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Interactions of Ergot Alkaloids with Anterior Pituitary D-2 Dopamine Receptors

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SUMMARY

The interactions of ergot alkaloids with bovine anterior pituitary D-2 dopamine receptors have been investigated with radioligand binding techniques and computer modeling procedures. Ergot alkaloids of the ergoline class are found to interact with the receptor in an agonist-specific fashion, exhibiting heterogeneous competition curves which can be resolved into high- and low-affinity components. In the presence of guanine nucleotides, however, only the low-affinity binding component is observable. In contrast, ergot alkaloids possessing a cyclic peptide side chain (ergopeptines) interact with the receptor in a homogeneous fashion, exhibiting competition curves which are monophasic and unaffected by guanine nucleotides. Ergopeptines thus display the binding characteristics of antagonist ligands even though they possess pharmacologically demonstrated agonist properties. These ergot alkaloid receptor binding characteristics determined from indirect competition experiments are also directly demonstrable using radiolabeled ergoline and ergopeptine ligands. The radiolabeled ergopeptine [^{3}H]dihydroergocryptine ([^{3}H]DHE) is shown to label identical binding sites with the same pharmacological characteristics as the antagonist [^{3}H]spiroperidol. However, the dissociation rate of [^{3}H]DHE from these binding sites is much slower than [^{3}H]spiroperidol and is inconsistent with its equilibrium-determined binding affinity. In addition, saturation experiments with radiolabeled antagonists and agonists and unlabeled ergopeptines indicate that ergopeptines interact with these receptor sites in a competitive fashion. A model is presented which may explain these unique binding properties of ergot alkaloid agonists to the pituitary D-2 dopamine receptor.

INTRODUCTION

Dopamine receptors can be classified into two major categories, D-1 and D-2, each possessing unique pharmacological and biochemical properties (1, 2). D-1 receptors potentiate adenylate cyclase activity upon agonist occupation, whereas D-2 receptors have been shown to attenuate this enzymatic activity. Dopaminergic ergot alkaloids have been demonstrated to exhibit selectivity for these subclasses of dopamine receptors. In general, dopaminergic ergots are highly potent agonists with full intrinsic activity at D-2 receptors whereas they are fairly weak antagonists or partial agonists at D-1 receptors (1, 2). The pituitary gland has been proposed to possess prototype D-2 dopamine receptors (1). In the anterior lobe, agonist occupancy of these receptors results in a diminished release of prolactin (3, 4) and a reduction of basal (5–9) and vasoactive intestinal peptide-induced (10) adenylate cyclase activity. Ergot alkaloids have been well established as potent inhibitors of prolactin secretion in all vertebrate species tested, including man (11–13). Their therapeutic potential has been well demonstrated in the treatment of hyperprolactinemia and pathological lactation in both women and men (13). More recently, several ergot derivatives have been shown additionally to possess agonist activity in diminishing anterior pituitary adenylate cyclase activity (5, 7, 10). Ergot alkaloids also exhibit agonist activity at the intermediate pituitary D-2 dopamine receptor, where they decrease α-melanocyte-stimulating hormone release and attenuate *beta*-adrenergic receptor-induced cyclic AMP production (14–17).

We have recently investigated the ligand binding properties of D-2 dopamine receptors on anterior pituitary membranes using radioligand binding techniques and computer-assisted data analysis (18). The binding of dopaminergic antagonists to the D-2 receptor displays mass-action properties consistent with a bimolecular reaction, whereas dopamine agonists exhibit heterogeneous binding properties indicative of two receptor binding

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states of high and low affinity. In the presence of guanine nucleotides, however, the high-affinity state is undetectable, suggesting that guanine nucleotides promote a conversion of the high-affinity state to the low-affinity state. However, we observed that the ergot alkaloid bromocryptine did not differentiate between the high- and low-affinity D-2 receptor binding states (18). Bromocryptine thus exhibits the binding characteristics of an antagonist even though it is a potent, highly efficacious agonist at D-2 receptors. In contrast, the ergot alkaloid pergolide was observed to demonstrate the receptor binding characteristics of an agonist, in keeping with its pharmacologically demonstrated agonist properties at the pituitary D-2 receptor (18). These preliminary findings with ergot alkaloid agonists might suggest that a drug's efficacy is not correlated with its ability to differentiate between the two D-2 receptor binding states.

In the present investigation, we extend our preliminary findings and examine the D-2 receptor binding characteristics of a large number of ergot alkaloid agonists in order to obtain a complete structure-activity relationship. Ergot alkaloids can be divided into two main classes, the ergopeptines and the ergolines (see Experimental Procedures). In addition, we investigate several potential molecular mechanisms regarding the inability of certain ergot alkaloids to discriminate between the high- and low-affinity receptor binding states of the anterior pituitary D-2 dopamine receptor.

EXPERIMENTAL PROCEDURES

Tissue preparation. Fresh steer pituitary glands were obtained from a local slaughterhouse. The glands were bisected and the anterior lobes were removed and used fresh or stored frozen (−70°) for up to 1 week. Anterior pituitary halves were finely minced then homogenized in 50 mM Tris-HCl buffer (pH 7.7 at 25°) (hereafter referred to as Tris buffer), at 100 mg of tissue per milliliter with a Tekmar homogenizer. The homogenate was centrifuged twice (for 10 min at 50,000 × *g*) at 4° with resuspension of the intermediate pellet in fresh Tris buffer. The final membrane pellet was resuspended in 37° assay buffer to yield a final tissue concentration of 20 mg/ml (wet weight).

Radioligand binding assays. The assay buffer employed contained 50 mM Tris buffer, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5.7 mM ascorbic acid. As documented elsewhere (19), the inclusion of ascorbic acid was necessary to demonstrate dopamine receptor-specific agonist-receptor interactions. The membrane-assay buffer suspension was added to triplicate glass test tubes (12 × 75 mm) containing radioligands and other unlabeled drugs to yield a final assay volume of 1 ml. Unless indicated otherwise, the binding experiments were incubated for 15 min at 37° with the exception of [^{3}H]DHE experiments, which were incubated for 30 min at 37°. The binding reactions were terminated by rapid filtration over GF/C glass-fiber filters (Whatman) under vacuum. The filters were subsequently rinsed with 15 ml of ice-cold 50 mM Tris buffer. Radioactivity trapped on the filters was counted by liquid scintillation spectroscopy at an efficiency of 45%. Nonspecific binding of all radioligands was determined in the presence of 1 μM (+)-butaclamol. At their K_D concentrations, the percentage of the specific binding to the total binding was 80–90% for [^{3}H]spiroperidol (~2000 cpm), 60–70% for [^{3}H]NPA[3] (~1500 cpm), 50–60% for [^{3}H]DHE (~1300 cpm), and 70–80% for [^{3}H]lisuride (~1700 cpm). Competition experiments were performed using [^{3}H]spiroperidol concentrations between 0.22 and 0.49 nM, [^{3}H]NPA concentrations between 0.12 and 0.29 nM, and [^{3}H]DHE concentrations between 0.31 and 0.44 nM.

[3] The abbreviations used are: NPA, (−)-*N*-*n*-propylnorapomorphine; DHE, dihydroergocryptine; GppNHp, guanyl-5′-yl imidodiphosphate.

Data analysis. The computer analysis employed is a weighted nonlinear regression curve-fitting program (20) using a generalized model for ligand-receptor binding systems (21). All computations were performed using a VAX 11 computer. The exact treatment of experimental data has been previously described in detail (18). Briefly stated, competition curves are analyzed according to models for the mass-action binding of radioligand and competing drug to one or multiple receptor binding sites. Deviation of the observed points from their predicted values are weighted according to the reciprocal of the predicted variance. Statistical difference between models was tested by comparing their residual variances of fits to the data. Saturation curves were analyzed similarly with the data in this paper, presented in Scatchard coordinates. All experiments for which representative data are presented were replicated a minimum of three times with the results varying by less than 20%.

Materials. [^{3}H]Spiroperidol (25–35.9 Ci/mmole), [^{3}H]NPA (68.9–75 Ci/mmole), and [^{3}H]DHE (21.9–25 Ci/mmole) were obtained from New England Nuclear Corporation. [^{3}H]Lisuride (26.4 Ci/mmole) was a gift of Schering AG. GppNHp was purchased from Sigma Chemical Company. The following drugs were gifts from the following pharmaceutical companies: apomorphine, Merck & Company (Rahway, N. J.); butaclamol, Ayerst Laboratories (Rouses Point, N. Y.); spiroperidol, Janssen Pharmaceuticals (Beerse, Belgium); pergolide and lergotrile, Eli Lilly and Company (Indianapolis, Ind.); lisuride, Schering (Bloomfield, N. J.). All other ergot alkaloids used in this study were generously supplied by Sandoz Pharmaceuticals (Basel, Switzerland).

Structural formulae of the ergot alkaloids. Ergot alkaloids can be divided into two broad categories, the ergopeptines and the ergolines, both of which contain the tetracyclic ergoline nucleus (22). Ergopeptines are of high molecular weight and consist of *d*-lysergic acid which is linked by an amide bridge to a cyclic peptide component. Ergolines are of lower molecular weight and are derivatives of the tetracyclic skeleton of lysergic acid. The structural formulae of all of the ergopeptines and ergolines utilized in this study are indicated in Fig. 1. The major structural differences between the ergopeptines are due to their substituents at the R_1 and R_2 positions (Fig. 1). Conversely, the ergolines differ mainly in their R_1 substitutions (Fig. 1). All of the ergopeptines and ergolines in Fig. 1 have been shown to exhibit agonist activity at either the anterior pituitary D-2 dopamine receptor (5, 7, 11–13, 23) or at D-2 receptor systems in the central nervous system (24–26).

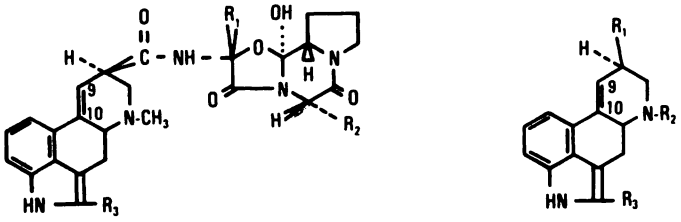
RESULTS

Interactions of ergolines and ergopeptines with [^{3}H]spiroperidol and [^{3}H]NPA binding. Figure 2 shows representative ergoline competition experiments with the radiolabeled antagonist [^{3}H]spiroperidol and the agonist [^{3}H]NPA. The ergoline CF25397/[^{3}H]spiroperidol curve exhibits heterogeneous characteristics (pseudo-Hill coefficient < 1), with computer analysis indicating that the data are best explained by assuming a two-state binding model. The dissociation constants for the high-affinity (R_H) and the low-affinity (R_L) agonist binding states have been designated K_H and K_L, respectively. In the presence of the guanine nucleotide GppNHp, however, the high-affinity component of the CF25397/[^{3}H]spiroperidol curve is abolished, resulting in a rightward shift and steepening (pseudo-Hill coefficient = 1) of the curve. Computer modeling now indicates the presence of a single homogeneous binding state whose affinity is similar to that of the low-affinity state observed in the control curve (Fig. 2). Since guanine nucleotides exert no direct effects on the binding of [^{3}H]spiroperidol (18), these data suggest that the high-affinity state (R_H) is converted to the low-affinity state (R_L) in the presence of guanine nucleotides. We have previously shown that low concen-

trations of [^{3}H]NPA selectively label the high-affinity D-2 receptor binding state (18). It is thus not surprising that the CF25397/[^{3}H]NPA competition curve is homogeneous and fits best to a single binding state whose affinity agrees well with the K_H value from the [^{3}H] spiroperidol curve (Fig. 2).

Illustrated in Fig. 3 are representative ergopeptine/[^{3}H]spiroperidol competition experiments using the ergopeptine ergosine. In contrast to the ergolines, the ergosine/[^{3}H]spiroperidol curve is steep (pseudo-Hill coefficient = 1) and is best described by a single binding site model (Fig. 3). Furthermore, the addition of GppNHp has no significant effect on the ability of ergosine to compete with [^{3}H]spiroperidol binding (Fig. 3).

The computer-derived parameters for all of the ergoline and ergopeptine competition experiments have been compiled in Table 1. All of the ergoline agonists demonstrate the ability to differentiate between the high- and low-affinity receptor binding states. In each case the affinities derived from [^{3}H]NPA binding are statistically indistinguishable from the K_H parameters derived from [^{3}H]spiroperidol binding. Similarly, the [^{3}H]spiroperidol competition curves are shifted by guanine nucleotides such that their single affinities are indistinguishable from the control K_L values. Furthermore, with the exception



Ergopeptine Skeleton

Ergoline Skeleton

Ergopeptines	R_1	R_2	R_3
Ergocornine	$CH(CH_3)_2$	$CH(CH_3)_2$	H
Ergocristine	$CH(CH_3)_2$	$CH_2C_6H_5$	H
Ergosine	CH_3	$CH_2CH(CH_3)_2$	H
Ergotamine	CH_3	$CH_2C_6H_5$	H
*Dihydroergotamine	CH_3	$CH_2C_6H_5$	H
*Dihydroergocryptine	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	H
*Bromocryptine	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	Br
Ergolines			
Ergometrine	$CONHCH(CH_3)CH_2OH$	CH_3	H
Lisuride	$NHCON(C_2H_5)_2$	CH_3	H
CF 25397	$CH_2SC_5H_4N$	CH_3	H
*CH 29717	$NHSO_2N(CH_3)_2$	CH_3	H
*CQ 32084	$NHSO_2N(C_2H_5)_2$	CH_3	H
*CM 29712	CH_2CN	CH_3	H
*Lergotrile	CH_2CN	CH_3	Cl
*Pergolide	CH_2SCH_3	$CH_2CH_2CH_3$	H

FIG. 1. *Structural formulae of the ergot alkaloids utilized*

The ergot alkaloids are divided into two classes, the ergopeptines and the ergolines, with the backbone skeletons and different substituents of each being designated. Those compounds marked with an *asterisk* are reduced at the 9–10 double bond.

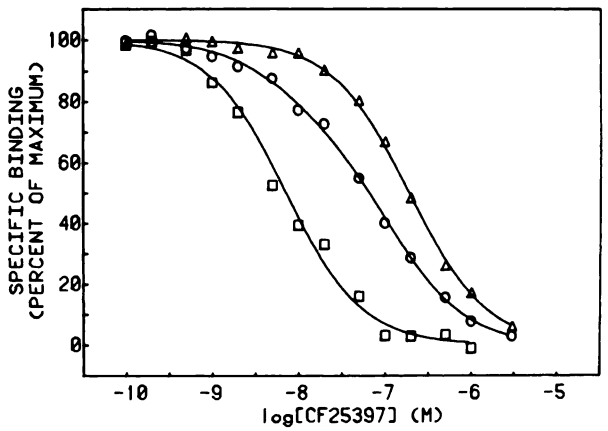


FIG. 2. *Computer-fitted CF25397/[^{3}H]ligand competition curves*

The data points shown were experimentally determined whereas the drawn lines represent the computer-modeled best fit to the data. The CF25397/[^{3}H]NPA curve (□) exhibits a pseudo-Hill coefficient of 0.94 and fits best to one binding site with an affinity of 5.3 nM. The control CF25397/[^{3}H]spiroperidol curve (○) has a pseudo-Hill coefficient of 0.66 and is best fitted to two binding sites with the following binding parameters: K_H = 5.9 nM, K_L = 80 nM, and $\%R_H$ = 47. In the presence of 0.1 mM GppNHp (△), the curve's pseudo-Hill coefficient increases to 1.0 and the curve models best to a single binding site with an affinity (K_L) of 75 nM.

of lergotrile and ergometrine, the high- and low-affinity receptor binding states are induced or detected in about equal proportions. These ergoline binding characteristics are qualitatively similar to those for classical dopamine agonists previously demonstrated (18). An important exception, however, is the ratio of affinities for the two receptor binding states. Catecholamine dopamine agonists exhibit K_L/K_H ratios approaching 100 (18). However, the ergolines demonstrate much less selectivity,

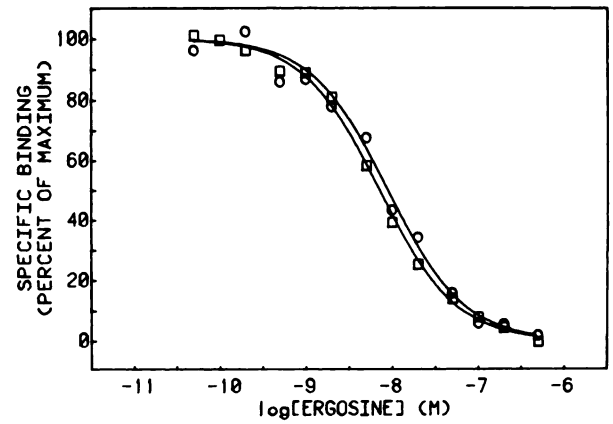


FIG. 3. *Computer-modeled ergosine/[^{3}H]spiroperidol competition curves.*

The control curve (□) represents the fit to a single binding site with a pseudo-Hill coefficient of 0.9 and a dissociation constant (K_D) of 3.8 nM. With the addition of 0.1 mM GppNHp (○), the curve is also sufficiently fit using a one-site binding model and exhibits a pseudo-Hill coefficient of 0.95 and a K_D of 4.1 nM. When the two curves are analyzed simultaneously, there is no significant ($p > 0.1$) difference between the two K_D values.

TABLE 1

Computer-derived parameters for ergot alkaloid competition experiments using [^{3}H]NPA and [^{3}H]spiroperidol

Competition experiments were performed and analyzed as described in Figs. 2 and 3. K_{NPA} refers to the single dissociation constant observed in competing for [^{3}H]NPA binding. K_H, K_L, and K_G represent dissociation constants for competing with [^{3}H]spiroperidol, with K_G designating the single dissociation constant observed in the presence of 0.1 mM GppNHp. All dissociation constants are in nanomolar units. $\%R_H$ indicates that fraction of the total [^{3}H]spiroperidol binding sites observed in the high-affinity state. The data are presented as mean ± standard error; the number of experimental replications is indicated by n. In some experiments, incubation times were increased to 30 min with no difference in the results. Using Student's t-test, there was no significant ($p > 0.05$) difference between any pair of K_{NPA} and K_H or K_L and K_G values.

Drug	K_{NPA}	K_H	K_L	K_G	$\%R_H$	n	K_L/K_H
Pergolide	3.8 ± 0.59	4.1 ± 1.6	137 ± 14	120 ± 47	45 ± 3.8	3	33
Lergotrile	13.0 ± 1.7	10 ± 1.2	370 ± 67	420 ± 26	31 ± 2	5	34
CM 29712	83 ± 15	90 ± 31	1230 ± 99	1220 ± 39	46 ± 4.5	3	14
CQ 32084	5.0 ± 0.7	6.3 ± 0.9	71 ± 10	69 ± 6	49 ± 5	3	11
CH 29717	8.8 ± 2.6	10 ± 3.2	180 ± 69	170 ± 48	46 ± 5	3	18
CF 25397	5.4 ± 1.4	6.5 ± 2.5	78 ± 11	75 ± 2	51 ± 2.4	4	12
Lisuride	0.5 ± 0.03	0.62 ± 0.1	4.8 ± 0.74	4.0 ± 0.17	51 ± 1	5	8
Ergometrine	87 ± 12	73 ± 21	2200 ± 320	2100 ± 630	32 ± 2	3	31
Ergocornine	4.2 ± 0.62	5.6 ± 1.2[a]		5.1 ± 1.2	—[a]	2	—
Ergocristine	4.9 ± 0.62	5.3 ± 1.8[a]		4.6 ± 0.7	—[a]	2	—
Ergosine	2.4 ± 0.1	2.6 ± 0.4[a]		3.0 ± 0.1	—[a]	3	—
Ergotamine	1.6 ± 0.57	1.1 ± 0.32	12.9 ± 1.8	11.2 ± 0.64	55 ± 6	3	12
Dihydroergotamine	1.4 ± 0.25	1.8 ± 0.5	17.9 ± 1.2	16.9 ± 1.6	51 ± 10	3	10
Dihydroergocryptine	0.91 ± 0.1	1.3 ± 0.14[a]		1.2 ± 0.24	—[a]	3	—
Bromocryptine	2.4 ± 0.33	3.2 ± 0.64[a]		3.4 ± 0.85	—[a]	3	—

[a] These compounds were found not to discriminate between the high- and low-affinity states of the D-2 receptor and thus exhibit single dissociation constants (i.e., $K_H = K_L$). As such, the $\%R_H$ is an undeterminable parameter in these experiments. See text for discussion.

with K_L/K_H ratios ranging between 8 and 34 (Table 1). In contrast to the ergolines, ergopeptine agonists, in general, do not discriminate between the two receptor binding states (Table 1). For these agonists, the homogeneous affinities in competing for [^{3}H]NPA binding and for [^{3}H]spiroperidol binding in the absence and presence of GppNHp are identical. There are, however, two ergopeptines which are exceptional, ergotamine and dihydroergotamine, which, interestingly, exhibit binding characteristics similar to those of the ergolines (Table 1).

Direct binding studies with radiolabeled ergopeptines and ergolines. The availability of both ergopeptine and ergoline compounds radiolabeled to a high specific activity has enabled us to examine their receptor binding properties directly. The radiolabeled ergopeptine, [^{3}H]DHE, has been previously characterized as labeling dopaminergic receptors in anterior pituitary membrane preparations (12, 27). As shown in Fig. 4, the binding of [^{3}H]DHE to anterior pituitary membranes is homogeneous and saturable, exhibiting a maximal binding capacity (B_{max}) identical with that for [^{3}H]spiroperidol. Furthermore, the binding of [^{3}H]DHE is unaffected by guanine nucleotides (Fig. 4). [^{3}H]DHE's binding characteristics are thus "antagonist-like" and similar to those exhibited in the unlabeled DHE competition experiments (Table 1).

The radiolabeled ergoline, [^{3}H]lisuride, has been shown previously to label dopaminergic receptors as well as serotonergic and *alpha*$_2$-adrenergic receptors in the central nervous system (28–30). There are no known *alpha*$_2$-adrenergic or serotonergic receptors in the anterior pituitary, however, and preliminary experiments have indicated that [^{3}H]lisuride binding to bovine anterior pituitary membranes is exclusively dopaminergic (data not shown). Saturation isotherms for this radiolabeled ergoline are shown in Fig. 5. In the absence of nucleotides, the [^{3}H]lisuride isotherm is curvilinear when plotted in Scatchard coordinates. Computer analysis of these data indicates the presence of two binding components present in approximately equal proportions in the membranes (Fig. 5). With the addition of GppNHp, the [^{3}H]lisuride isotherm becomes homogeneous, indicating a uniform population of binding sites whose affinity is similar to the low-affinity sites defined in the control isotherm (Fig. 5). It is important to note that the total receptor binding capacity of [^{3}H]lisuride is similar to that for [^{3}H]spiroperidol and [^{3}H]DHE (Figs. 4 and 5). Fur-

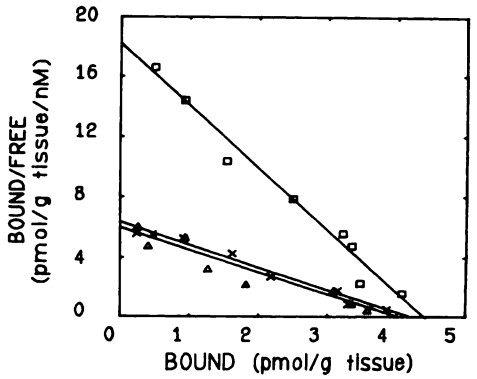


FIG. 4. *Saturation isotherms for [^{3}H]spiroperidol and [^{3}H]DHE binding to anterior pituitary membranes*

All saturations were performed using the same membrane preparation. [^{3}H]Spiroperidol concentrations varied between 0.043 and 5.6 nM and the resulting isotherm (□) exhibits a K_D and B_{max} of 0.24 nM and 4.3 pmoles/g of tissue (wet weight), respectively. The [^{3}H]DHE saturations were conducted with concentrations ranging from 0.052 to 8.1 nM. The control isotherm (△) exhibits K_D and B_{max} values of 0.67 nM and 4.0 pmoles/g of tissue. In the presence of 0.1 mM GppNHp (×), the [^{3}H]DHE isotherm shows a K_D of 0.66 nM and a B_{max} of 4.1 pmoles/g of tissue.

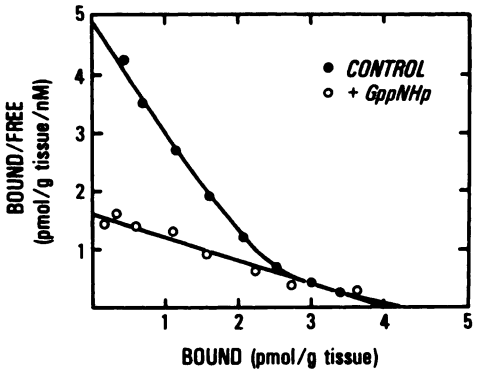


FIG. 5. *[³H]Lisuride saturation isotherms in bovine anterior pituitary membranes*

Both isotherms were conducted in the same membrane preparation using [³H]lisuride concentrations from 0.1 to 14.2 nM. The control isotherm (●) was found to fit optimally to two binding sites with the following parameters: K_H = 0.40 nM, K_L = 2.6 nM, R_H = 1.9 pmoles/g of tissue (47% of total), and R_L = 2.2 pmoles/g of tissue (53% of total). In the presence of 0.1 mM GppNHp (○), the [³H]lisuride saturation isotherm fits best to a single site with a K_D of 2.4 nM and a B_{max} of 4.0 pmoles/g of tissue.

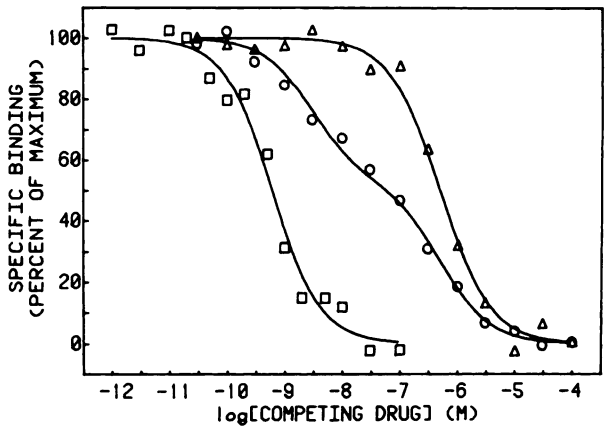


FIG. 6. *Computer-modeled [³H]DHE competition experiments*

The one-site spiroperidol/[³H]DHE curve (□) has a pseudo-Hill coefficient of 0.95 and an affinity of 0.34 nM. The control apomorphine/[³H]DHE competition curve (○) assumed a two-site fit with a pseudo-Hill coefficient of 0.47 and the following parameters: K_H = 3.7 nM, K_L = 333 nM, and $\%R_H$ = 52. With 0.1 mM GppNHp (△) the apomorphine curve assumes a one-site fit with a pseudo-Hill coefficient of 1.2 and an affinity (K_L) of 315 nM.

thermore, the dissociation constants for the high- and low-affinity components of [³H]lisuride binding agree reasonably well with the K_H and K_L values for lisuride shown in Table 1. It is thus reasonable to assume that the two binding components of the ergoline [³H]lisuride represent the high- and low-affinity receptor binding states of the D-2 receptor.

Mechanisms for ergopeptine-receptor interactions. The data presented thus far indicate that, in general, ergopeptine agonists exhibit the binding characteristics of antagonist ligands. In order to further investigate and define the binding characteristics of ergopeptine ligands, we performed competition experiments with [³H]DHE. Figure 6 shows [³H]DHE competition experiments involving the unlabeled antagonist, spiroperidol, and the agonist, apomorphine. Spiroperidol competes with [³H]DHE binding in a homogeneous fashion with an affinity identical with that directly determined with [³H]spiroperidol (Figs. 4 and 8) (18). In contrast, the apomorphine/[³H]DHE competition curve is clearly biphasic, exhibiting high (R_H) and low (R_L) affinity competition components (Fig. 6). In the presence of GppNHp only the R_L component is present. These data are qualitatively and quantitatively identical with apomorphine/[³H]spiroperidol competition experiments in anterior pituitary membranes (18). The apomorphine K_L/K_H ratio of about 100 in Fig. 6 is noteworthy.

Figure 7 shows [³H]DHE competition experiments with the ergot alkaloids bromocryptine and pergolide. Bromocryptine competes for [³H]DHE binding in a homogeneous, uniphasic fashion, whereas pergolide displays a competition curve that is optimally computer-fitted to two binding sites. Only the low-affinity binding site is observed for pergolide in the presence of GppNHp (Fig. 7). The binding parameters for bromocryptine and pergolide derived in Fig. 7 and in good agreement with the values for these drugs in competing with [³H]spiroperidol binding (Table 1) (18). The data in Figs. 4, 6, and 7 as well as previous characterizations of anterior pituitary [³H]DHE binding (12, 27, 31) thus suggest that the agonist [³H]DHE labels, in an identical fashion, the same receptor sites as the antagonist, [³H]spiroperidol.

One possible explanation for why ergopeptine agonists exhibit the binding characteristics of antagonist ligands is that they interact with the receptor in an irreversible fashion. Indeed, Bannon *et al.* (32) have suggested this possibility based on the long duration of bromocryptine's agonist action *in vivo* and on subsequent radioligand binding experiments. In order to test this hypothesis, we initially performed experiments to evaluate the competitive/noncompetitive nature of ergopeptine binding. Shown in Fig. 8A is a [³H]spiroperidol saturation experiment conducted in the presence and absence of DHE. The presence of DHE results in a decrease in the apparent affinity (increase in K_D) of [³H]spiroperidol with no

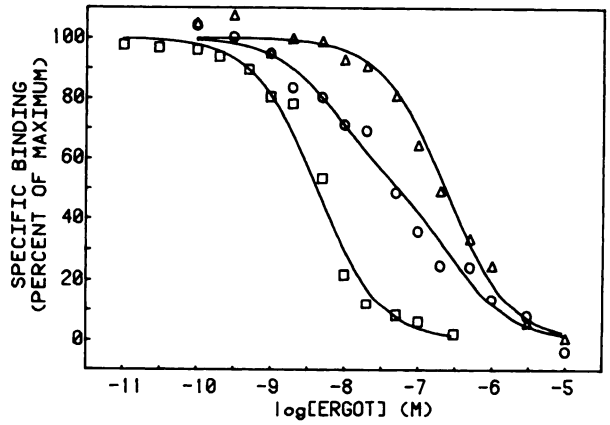


FIG. 7. *Ergot/[³H]DHE computer-fitted competition curves*

The bromocryptine/[³H]DHE curve (□) represents a single binding site with a K_D of 2.9 nM and a pseudo-Hill coefficient of 1.3. The pergolide/[³H]DHE curve (○) is best explained by two binding sites with the following parameters: K_H = 5.5 nM, K_L = 208 nM, $\%R_H$ = 48, and pseudo-Hill coefficient = 0.43. The 0.1 mM GppNHp curve (△) is fitted best to one site with a K_D of 161 nM and a pseudo-Hill coefficient of 0.92.

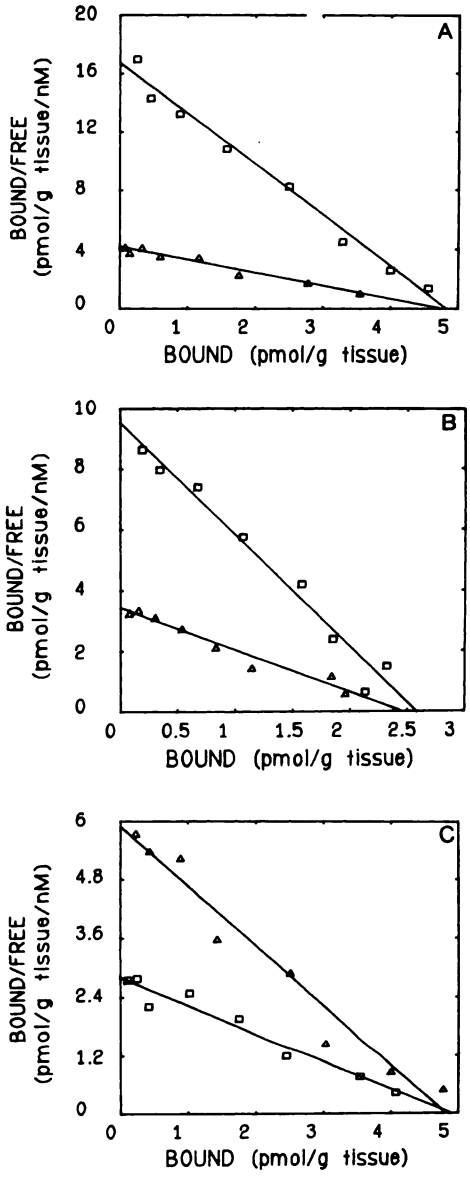


FIG. 8. *[³H]Ligand saturation experiments in the presence and absence of unlabeled ergopeptines*

Each pair of saturation isotherms was conducted in the same membrane preparation. A, [³H]Spiroperidol isotherms were obtained using [³H]spiroperidol concentrations from 0.024 to 3.58 nM. The control isotherm (□) shows a K_D value of 0.29 nM and a B_{max} value of 4.8 pmoles/g of tissue. In the presence of 4 nM DHE (△) the K_D and B_{max} parameters are 1.1 nM and 4.7 pmoles/g of tissue, respectively. B, [³H]NPA saturation experiments were conducted with [³H]NPA concentrations from 0.029 to 3.72 nM. The K_D and B_{max} parameters for the isotherms are as follows: control (□), K_D = 0.27 nM, B_{max} = 2.6 pmoles/g of tissue; + 3 nM DHE (△), K_D = 0.72 nM, B_{max} = 2.45 pmoles/g of tissue. C, [³H]DHE saturation experiments were conducted with concentrations ranging from 0.048 to 10.1 nM. The parameters are as follows: control (△), K_D = 0.8 nM, B_{max} = 4.8 pmoles/g of tissue; + 3 nM bromocryptine (□), K_D = 1.8 nM, B_{max} = 4.9 pmoles/g of tissue.

change in binding capacity. Similar results were obtained when [³H]NPA saturation experiments were performed (Fig. 8B). Furthermore, when [³H]DHE saturation isotherms were conducted in the presence of bromocryptine there was also a decrease in apparent affinity but no change in binding capacity for [³H]DHE (Fig. 8C). Preincubation with the ergopeptines led to results identical with those in Fig. 8 (data not shown). These data thus suggest that ergopeptines do *not* bind in an irreversible or noncompetitive fashion but instead exhibit the characteristics of competitive ligands.

To evaluate the reversibility of ergopeptine ligands in a more direct fashion, [³H]DHE kinetic experiments were performed. As seen in Fig. 9A, [³H]DHE binding achieves equilibrium by 30 min. The association data are replotted in the *inset* (Fig. 9A), and a pseudo-first order association rate constant, k_{obs}, is determined from the slope of the line, which equals 0.16 min^{-1}. The dissociation of [³H]DHE was evaluated by adding excess bromocryptine after equilibrium was achieved (Fig. 9B). [³H]DHE binding is completely reversible, with the dissociation occurring as a first-order process. The dissociation rate constant was determined to equal 0.0071 min^{-1} (Fig. 9B). Other unlabeled compounds, such as spiroperidol and apomorphine, that were used to measure dissociation gave similar results (data not shown). The second-order

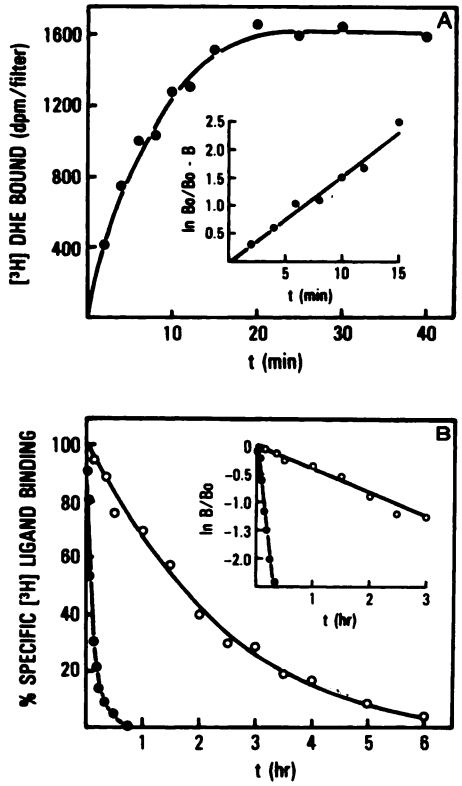


FIG. 9. *[³H]DHE and [³H]spiroperidol kinetic experiments*

A, The association of [³H]DHE was examined by adding 0.38 nM [³H]DHE to membranes previously equilibrated at 37° and sampling the specific binding at the indicated time points. The *inset* shows a first-order kinetic plot of these data, where B represents the amount bound at time t and B_o represents the amount bound at equilibrium. B, Dissociation experiments were performed by allowing [³H]DHE (0.38 nM) and [³H]spiroperidol (0.21 nM) to reach equilibrium and adding excess bromocryptine (20 μM final) and (+)-butaclamol (20 μM final) to the [³H]DHE (○) and the [³H]spiroperidol (●) experiments, respectively. *Inset*, first-order rate plot of the data, where B represents the amount specifically bound at time t and B_o is the amount bound at t = 0. The first-order dissociation rate constants (k_2) can be determined from this plot and equal 0.0071 min^{-1} for [³H]DHE and 0.13 min^{-1} for [³H]spiroperidol.

association rate constant, k_1, can be calculated from the equation $k_1 = (k_{obs} - k_2/[^3H]DHE)$ and equals 0.40 nM^{-1} min^{-1}. The ratio of the rate constants (k_2/k_1) provides a kinetic estimate of the equilibrium dissociation constant (K_D) for [^{3}H]DHE binding. From the data in Figs. 9A and 9B, the kinetically estimated K_D is 18 pM, which is approximately 50-fold higher than values determined either directly (Figs. 4 and 8C) or indirectly (Table 1). Thus, although DHE is a competitive and reversible ligand, its interactions with the receptor exhibit anomalous kinetics. This is additionally suggested by a comparison of the [^{3}H]DHE and [^{3}H]spiroperidol dissociation experiments shown in Fig. 9B. [^{3}H]Spiroperidol dissociates from the receptor almost 20-fold faster than [^{3}H]DHE and exhibits kinetics in accordance with its equilibrium-determined K_D (33).

DISCUSSION

A major question concerning the ergopeptine agonist-receptor binding characteristics is how they can be incorporated within our previously proposed (18) ternary complex model for the D-2 dopamine receptor. This model suggests that there are two possible steps of receptor-ligand interaction:

$$L + R \rightleftharpoons LR \quad (1)$$

$$LR + X \rightleftharpoons LRX \quad (2)$$

Both antagonist and agonist ligands participate in the first binding step, leading to the formation of a binary complex. Agonists, however, are proposed to induce a conformational change in the receptor, allowing the complexation of a third membrane component. Agonists are bound with higher affinity to this ternary complex (R_H) than the low-affinity binary complex (R_L). This model has also been proposed for the *beta*-adrenergic receptor systems (34, 35), where the third component has been identified as a guanine nucleotide-binding regulatory protein (36). Guanine nucleotide association with this protein displaces the equilibrium of these systems such that only the first binding step is observed.

In the context of the above model, several explanations for the anomalous binding characteristics of ergopeptines can be considered. One possibility may be that the radioligand binding sites under investigation in the anterior pituitary bear no relationship to the pharmacologically identified D-2 receptors. In this case, the "non-discriminating" ergopeptines would actually be antagonists at the D-2 receptor labeled with radioligands. This explanation is unlikely since there is excellent agreement between the affinities determined for ligands in radioligand binding studies and those obtained in pharmacological investigations (12, 18, 37). Thus, the receptors identified in these two experimental paradigms appear to be identical.

Another explanation, mentioned earlier, may be that the ergopeptines bind to the receptor in an irreversible fashion. This hypothesis seems to be discounted by the experiments shown in Figs. 8 and 9. In Fig. 8, the ergopeptine DHE was shown to interact in a competitive fashion, whereas in Fig. 9 [^{3}H]DHE binding was shown to be completely reversible. These data appear to conflict with the observations of Bannon *et al.* (32), who observed that 2 hr after a peripheral injection of bromocryptine there was a decrease in the binding capacity of [^{3}H]spiroperidol to rat striatal membranes. However, since bromocryptine was administered *in vivo* it is possible that the observed loss in binding was due to an agonist-induced receptor down-regulation (38).

Other conceivable mechanisms for ergopeptine binding include the possibility that they do not bind at the same "active" site on the receptor as other ligands or that they bind to more than one receptor site. The former possibility appears to be excluded by the observation that [^{3}H]DHE and [^{3}H]spiroperidol bind to sites which qualitatively and quantitatively exhibit the same pharmacological specificity. The latter possibility is ruled out by the fact that [^{3}H]DHE and [^{3}H]spiroperidol possess the same binding capacity (Fig. 4).

Finally, one may speculate that, although ergopeptines bind at the same site on the receptor as other ligands, their cyclic peptide side chain undergoes an additional binding reaction with adjacent receptor sites. This latter reaction is proposed to provide a major portion of the ergopeptine-receptor binding energy. In this model, ergopeptines would exert their agonist activity by inducing ternary complex formation; however, in contrast to low molecular weight agonists, little additional binding energy is derived from this reaction. Thus, since ergopeptines exhibit the same affinity for the receptor whether or not it is coupled to X, guanine nucleotides would not be predicted to affect their binding affinity. This model is also consistent with the extremely slow dissociation of [^{3}H]DHE from its binding sites which, presumably, is limited by the dissociation of the peptide side chain. These observations could account for several reports in the literature documenting extremely long-lasting pharmacological effects of ergopeptine agonists on dopamine receptors (32, 39–41).

This proposed model for ergopeptine binding is additionally consistent with several experimental observations. First, the ability of ergot agonists to discriminate between the high- and low-affinity receptor states is generally correlated with the possession of a high molecular weight peptide side chain. For instance, the ergopeptines ergocornine, ergocristine, and ergosine are structurally homologous with the ergolines ergometrine, lisuride, and CF25397 (Fig. 1). All of these compounds possess the same tetracyclic ergoline nucleus containing the proposed dopaminergic pharmacophore (42–45) which presumably interacts with the "active site" on the receptor. However, in contrast to the ergolines, the ergopeptines do not differentiate between the two receptor binding states. Second, even though the ergoline compounds were able to differentiate between R_H and R_L, they did not exhibit as great a selectivity as the smaller catecholamine agonists (Table 1; Fig. 6) (18). Thus, the ergolines can be placed in the middle of a general structural continuum in which the K_L/K_H ratio is dependent on the size of an agonist and thus the number of potential attachment points on the receptor. Finally, since dopaminergic ergot alkaloids are generally suggested to possess full intrinsic agonist activity at D-2 receptors, then those ergots which discriminate between R_H and R_L would be expected to

exhibit about 50% R_H as other full agonists (18). This prediction is generally fulfilled for both ergoline and ergopeptine agonists (Table 1).

Although our model can successfully explain the binding characteristics of most of the ergot alkaloids, there do appear to be some exceptions. For instance, it was interesting to observe that ergotamine and dihydroergotamine exhibited the binding characteristics of ergolines despite the fact that they possess cyclic peptide side chains (Fig. 1; Table 1). A simple explanation for this phenomenon is not immediately available; however, these two ergopeptines are the only ergots which have their particular substituents at both the R_1 and R_2 positions (Fig. 1). This substituted R_1 and R_2 combination may serve to place the peptide side chain in such a 3-dimensional conformation as to preclude an extremely avid binding reaction so that the binding characteristics of an ergoline are observed. Another exceptional finding was that both lergotrile and ergometrine exhibited about 30% R_H in their [^{3}H]spiroperidol competition curves (Table 1). We have previously suggested that the percentage of R_H may be correlated with the intrinsic activity of a dopaminergic agonist (46). These findings would thus appear in conflict with the proposal that dopaminergic ergot agonists exhibit full intrinsic activity at D-2 receptors. However, the efficacy of lergotrile and ergometrine has not yet been investigated in the bovine anterior pituitary D-2 receptor system. It will be especially important to examine the efficacy of these compounds in *in vitro* systems such as the dopaminergic-mediated inhibition of vasoactive intestinal peptide-induced adenylate cyclase activity in anterior pituitary membranes (10).

Our present findings suggest that the K_L/K_H ratio or the "fold shift" of agonist competition curves induced by guanine nucleotides is not correlated with, or predictive of, the intrinsic activity of a dopaminergic agonist. Previous reports have indicated a dissociation of *beta*-adrenergic agonist activity and the ability of GTP to affect agonist competitive curves in the rat reticulocyte and turkey erythrocyte systems, but these findings have been shown to be due to incomplete washout of GDP (47, 48). However, Lefkowitz and co-workers have demonstrated that an agonist's K_L/K_H ratio is highly correlated with its efficacy in the frog erythrocyte *beta*-adrenergic and the human platelet *alpha*$_2$-adrenergic receptor systems (34, 49, 50). In addition, the percentage of R_H was also correlated with the agonist's intrinsic activity at *beta*-adrenergic receptors (34, 49) but not at *alpha*$_2$-adrenergic receptors (50). These differences in the binding characteristics of *beta*-adrenergic, *alpha*$_2$-adrenergic, and D-2 dopaminergic catecholamine receptor systems may be reflective of structural differences in the receptors themselves or in other membrane components with which they interact.

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