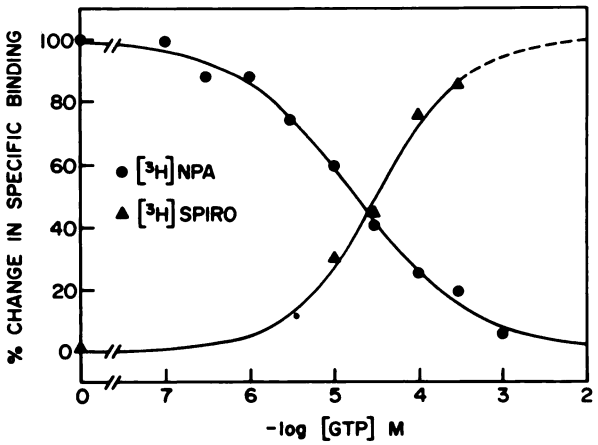

FIG. 6. *Dose-response relationship for the effect of guanine nucleotides (GTP) on ^3H-labeled agonist and ^3H-labeled antagonist binding*

Membranes were incubated with [^{3}H]*n*-propylapomorphine ([^{3}*H*]-*NPA*) (155 pM) (●) and [^{3}H]spiroperidol ([^{3}*H*]*SPIRO*) (160 pM) (▲) in the presence of increasing concentrations of GTP. The results are expressed in terms of percentage change in specific binding for each ligand. [^{3}H]*n*-Propylapomorphine binding was 14.6 pM in the absence of GTP and 2.1 pM in the presence of 1 mM GTP. [^{3}H]Spiroperidol was 32.3 pM in the absence of GTP and 42.4 pM in the presence of 1 mM GTP. The results of the experiment shown for each ligand were from different membrane preparations and are representative of three such experiments.

dramatic graphic difference can be observed when similar data are transformed in Scatchard plot. Control saturation curves are slightly curvilinear, whereas in the presence of guanine nucleotides the Scatchard plot gives a straight line (27). These data suggest that [^{3}H]spiroperidol may bind to two affinity forms of the receptor in roughly equal proportions and that in the presence of guanine nucleotides the heterogeneous population of sites with low and high affinity for antagonists is converted to a single population of antagonist high-affinity form.

As shown in Fig. 6, the effects of guanine nucleotides on agonist and antagonist binding in this system appear to be closely related. The data (Fig. 6) indicate that high-affinity [^{3}H]*n*-propylapomorphine binding displays the same sensitivity to GTP than [^{3}H]spiroperidol (EC_{50} 17 μM versus 32 μM, respectively).

DISCUSSION

The results presented here document that in the porcine anterior pituitary gland system both [^{3}H]spiroperidol and [^{3}H]*n*-propylapomorphine label sites with a dopaminergic specificity. Quantitative analysis of ligand-binding data suggests that, in the particulate preparations used, the dopamine receptor exists in two forms of approximately equal proportions that are discriminated by both agonists and antagonists and modulated by guanine nucleotides. It would appear from our data that the agonist high-affinity form, which can be directly labeled by [^{3}H]*n*-propylapomorphine and which amounts to about 50% of the receptor sites, is converted by guanine nucleotides to a form of the receptor which has lower affinity for agonists and for technical reasons is no longer labeled by direct agonist binding. Similarly, the same two forms of the receptor in these membranes appear to be discriminated by antagonists, although in a fashion reciprocal to agonists. Thus, in membranes, saturation binding data of [^{3}H]spiroperidol can be fitted to a model for two forms of the receptor with high and low affinities for the antagonist. However, guanine nucleotides convert the antagonist low-affinity form to a form of the receptor showing a single high affinity for the antagonist [^{3}H] spiroperidol. Figure 7 shows the schematics of a model for the dopamine receptor in this tissue which is consistent with the data presented here. The model indicates that in addition to GTP the two forms of the receptor can be interconverted by *N*-ethylmaleimide and heat treatment. The evidence for these data is the subject of the accompanying paper (28).

Several lines of evidence suggest that the two forms of the receptor discriminated by agonists correspond to those discriminated reciprocally by antagonists. First, for both agonists and antagonists, the two different affinity forms of the receptor population appear to be present in the same proportions. Second, both agonist and antagonist affinity forms appear to be modulated by guanine nucleotides in the same dose-related fashion with similar EC_{50} values (Fig. 6) and similar nucleotide specificity.

It is apparent that agonists discriminate much more effectively the two affinity forms of the receptor than do antagonists. As shown in Table 1, the ratio of K_L/K_H for all four agonists shown varied from about 30- to 200-fold. This difference in affinity of the agonists for the two forms of the receptor is sufficient in every case to permit observation of a biphasic agonist competition curve for [^{3}H]spiroperidol binding. On the other hand, the discrimination of both affinity forms by the antagonist [^{3}H] spiroperidol is much more subtle. Analysis of detailed [^{3}H]spiroperidol saturation curves indicates that a 10-fold difference can be calculated between the low- and

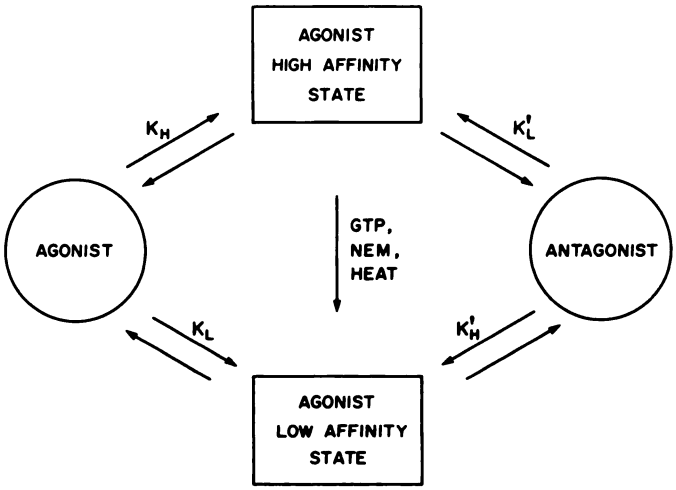

FIG. 7. *Proposed reciprocal model for agonist and antagonist binding to the porcine anterior pituitary dopaminergic receptor*

Both agonists and, to a lesser degree, antagonists discriminate two forms of the receptor population, each form displaying a higher affinity for either the agonist or the antagonist. The agonist high-affinity state (antagonist low-affinity state) is convertible into the other form with guanine nucleotide and other treatments [e.g., *N*-ethylmaleimide (*NEM*), heat].

high-affinity forms for the antagonist (Fig. 5). This represents probably the maximal difference observed in this system, as the range of the differences in values obtained in many experiments varied from 2- to 10-fold between the two affinities for [^{3}H]spiroperidol. However, this modest difference is enough to produce a small but significant (1.5- to 2-fold) shift to the left of an antagonist competition curve for [^{3}H]spiroperidol in the presence of guanine nucleotides (27), but the difference is too small to be measurable from antagonist competition curves of [^{3}H]antagonist binding. The theoretical reasons for this have been presented by De Lean *et al.* (29). Thus, as mentioned under Results, antagonist competition curves for [^{3}H]spiroperidol appeared monophasic and of normal steepness (slope factor ~ 1).

In the course of these studies, experiments were performed to exclude the possibility that a lower-affinity accessory spirodecanone site described by Gorissen *et al.* (30) might be contributing to this apparent heterogeneity of affinity for [^{3}H]spiroperidol. These spirodecanone sites, which recognize only one part of the structure of the radioligand, are of much lower affinity (25–50 nM), and the addition to the binding assay of a compound specific for this accessory site (R 5260) (30) did not affect the results. It was also possible to eliminate the chance that the apparent increase in [^{3}H]spiroperidol binding might be due to the release of tightly bound endogenous agonist (27).

It is of interest to attempt to compare the results obtained in this study with the results of quantitative computer analysis of agonist-binding data in other systems. In the *beta*-adrenergic receptor system of the frog erythrocyte (15), agonists of differing intrinsic activity induced up to 90–95% of agonist high-affinity state of the receptor, whereas agonists of lower intrinsic activity (0.1–0.2) showed closer to 50–60% of the high-affinity state. In the studies reported here, although agonists of differing intrinsic activity were not used, the proportion of agonist high-affinity form of the receptor appears to be consistently around 50%. Still contrasting between the two systems is the observation that guanine nucleotides consistently produce a complete shift of *beta*-adrenergic agonist competition curves to the right (no residual high-affinity state) (15), whereas in the dopaminergic system a residual high-affinity form more often seems to remain even in the presence of maximal concentrations of nucleotides. Whether these observations represent an intrinsic difference between positively and negatively coupled systems must await further investigations. As mentioned above, the dopaminergic receptors of the pituitary gland (anterior and intermediate lobe) appear to be coupled to an attenuation of cyclic AMP levels or the enzyme adenylate cyclase (8, 9, 31–33).

The recognition by the use of appropriate data analysis that agonists bind to more than one affinity state of the dopaminergic receptor permits the reevaluation of the correlation that exists between the dissociation constants (K_D) for agonists calculated from binding data and the potency of the same agonists to elicit an inhibition of prolactin release from the pituitary mammotrophs. In an earlier study (7) we reported a good correlation between the relative potencies of a series of agonists for inhibiting prolactin release from rat anterior pituitary cells in culture and the ability of these agonists to compete for the binding of [^{3}H]dihydroergocryptine to bovine anterior pituitary membranes. However, potencies for agonists such as dopamine and apomorphine from prolactin release data were about 10- to 20-fold lower than dissociation constants from [^{3}H]dihydroergocryptine binding data. It is apparent from the data presented here (Table 1) that the dissociation constant (K_H) of the high-affinity state for dopamine and apomorphine correlate much more closely with the potency obtained from prolactin release data (cf. table 1, ref. 7) (dopamine, K_D = 35 nM versus K_H = 66 nM); apomorphine, K_D = 3 nM versus K_H = 4.7 nM). Therefore, it is reasonable to speculate that the physiological response to dopamine, i.e., inhibition of prolactin release, is a reflection of the interaction of dopaminergic agonists or of dopamine itself with the high-affinity state of the receptor in the anterior pituitary gland.

Burt *et al.* (4) originally proposed that a single dopamine receptor in brain might interconvert between conformations which have high affinities for agonists and antagonists, respectively. This proposal was based on the fact that dopaminergic agonists were much more potent than antagonists at competing for ^{3}H-labeled agonist binding sites, whereas antagonists were more potent than agonists at competing for ^{3}H-labeled antagonist binding sites. In retrospect, however, since agonist and antagonist ligands label more than one receptor subtype in brain (34–36), it would have been difficult to reach a definite conclusion on this point. In the bovine anterior pituitary gland a different situation was encountered because a single receptor subtype appears to exist in that tissue. Binding of the agonist [^{3}H]dihydroergocryptine was potently competed for by both agonists and antagonists (7), suggesting that the above thesis of the agonist versus antagonist state of the receptor might not fit these experimental data. The results presented in this paper on the binding of [^{3}H]*n*-propylapomorphine and [^{3}H]spiroperidol are consistent with our earlier report (7) inasmuch as both agonists and antagonists are very potent competitors of both ^{3}H-labeled agonist and ^{3}H-labeled antagonist binding. Moreover, [^{3}H]*n*-propylapomorphine and [^{3}H]spiroperidol bind to the particulate preparations of porcine anterior pituitary gland with the same specificity, suggesting that they label the same receptor.

The data presented here are consistent with the presence of two affinity forms of the receptor which are modulated by guanine nucleotides. These two forms can be discriminated reciprocally by both agonists and antagonists. We believe that this situation represents an attractive alternative to the model of Seeman (34) of two distinct receptors (D_2 and D_4 receptors) to explain high-affinity agonist binding or nanomolar sensitivity of the inhibition of prolactin release to dopamine in this tissue. Pharmacologically, our data in the porcine anterior pituitary system appear consistent with the terminology proposed by Meunier and Labrie (9) (DA-, for dopaminergic receptors of the anterior and intermediate lobe of the pituitary gland which appear to be coupled negatively to adenylate cyclase). Dopamine receptor interactions with adenylate cyclase in the porcine system, however, have not yet been critically examined.

As documented here, the modulation of the two affinity

forms of the receptor by guanine nucleotides suggests the involvement in the mechanism of dopaminergic ligand binding of a protein entity capable of binding nucleotides and affecting the binding of ligands to the receptor. By analogy with several other systems in which nucleotide effects on binding have been reported (17)—and in particular with the *beta*-adrenergic receptor system, where this phenomenon has been biochemically documented to involve the interaction of a guanine nucleotide-binding protein with the receptor (37, 38)—we speculate that these nucleotide-sensitive multiple-affinity forms of the dopamine receptor of porcine anterior pituitary gland are due to the interaction of the receptor with a guanine nucleotide-binding protein. The studies reported in the accompanying paper (28) were performed in an attempt to obtain more compelling evidence for the involvement of such a nucleotide-binding protein with the dopamine receptor of porcine anterior pituitary gland.

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