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DOPAMINE RECEPTOR OF THE PORCINE ANTERIOR PITUITARY GLAND

SOLUBILIZATION AND CHARACTERIZATION

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Abstract—In the anterior pituitary gland, dopamine controls the release of prolactin from the mammotrophs. The dopamine receptors in the porcine gland have been shown to exist in two different affinity states of equal proportion, one bearing high affinity for agonists and labeled by ^{3}H-agonist-ligands and the other displaying low affinity for agonists. Both forms of the receptor can be labeled by ^{3}H-antagonist-ligands. Dopamine receptors from porcine anterior pituitary membranes can be solubilized with retention of their ability to interact with specific dopaminergic ligands. Treatment of membrane preparations with 1% digitonin resulted in the solubilization of 20–25% of the specific binding sites labeled by [^{3}H] spiroperidol with a specific activity of about 100 fmoles/mg. The receptor was a glycoprotein as assessed by the interaction of these binding sites with agarose-immobilized lectin. [^{3}H]Spiroperidol binding in solubilized preparations was saturable, of high affinity (K_D = 570 pM), and to a single class of stereoselective binding sites. Agonist competition for [^{3}H]spiroperidol binding indicated that, whereas the solubilized receptor retained its dopaminergic specificity, the high-affinity interactions of the receptor with agonists present in membranes and sensitive to guanine nucleotides were lost in solubilized preparations. Thus, the K_D values calculated from the agonist competition curves for [^{3}H]spiroperidol corresponded to the agonist affinities for the low-affinity state of the receptor documented in membranes. However, high-affinity agonist binding and its sensitivity to guanine nucleotides were preserved when the membrane-bound receptor was prelabeled with the agonist [^{3}H]*N-n*-propylnorapomorphine prior to solubilization. These results suggest that a component that confers agonist high-affinity binding and guanine nucleotide responsiveness to the receptor is lost during solubilization unless a stable complex is formed with the agonist prelabeled receptor prior to solubilization.

In the pituitary gland, dopamine plays an important physiological role. In the anterior lobe of the gland, the binding of dopaminergic agonists to a specific receptor has been shown to inhibit prolactin secretion with a typical dopaminergic specificity [1]. In the intermediate lobe of the rat pituitary gland, dopamine has been shown to control the release of α-melanocyte stimulating hormone [2]. Recently it has been possible to demonstrate that occupancy of the dopamine receptor in both the anterior and intermediate lobes of the pituitary gland results in the inhibition of adenylate cyclase [3–7] or decreased levels of cyclic AMP [8]. Guanine nucleotides were shown originally in brain [9] and subsequently in the anterior and intermediate pituitary [10–15,†] to modulate the interaction of dopaminergic ligands with the dopamine receptor. As is observed in numerous other receptor systems [16], guanine nucleotides decrease the apparent affinity of a form of the receptor that displays high affinity for agonists, thus converting the receptor site to a unique form that interacts with agonists with low affinity [17]. Thus, it has been postulated that the guanine nucleotide sensitive agonist high-affinity state of the receptor may be due to an association of the dopamine receptor in the anterior pituitary with a putative guanine nucleotide binding protein [12–15]. Therefore, increasing evidence suggests that the mechanism by which dopamine causes inhibition of hormone release in the pituitary gland may be, at least in part, mediated by a guanine nucleotide binding protein–agonist–receptor complex which promotes the inhibition of adenylate cyclase.

A prerequisite for the further characterization of these components and the biochemical documentation of their postulated interactions is the solubilization of these components from the membrane in an active form as a single component or as a complex of the various components. Thus, for the receptor component, solubilization in an active form implies retention of the ability of the receptor to interact with specific ligands. Several reports have appeared already documenting the solubilization of dopaminergic binding sites from brain tissue [18–23] using detergents such as digitonin, 3′-[(3-cholamidopropyldimethylamino) dimethyl-aminio]-1-propranesulfonate (CHAPS), and lysolecithin or chaotropic agents. We report here that the dopamine receptor of the porcine anterior pituitary can be solubilized, using the detergent digitonin, with retention of its

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binding properties. Moreover, the high-affinity interactions of the receptor with agonists and its sensitivity to guanine nucleotides can be preserved by stabilization of the complex prior to solubilization by the presence of an agonist. Characterization of the soluble receptor by binding to agarose-immobilized lectins revealed that the receptor is a glycoprotein.

MATERIALS AND METHODS

[^{3}H]Spiroperidol (33.2 Ci/mmole) and [^{3}H]*N*-*n*-propylnorapomorphine (NPA) (60 Ci/mmole) were purchased from the New England Nuclear Corp. (Boston, MA). (±)ADTN* was a gift from Dr. J. D. McDermed, Burroughs Wellcome Co. (Research Triangle Park, NC); all other compounds and reagents were obtained from sources previously described [13]. Digitonin was obtained from the Gallard–Schlesinger Chemical Manufacturing Co. (Carle Place, NY). Sephadex G-50 fine was from Pharmacia Fine Chemicals (Uppsala, Sweden). Agarose-immobilized lectins were obtained from the E-Y Laboratories (San Mateo. CA).

Membrane preparation and receptor solubilization. Membrane preparations were obtained according to procedures published previously [13]. Soluble receptor preparations were prepared according to the following procedure. A membrane preparation, which was stored frozen, was centrifuged at 30,000 *g* for 10 min, and the pellet was resuspended in one-third the original volume of the membrane suspension with cold (4°) 100 mM NaCl/2 mM $MgCl_2$/25 mM Tris–HCl/1% digitonin (w/v)/0.1% ascorbate (pH 7.4 at 4°). The ratio of membrane protein to detergent was 9 mg of protein to 10 mg of digitonin per ml of solution. The suspension was stirred slowly at 4° or at 25° for 30 min and centrifuged at 40,000 *g* for 45 min. The amount of solubilized binding activity was not reduced by centrifugation at 100,000 *g* for 60 min or by filtration through a 0.2 μm Millipore filter, indicating that the receptor was functionally solubilized. The supernatant fraction containing the soluble receptor was kept at 4° and used immediately.

Binding assays. Membrane radioligand binding assays were performed as described previously on preparations containing 3.6 ± 1.0 mg protein/ml [13].

Soluble receptor binding assays were performed as follows. Solubilized receptor preparation (200 μl, 0.74 mg/200 μl) was incubated with 1–2 nM [^{3}H]-spiroperidol in the presence of the indicated agents in a total assay volume of 0.5 ml of 100 mM NaCl/2 mM $MgCl_2$/25 mM Tris–HCl/0.1% ascorbate (pH 7.4 at 4°) for 18 hr at 4° unless otherwise specified. Bound ligand was separated from free ligand by Sephadex G-50 chromatography [24]. Bound ligand was collected in a volume of 1 ml, and the radioactivity was counted by scintillation spectrometry. Nonspecific binding was determined in the presence of 1 μM (+)butaclamol. Saturation and competition binding data were analyzed by a nonliner least-squares curve-fitting procedure based on a generalized model for complex–ligand–receptor systems according to the law of mass action [25].

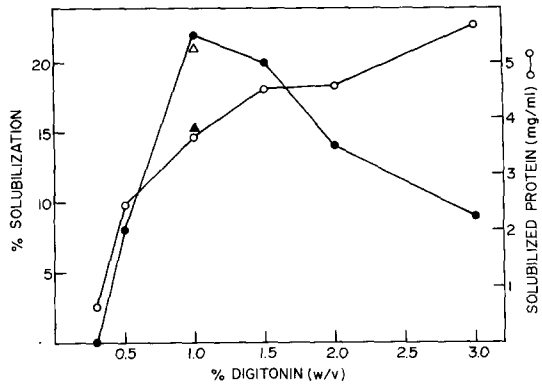

Fig. 1. Extent of solubilization of [^{3}H]spiroperidol binding sites from porcine anterior pituitary membranes as a function of digitonin concentration. Anterior pituitary membranes were solubilized at 25° (●, ▲) or 4° (△) in the presence (●, △) or absence (▲) of 100 mM NaCl with the indicated concentrations of digitonin according to the procedures described in Materials and Methods. [^{3}H]Spiroperidol binding was determined in a soluble binding assay with a saturating concentration (2 nM) of [^{3}H]spiroperidol. Nonspecific binding was determined in the presence of 1 μM (+)butaclamol. Protein concentration was determined by the amido Schwarz assay using bovine serum albumin as a standard. Each point was the average of duplicate determinations. The experiment is representative of two experiments.

Protein determinations. Protein concentration was determined by the methods of Lowry and the amido Schwarz assay [26] using bovine serum albumin as a standard.

RESULTS

Solubilization of dopamine receptor binding activity from porcine anterior pituitary membranes. The ability of the detergent digitonin to solubilize [^{3}H]spiroperidol binding sites is shown in Fig. 1. Solubilization reached a maximum of 22% at 1% digitonin with a specific binding of 101 fmoles/mg. Although the solubilization at 1.5% digitonin was similar (21%), the specific binding was lower (74 fmoles/mg). When membranes were labeled with 2 nM [^{3}H]spiroperidol prior to solubilization, the results were similar to those obtained in the absence of prelabeling. Solubilization at 4° vs 25° gave similar results (Fig. 1). Assay of the insoluble membrane fraction after solubilization with 1% digitonin for residual [^{3}H]spiroperidol binding showed that only 10–15% of the receptor binding activity remained, suggesting that up to 60% of the receptor activity might have been inactivated. This is supported by the fact that at higher concentrations of digitonin fewer receptor binding sites were solubilized.

Several conditions were explored in an effort to improve the efficiency of digitonin in solubilizing the membranes. Altering the physical conditions during solubilization by homogenizing, sonicating, or solubilizing at 25° instead of 4° had little or no effect on the yield of receptor. Additions and combination of

* ADTN = 2-amino-6,7-dihydropytetrahydronaphthalene.

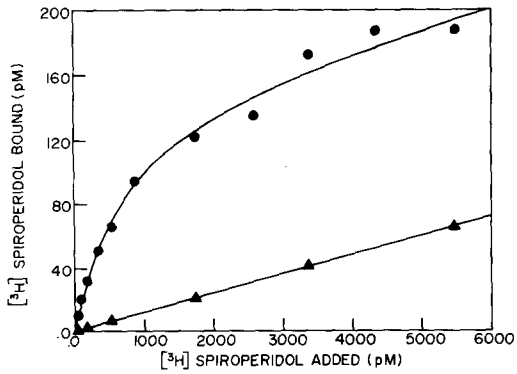

Fig. 2. Saturation isotherm of [^{3}H]spiroperidol binding to soluble preparations of porcine anterior pituitary membranes. Anterior pituitary membranes were solubilized in 1% digitonin, and 200 μl of soluble receptor preparation (0.74 mg) was incubated with increasing concentrations of [^{3}H]spiroperidol in a total assay volume of 0.5 ml for 18 hr at 4°. Bound [^{3}H]spiroperidol was separated from free by a Sephadex G-50 column assay. Total [^{3}H]spiroperidol binding is represented by the closed circles (●), and nonspecific binding (▲) was determined in the presence of 1 μM (+)butaclamol. The data were analyzed by a nonlinear least-squares fitting according to Ref. 25, and the line represents the best fit to a single site. The experiment was performed in duplicate and is representative of three experiments.

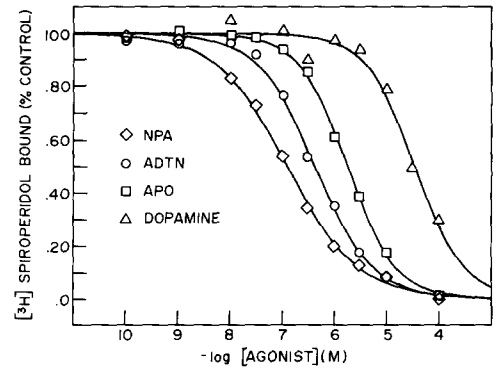

Fig. 3. Ability of a series of dopaminergic agonists to compete for the binding of [^{3}H]spiroperidol to soluble preparations of porcine anterior pituitary membranes. Increasing concentrations of agonists were incubated with 2 nM [^{3}H]spiroperidol in a soluble binding assay according to the procedure described in Materials and Methods. In the experiment, 100% binding corresponded to 210 pM or 142 fmoles/mg. Curves are computer best fit curves according to the procedure described in Materials and Methods [25]. Each curve was performed in duplicate and is representative of two experiments.

various agents to digitonin [250 mM sucrose, 10% glycerol, 1 mM dithiothreitol (DTT), 15 mM EDTA, 5 mM phospholipids, 5 mM $CaCl_2$ or 20% ammonium sulfate] decreased or had no effect on solubilization. The only addition that improved the percent solubilization was 100 mM NaCl (Fig. 1). Triton X-100, lubrol PX, cholate and deoxycholate failed to solubilize receptor, and the residual sites in the membrane fraction after solubilization were low, suggesting inactivation of the receptors. Solubilization with CHAPS, according to the procedure of Lew *et al.* [20] and with lysolecithin [21] resulted in 18 and 20% solubilization, respectively, but did not offer a distinct advantage over digitonin.

Equilibrium binding of [^{3}H]*spiroperidol to solubilized preparations.* Binding of [^{3}H]spiroperidol to soluble preparations at 4° reached equilibrium in approximately 6 hr (data not shown). For convenience, soluble binding assays were incubated overnight (~18 hr). An equilibrium binding isotherm of [^{3}H]spiroperidol is shown in Fig. 2. Computer based analysis of this data yielded a K_D of 570 ± 55 pM for [^{3}H]spiroperidol with a concentration of receptor in the assay of 143 ± 5.4 pM. The ligand appeared to bind to a homogeneous population of sites with a mean specific activity of about 100 fmoles/mg of soluble protein preparation. At saturation, nonspecific binding measured in the presence of 10^{-6} M (+) butaclamol was only 25% of total binding.

Specificity of [^{3}H]*spiroperidol binding to solubilized preparations and comparison to the membrane-bound receptors.* Agonist competition curves for [^{3}H] spiroperidol binding to soluble preparations are shown in Fig. 3. A typical dopaminergic order of potency is apparent: NPA > ADTN > apomorphine > dopamine. However, for the most potent agonists such as NPA and ADTN, as well as for antagonists, competition curves of these agents for [^{3}H]spiroperidol binding revealed a low-affinity binding site (cf. Fig. 3). This site constituted 15–20% of the total [^{3}H]spiroperidol binding. Thus, competition curves of unlabeled spiroperidol for [^{3}H] spiroperidol were biphasic with 80% of the sites having high affinity (0.5 nM) for spiroperidol and 20% having low affinity (20 nM) for spiroperidol. On further examination, the binding characteristics of these sites appeared to correspond to the anesthetic, spirodecanone, binding sites documented in soluble rat striatum binding by Gorissen *et al.* [27]. These sites were identified as sites, labeled by [^{3}H]spiroperidol, that were displaceable by 10^{-5} M R5260 and 10^{-4} M (+)butaclamol but not by 10^{-6} M (+)butaclamol. Therefore, the dissociation constants estimated by quantitative analysis and given in Table 1 were determined from the high-affinity portion of agonist and antagonist competition curves for [^{3}H]spiroperidol binding to soluble preparations.

As shown in Table 1, the dissociation constants for agonist binding correlate closely with the low-affinity agonist dissociation constants for the membrane bound receptor. In solubilized preparations, ADTN was slightly more potent than apomorphine whereas the opposite was found in the membranes. The overall potency of ADTN in membrane preparation was slightly higher than that for apomorphine; however, the calculated low-affinity dissociation constants (K_L) for both agents are virtually identical [13].

The dissociation constant for the antagonist [^{3}H] spiroperidol, calculated from direct binding or competition curves, was slightly higher than that obtained with membrane bound receptor (Table 1). This may stem from the interaction of the ligand with the detergent. The difference of 900-fold between the

Table 1. Comparison of the dissociation constants of dopaminergic agonists and antagonists in soluble and membrane preparations of porcine anterior pituitary gland*

Ligand	Soluble	Membrane	
	K_D (nM)	K_L (nM)	K_H (nM)
Agonist			
NPA	19 ± 2.6	18 ± 3.9	0.081 ± 0.022
Apomorphine	310 ± 93	160 ± 61	4.7 ± 2.4
(±)ADTN	54 ± 25	190 ± 59	2.3 ± 0.8
Dopamine	5700 ± 1600	4300 ± 2700	66 ± 37
Antagonist			
Spiroperidol	0.630 ± 0.079	0.130 ± 0.016	
(+)Butaclamol	2.70 ± 0.45	1.90 ± 0.15	
(−)Butaclamol	2500 ± 660		

* The K_D values for the agonist binding to soluble receptor preparations were calculated from the competition curves shown in Fig. 3. The antagonist K_D values for soluble receptor binding were calculated from 11-points competition curves. As described in Results, antagonist competition curves for [^{3}H]spiroperidol binding to the soluble preparations were biphasic as they showed a non-dopaminergic low-affinity site (spirodecamone-like site blocked by R-5260). Thus, this site was ignored in the analysis of binding, and the K_D values presented were calculated from the higher affinity site. For the agonists NPA and ADTN the same procedure was applied. The agonist K_D values for membrane bound receptor are the dissociation constants for the agonist high-affinity state of the receptor "K_H" and the agonist low-affinity state "K_L" determined previously [13]. The antagonist K_D values for membrane bound receptor were also determined previously [13]. Comparison of the K_D values in membranes at 25° vs 4° did not differ by more than 2- to 3-fold. All K_D values were determined by computer assisted methods as described in Ref. 25.

K_D values for (+)butaclamol and (−)butaclamol indicates that the soluble receptor retained its stereoselectivity.

Effect of guanine nucleotides on agonist competition of [^{3}H]spiroperidol binding to solubilized preparations. As shown in Fig. 4, the presence of 100 μM Gpp(NH)p had no effect on the ability of NPA to compete for [^{3}H]spiroperidol binding to solubilized receptor preparations. The calculated K_D values for the agonist and receptor concentration in the presence and absence of Gpp(NH)p were 19 ± 2.6 nM, 210 ± 7.3 pM and 23 ± 2.4 nM, 220 ± 5.8 pM respectively. As expected from these results, no high-affinity direct [^{3}H]NPA binding could be demonstrated in solubilized preparations. These results suggest that, upon solubilization, the interaction of the

Table 2. Solubilization of [^{3}H]agonist and [^{3}H]antagonist prelabeled dopamine receptor from porcine anterior pituitary membranes*

Experiment	Ligand in membrane prelabeling			
	[^{3}H]NPA (fmoles)	[^{3}H]SPIRO (fmoles)	[^{3}H]SPIRO +1 nM NPA (fmoles)	High-affinity form (%)
1	189	465	330	41
2	165	399	270	42
3	108	180		59

* Membranes were incubated for 60 min at 25°, as described in Materials and Methods, with 0.8–1 nM [^{3}H]NPA; 0.7–2 nM [^{3}H]spiroperidol; and 0.7–2 nM [^{3}H]spiroperidol and 1 nM unlabeled NPA in the absence and presence of 1 μM (+)butaclamol. The concentrations of agonist were sufficient to saturate only the agonist high-affinity form of the receptor while the antagonist concentration was sufficient to prelabel the entire population of receptor. After incubation, the membranes were centrifuged and solubilized according to the procedure described in Materials and Methods at 4°. Residual free ligand was separated from bound by Sephadex G-50 chromatography of the solubilized preparations. The results are expressed in fmoles of specific binding in 1 ml of solubilized preparation in each respective experiment. Percent agonist high-affinity form is expressed as the percent of the number of [^{3}H]NPA sites compared to the total number of sites labeled and solubilized with [^{3}H]spiroperidol. Abbreviations: NPA = *n*-propylapomorphine; SPIRO = spiroperidol.

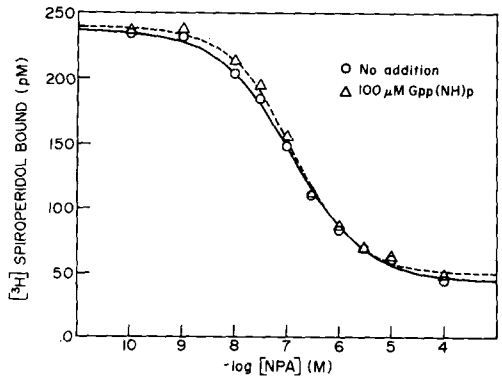

Fig. 4. Effect of the guanine nucleotide Gpp(NH)p on competition of NPA for [^{3}H]spiroperidol binding to soluble receptor preparations. Soluble preparation was incubated as described under Materials and Methods with [^{3}H]spiroperidol (2 nM) and increasing concentrations of NPA in the absence (○———○) and presence (△ - - - △) of 100 μM Gpp(NH)p. The experiment shown was performed in duplicate and is representative of three such experiments.

receptor with the putative guanine nucleotide binding protein was lost. However, as shown below, when the receptor was labeled with the agonist [^{3}H]NPA prior to solubilization and then solubilized, an agonist–receptor complex which remained sensitive to nucleotide was solubilized with digitonin.

Effect of receptor prelabeling on the proportion of agonist high- and low-affinity states solubilized. Three equal portions of membranes were labeled with 1 nM [^{3}H]NPA, 2 nM [^{3}H]spiroperidol, and 2 nM [^{3}H]spiroperidol and 1 nM NPA. The prelabeled membranes were then solubilized with 1% digitonin at 4° for 30 min. The solubilized receptor preparations were then chromatographed on G-50 columns and the bound radioligand was determined. Since as shown previously [13] [^{3}H]NPA at a concentration of 1 nM labels the proportion of membrane-bound receptor that exists in the agonist high-affinity state (K_D = 150 pM), it represents a measure of the proportion of total high-affinity agonist complex solubilized. [^{3}H]Spiroperidol labels both agonist high- and low-affinity states of the receptor in the membrane and represents a measure of the total receptor solubilized. Labeling the receptor in the membranes with [^{3}H]spiroperidol in the presence of 1 nM unlabeled NPA approximates the proportion of low-affinity agonist form of the receptor solubilized.

The results presented in Table 2 from three separate experiments indicate that approximately equal proportions of the agonist high- and low-affinity states were solubilized when the membranes were prelabeled in the presence of agonist. This is presumably a reflection of the status of the receptor in the membrane and suggests that once the agonist–receptor complex is formed it is stable to the detergent solubilization.

Sensitivity of membrane-bound and solubilized agonist-receptor complex to guanine nucleotides. As shown in Fig. 5A, at 25° addition of Gpp(NH)p (10 and 100 μM) to membrane-bound receptor prelabeled with [^{3}H]NPA greatly increased the rate of dissociation of the radioligand as compared with the

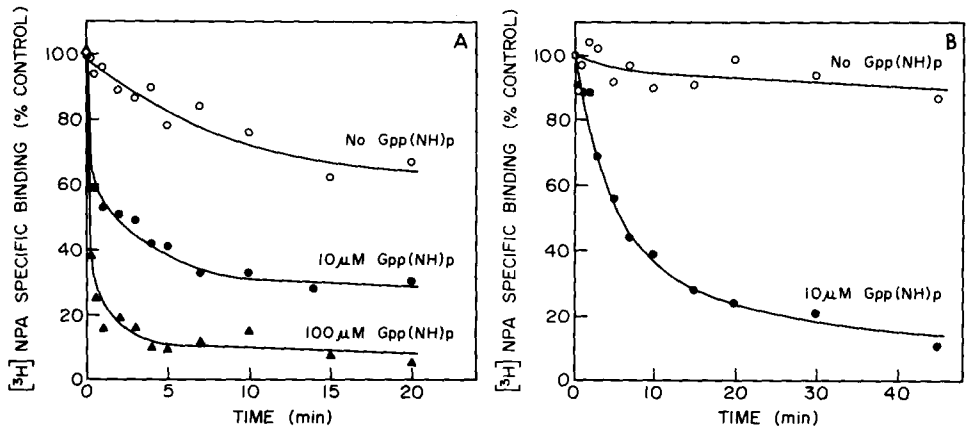

Fig. 5. Effects of guanine nucleotides on the rates of dissociation of [^{3}H]NPA binding from membrane-bound and solubilized receptor preparations. (A) Membranes were incubated with 500 pM [^{3}H]NPA for 60 min at 25° to achieve equilibrium in the presence of 0.1% ascorbate. Dissociation was initiated by the addition at time 0 of an excess of (+)butaclamol (10 μM) or (+)butaclamol plus 10 and 100 μM Gpp(NH)p. Samples were filtered at 25° on GF/B filters at the time intervals shown. Nonspecific binding was determined by parallel incubations containing 10 μM (+)butaclamol from the beginning of the incubation. The experiment, performed in duplicate, is representative of three such experiments. (B) Membranes were incubated as above with [^{3}H]NPA to achieve equilibrium. After the incubation, membranes were centrifuged and resuspended in 1% digitonin, 25 mM Tris–HCl, 100 mM NaCl, 2 mM $MgCl_2$, 0.1% ascorbate, pH 7.4, at 4°, stirred for 30 min on ice, and centrifuged at 40,000 *g* for 45 min to obtain the solubilized labeled receptor preparation. Dissociation of the ligand was started by adding at time 0 excess (+)butaclamol (10 μM) or (+)butaclamol and 100 μM Gpp(NH)p. Incubations were continued at 4° for the time intervals indicated, and binding was determined by Sephadex G-50 chromatography. The results are representative of two similar experiments.

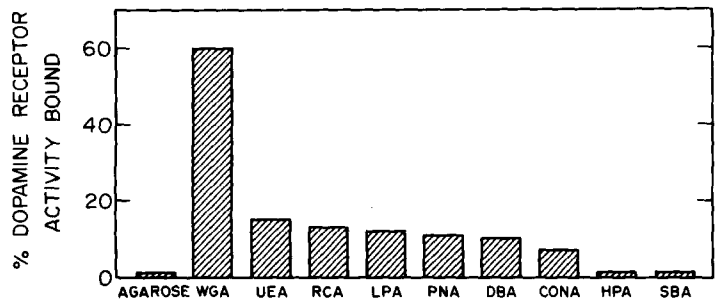

Fig. 6. Binding of soluble preparation of anterior pituitary membranes to agarose-immobilized lectins. Anterior pituitary membranes were solubilized with 1% digitonin in a buffer containing 100 mM NaCl/2 mM $MgCl_2$/2 mM $CaCl_2$/2 mM $MnCl_2$/25 mM Tris–HCl (pH 7.4) (Buffer B). Soluble preparations (0.5 ml) were incubated with each lectin agarose gel (0.2 ml) (previously equilibrated with Buffer B and 0.1% digitonin) for 2 hr at 4°. After centrifuging the suspended agarose gel, the supernatant fraction was carefully removed, and the amount of unbound receptor was determined with 2 nM [^{3}H]spiroperidol in a soluble binding assay. Nonspecific binding was determined in the presence of 10^{-6} M (+)butaclamol. Agarose gel containing no immobilized lectin was used to determine receptor binding to the agarose that was not due to lectin binding. The ability of the bound receptor to be eluted from the wheat germ agglutinin agarose gel was determined by first washing the gel with three 5-ml portions of Buffer B + 0.1% digitonin and then eluting with 0.1 M *N*-acetylglucosamine in Buffer B + 0.1% digitonin. The source of the lectins, their abbreviation, and their specificities are given below: *Triticum Vulgaris* (WGA), $\beta(1\rightarrow 4)$-D-GlcNAc, sialic acid; *Ulex Europaeus* (UEA), α-L Fucose; *Ricimus Communis* (RCA), β-Gal; *Limulus Polyphemus* (LPA), sialic acid; *Arachis Hypogaea* (PNA), D-Gal-$\beta(1\rightarrow 3)$-D-GalNAc > D-$GalNH_2$ = α-D-Gal; *Dolichos Biflorus* (DBA), α-D-GalNAc; Concanavalin A (CON-A), α-D-Man > α-D-Glc > α-D-GlcNAc; *Helix Pomatia* (HPA), α-D-GalNAc, α-D-GlcNAc, α-D-Gal; and Glycine Max (SBA), α-D-GalNAc > β-D-GalNAc $\gg$ α-D-Gal. The results shown represent the average of two experiments performed in duplicate.

addition of 10 μM (+)butaclamol alone. This increase in the rate of [^{3}H]NPA dissociation could also be demonstrated with solubilized receptors that had been labeled in the membrane with [^{3}H]NPA prior to solubilization (Fig. 5B). This indicates that in the membranes the interaction between the receptor and the putative component of the system that confers nucleotide sensitivity to agonist binding can be preserved in solubilized preparations as long as the agonist–receptor high-affinity complex has been formed prior to solubilization. Moreover, just as guanine nucleotides presumably convert the membrane-bound agonist high-affinity receptor complex to a receptor form having low affinity for agonists [13], Gpp(NH)p is capable of effecting the same interconversion in solubilized receptor preparations.

Solubilized receptor binding to agarose-immobilized lectins. Of the nine lectin-agarose gels tested, wheat germ agglutinin (WGA) agarose was the most efficient in binding solubilized receptor. As shown in Fig. 6, WGA-lectin agarose bound 60% of the receptor applied to it, while all other lectin-agarose gels bound only 15% or less. Moreover, 53% of the receptor that was bound to the WGA-lectin agarose could be eluted with 0.1 M *N*-acetylglucosamine (not shown). In experiments in which the receptor was prelabeled in membrane preparations with the agonist [^{3}H]NPA and the antagonist [^{3}H]spiroperidol prior to solubilization, the binding of the soluble receptor to WGA-lectin agarose was unaffected.

DISCUSSION

Solubilization of a membrane-bound hormone receptor in a form that can still interact specifically with ligands represents the first necessary step toward the eventual biochemical characterization of this protein. Although [^{3}H]spiroperidol binding sites have been solubilized previously from brain tissue, this report represents the first to describe that dopamine receptors have been solubilized and characterized from the anterior pituitary gland. The anterior pituitary gland represents an attractive system to study the biochemical characterization of this receptor since this tissue presumably contains only one subtype of the dopamine receptor (D_2) [13–15, 28]. Despite the fact that ligands such as [^{3}H]spiroperidol can bind to D_2 receptors, the presence of several subtypes of these receptors in tissues such as the brain can complicate the interpretation of results.

The maximum percent solubilization from pituitary membranes was routinely 20–25% of the total sites present. This is comparable to that obtained with digitonin in preparations from caudate nucleus [18, 19]. To determine that the solubilized receptors, despite their relatively low yield, were representative of the membrane-bound receptors, it was necessary to perform a thorough characterization of the soluble preparations. Saturation binding isotherms for [^{3}H]spiroperidol indicate that the binding was saturable to a single site with high affinity. The K_D of [^{3}H]spiroperidol for the soluble receptor was 570–630 pM (Fig. 2, Table 1) which is somewhat higher than the K_D of 50–150 pM obtained for binding to particulate preparations [13]. Although the soluble K_D was the same or lower than those values published for the caudate nucleus preparations [20, 29]. The solubilized receptor sites retained their stereoselectivity and specificity as the order of potency of agonist

binding to the soluble preparations was similar to that in the particulate preparations.

It has been documented that agonist competition curves for [^{3}H]spiroperidol binding in particulate preparations are biphasic, and high- and low-affinity forms of the receptor for agonists are present [12–15]. In solubilized preparations only the low-affinity form was detected for the competition of agonists for soluble [^{3}H]spiroperidol binding (cf. Figs. 3 and 4, Table 1). Moreover, as shown in Fig. 4, the presence of guanine nucleotides had no effect on the NPA competition curve for soluble [^{3}H]spiroperidol binding. Therefore, it appears that after solubilization the ability of agonists to interact with high affinity with the receptor and the ability of guanine nucleotides to modulate this affinity were lost. This is a common finding in several solubilized receptor systems [30].

However, if the agonist high-affinity state of the receptor were labeled prior to solubilization by incubation of membranes with [^{3}H]NPA, then this form of the receptor appeared to be stable to solubilization. As suggested by the data in Table 2, the high- and low-affinity states of the receptor were solubilized in roughly the same proportion as that documented in membrane preparations (i.e. about 50%). In addition, the stabilized soluble agonist high-affinity form of the receptor retained its sensitivity to guanine nucleotides as evidenced by the ability of guanine nucleotides to accelerate the dissociation of [^{3}H]NPA in solubilized preparations in a manner similar to the membrane-bound receptors.

These results are consistent with the formulation postulated previously [12–15], that the receptor can exist as two distinct forms that can be discriminated by agonists. In addition, these data indicate that, whereas the receptor can be solubilized with an apparent retention of its binding specificity, high-affinity interactions of the receptor with agonists are not present in solubilized preparations unless stabilized prior to solubilization by the presence of an agonist. The same observations have been reported recently for the D_2 receptor solubilized from canine caudate nucleus [23]. The results obtained so far are entirely analogous to the beta-adrenergic system [31] where the component required for high-affinity agonist interactions with the receptor has been documented to be a guanine nucleotide binding protein. These results are also comparable to those from the α_2-adrenergic receptor of the human platelet, a receptor coupled negatively to adenylate cyclase [32–34].

The anterior pituitary dopamine receptor appears to be a glycoprotein as shown by the ability of lectins to bind to the receptor. Because the receptor binds to WGA-lectin agarose, the receptor is likely to have oligosaccharide substituents of the complex type since WGA-lectin will prefentially interact with *N*-acetylglucosamine and sialic acid [35, 36]. The absence of significant interaction of the receptor with the other lectins may be due to the presence of the detergent. The fact that the agonist–receptor complex can be stabilized to withstand solubilization and can bind to WGA-lectin agarose suggests that it may be possible to isolate and further characterize this agonist–receptor complex.

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