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Solubilization and Reconstitution of Dopamine D_1 Receptor from Bovine Striatal Membranes: Effects of Agonist and Antagonist Pretreatment

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The bovine striatal dopamine D_1 receptor was solubilized with a combination of sodium cholate and NaCl in the presence of phospholipids, following treatment of membranes with a dopaminergic agonist (SKF-82526-J) or antagonist (SCH-23390). The solubilized receptors were subsequently reconstituted into lipid vesicles by gel-filtration. A comparison of ligand-binding properties shows that the solubilized and reconstituted receptors bound [^{3}H]SCH-23390 to a homogeneous site in a saturable, stereospecific and reversible manner with a K_d of 0.95 and 1.1 nM and a B_{max} of 918 and 885 fmol/mg protein respectively for agonist- and antagonist-pretreated preparations. These values are very similar to those obtained for membrane-bound receptors. The competition of antagonists for [^{3}H]SCH-23390 binding exhibited a clear D_1 dopaminergic order in the reconstituted preparation obtained from either agonist or antagonist-pretreated membranes, except that (+)butaclamol was about four-fold more potent than *cis*-flupentixol in displacing [^{3}H]SCH-23390 binding in preparation obtained from agonist-pretreated membranes compared to antagonist-pretreated membranes. The agonist/[^{3}H]SCH-23390 competition studies revealed the presence of a high-affinity component of agonist binding in both the reconstituted receptor preparations. The number of high-affinity agonist binding sites, however, is 40-80% higher in reconstituted preparation obtained from antagonist-treated membrane compared to that obtained from the agonist-treated membrane. In both the preparations, 100 μM guanylylimidodiphosphate (Gpp(NH)p) completely abolished the high-affinity component of agonist binding compared to partial abolition in the native membranes, indicating a close association of a G-protein with the solubilized receptors. Whether the receptor was solubilized following agonist or antagonist preincubation of the membranes, the receptor-detergent complex eluted from a steric-exclusion HPLC column with an apparent molecular size of 360,000. Preincubation of the solubilized preparations with Gpp(NH)p had virtually no effect on the elution profile suggesting a lack of guanine nucleotide-dependent dissociation of G-protein receptor complex.

KEY WORDS: [^{3}H]SCH-23390; dopamine D_1 receptor; bovine striatum; solubilization; guanine nucleotides.

INTRODUCTION

Central nervous system dopamine receptors have been classified into two subtypes, viz., D_1 and D_2, based on their affinity towards dopaminergic ligands and relationship to adenylate cyclase (1-3). The D_1 dopamine receptor is known to stimulate adenylate cyclase whereas the D_2 receptor is believed to be negatively coupled to the enzyme. By radioligand binding, both the receptors

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have been well-characterized in the membrane-bound form (4) and considerable progress has been made towards solubilization and purification of the dopamine D_2 receptor from various tissues (5-10). The cloning and expression of rat D_2 dopamine receptor cDNA has also been recently reported (11). However, compared to dopamine D_2 receptor, little biochemical information is available on the dopamine D_1 receptor, which has been recently demonstrated to interact with dopamine D_2 receptors in behavioral responses (12), and undergo changes in schizophrenia (13) and Parkinson's disease (14).

The availability of a novel benzazepine, SCH-23390 (R(+)8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol), as a selective antagonist of dopamine D_1 receptors (15) has given impetus to the studies related to biochemical characterization of the receptor. The radiolabelled derivatives of SCH-23390 have been shown to selectively label brain dopamine D_1 receptors with a high affinity and very low nonspecific binding (16,17). SCH-23390 derivatives have recently been used as photoaffinity crosslinking reagents, and as affinity chromatography matrices to partially purify the rat brain dopamine D_1 receptor (18). Studies on competition of antagonist binding sites reveal that the striatal dopamine D_1 receptor exists in high- and low-affinity states for the dopaminergic agonists (19-21), an observation that is common to many adenylate cyclase-coupled receptors including dopamine D_2 (22) as well as α_2- and β-adrenergic receptors (23,24). The high-affinity state of receptors is understood to arise from their coupling to a guanine nucleotide binding protein (G-protein) G_s or G_i, the stimulatory and inhibitory proteins respectively, or G_o and other members of the G-protein family (25). Thus the D_1 dopamine receptor, which mediates activation of adenylate cyclase, is believed to be linked to G_s (26). The guanine nucleotides, by interacting with the G-proteins, cause a reduction in the agonist high-affinity form of the dopamine D_1 receptor (21,27), similar to that observed for dopamine D_2 (6,28) as well as β-adrenergic (29) and α_2-adrenergic receptors (30).

In many instances, detergent solubilization of the receptors including dopamine D_2 and α-adrenergic receptors has been shown to result in a loss of sensitivity of agonist binding to guanine nucleotides unless the membranes are preoccupied with an agonist prior to solubilization (31,32,33). The dopamine D_1 receptor has recently been solubilized from rat and canine brains (33,34,35). The rat brain dopamine D_1 receptor was solubilized with sodium cholate following agonist preoccupancy of the membranes and it was shown that reconstitution of the receptors in lipid vesicles leads to a potentiation of high affinity agonist sites that are sensitive to guanine nucleotides (35). The author reported that solubilization of antagonist-occupied receptor results in lower recovery of binding sites than the agonist-occupied receptors; however, no comparison was done with respect to the properties of agonist and antagonist binding sites. Thus, it is unknown whether the solubilization of antagonist-preoccupied receptor results in maintenance of receptor-G-protein coupling as manifested by guanine nucleotide modulation of agonist binding. The report of digitonin solubilization of canine dopamine D_1 receptor (34) demonstrates a considerable reduction in the affinity of the solubilized receptor towards [^{3}H]SCH-23390 and partial sensitivity to guanine nucleotides.

In the present communication, we have compared the molecular properties of cholate-solubilized and reconstituted dopamine D_1 receptor from agonist and antagonist-pretreated membranes. The results show that dopamine D_1 receptor from bovine striatum are solubilized with similar yields following appropriate agonist or antagonist preoccupancy. The solubilized receptors, under both conditions, display similar size on HPLC, but show some quantitative differences in agonist and antagonist binding.

EXPERIMENTAL PROCEDURE

Materials. [^{3}H]SCH-23390 (70-80 Ci/mmol) was purchased from Amersham, Canada. (+)Butaclamol, (±)SKF-38393, (±)ADTN and (−)NPA were obtained from Research Biochemicals Inc., Wayland, MA. Dopamine, propranolol, soybean phosphatidylcholine, Sephadex G-50, and bovine γ-globulin were purchased from Sigma Chemical Co., St. Louis, MO. Sodium cholate and guanylylimidodiphosphate (Gpp(NH)p) were purchased from Calbiochem and Boehringer Mannheim, respectively. Polyethylene glycol (6000) was from BDH Chemicals, Canada. Size exclusion HPLC column TSK 400 and Bio-Bead SM-2 were from BioRad, Richmond, CA. The following drugs were gifts: SKF-82526-J, Smith, Kline and French, PA; haloperidol, McNeil, Canada; spiroperidol, and ketanserin, Janssen Pharmaceutica, Belgium; *Cis-* and *Trans*-isomers of flupentixol, Lundbeck, Denmark; fluphenazine, Squibb, Montreal; sulpiride, Ravizza; phentolamine, Pfizer, N.Y.; YM-09151-2, Yamanouchi, Japan; and SCH-23390, Scherring Corp., Bloomfield, N.J.

Methods

i) Solubilization and Reconstitution of Receptor. Bovine striatal membranes were prepared as previously described (36). The striatal membrane preparation (12-14 mg/ml) was diluted with 2 volumes of Tris-EDTA buffer (50 mM Tris-HCl, 1mM EDTA, pH 7.4) containing 5 mM $MgCl_2$ and 0.1% ascorbate (Tris-ascorbate buffer) and incubated with 10 μM SKF-82526-J or 10 μM SCH-23390 for 30 min at 37°C. The membranes were then centrifuged at 12,000 *g* for 15 min, washed twice with 5 volumes of the Tris-ascorbate buffer, and resuspended in original volume of the same buffer. To solubilize, the preincubated

membrane was diluted with an equal volume of a detergent solution containing the following (final concentrations in membrane-detergent mixture) sodium cholate, 0.5%; NaCl, 1 M; dithiothreitol, 0.1 mM; phenylmethylsulphonylfluoride, 0.1 mM; benzamidine, 0.1 mM; and crude soybean phosphatidylcholine, 1 mg/ml in Tris-ascorbate buffer. The mixture was stirred gently in an ice-bath for 30 min. The mixture was centrifuged at 130,000 *g* for 60 min and the upper 80% of the clear supernatant, carefuly aspirated, was taken as solubilized receptors. To reconstitute, the solubilized receptors were applied to a Sephadex G-50 column equilibrated with Tris-EDTA buffer. The column was eluted with the same buffer and the turbid void volume fractions were collected.

ii) Receptor Binding Assay. The binding of [^{3}H]SCH-23390 to solubilized and reconstituted receptor was performed in triplicate in 1.0 ml assay volume containing Tris-EDTA buffer (pH 7.4). For [^{3}H]SCH-23390/agonist competition experiments, the incubation medium additionally contained 5 mM $MgCl_2$ and 0.1% ascorbate. Non-specific binding was determined in parallel assays in the presence of 1 μM *cis*-flupentixol. The reaction was started by adding 250 μl of the receptor preparation (100-150 μg protein) and was carried out for 90 min at 25°C. At the end of the incubation period, the tubes were placed on ice and 50 μl of ice-cold bovine γ-globulin (20 mg/ml) in Tris-EDTA buffer and 500 μl of polyethylene glycol (30%, w/v in Tris-EDTA buffer) was added to precipitate the receptor-ligand complex. The tubes were vortexed and after 10 min of incubation on ice, the contents of the tubes were filtered on GF/C filters (Whatman). The filters were washed four times with 5 ml of ice-cold polyethylene glycol (10% w/v in Tris-EDTA buffer). The binding of [^{3}H]SCH-23390 to bovine striatal membranes was performed as described above, except that at the end of the incubation period, the membranes were filtered directly on GF/B filters (Whatman) without the addition of bovine γ-globulin and polyethylene glycol, and that the filters were washed with cold, Tris-EDTA buffer only. The radioactivity bound to the filters was determined by placing them in 5 ml of scintillation cocktail (PCS; Packard) and counting, after overnight equilibration, in a Beckman Scintillation Counter (Model 5800) at about 50% efficiency.

iii) Sucrose-Density Gradient Centrifugation. For sucrose-density gradient centrifugation, the receptors were solubilized as described above from agonist or antagonist-pretreated membranes. Two ml samples of the solubilized preparations were loaded on to 11 ml of continuous sucrose density gradient (10-30% sucrose in Tris-EDTA buffer containing 0.5% sodium cholate and 250 mM NaCl) and centrifuged in s.w. 40 rotor of the Beckman ultracentrifuge (Model L565) at 97,500 *g* for either 18 h or 40 h. The samples were loaded either at the top or the bottom of the gradients. For bottom-loading, sucrose was dissolved in the reconstituted preparation at a final concentration of 30% w/v and the mixture was placed at the bottom with a syringe. At the end of centrifugation, 1 ml fractions were collected manually and assayed for sucrose concentration and [^{3}H]SCH-23390 binding. It was separately determined that sucrose, up to a final concentration of 10%, did not interfere with the binding assays; therefore, no correction was made for the presence of different amounts of sucrose in the assay.

iv) High Performance Liquid Chromatography of the Solubilized Receptor on Size-Exclusion Column. For HPLC on size-exclusion column, the dopamine D_1 receptor was solubilized after preincubating the striatal membranes with 10 μM SKF-82526-J or 10 μM SCH-23390. One ml sample of the solubilized preparation was injected, at room temperature, into a BioRad HPLC system equipped with a Bio-Sil TSK 400 (600 × 7.5 mm) size-exclusion column equilibrated with Tris-EDTA buffer (pH 7.1) containing 0.5% sodium cholate and 250 mM NaCl. This buffer system also served as the mobile phase. The flow rate was adjusted to 1 ml/min and the protein content of the eluate was continuously monitored at 280 nm using an LKB monitor. One ml fractions were collected and their specific [^{3}H]SCH-23390 binding determined after treating each fraction with Bio-Bead SM-2 (0.5 g/ml) in the presence of 0.5 mg/ml soybean phosphatidylcholine. The Bio-Bead procedure was used as an alternative to Sephadex G-50 for quick reconstitution of the receptor binding activity.

The HPLC column was calibrated with protein standards of known molecular weights (BioRad); bovine thyroglobulin (670,000), bovine gamma globulin (158,000), Chick Ovalbumin (44,000), horse myoglobin (17,000), and vitamin B-12 (1,350). Estimates of the molecular size of the receptor was determined graphically plotting the log Mr of the protein standards versus K_{av} where $K_{av} = (V_e-V_o)/(V_t-V_o)$.

v) Electron Microscopy of the Reconstituted Preparation. The reconstituted receptor preparation was pelleted by centrifugation at 130,000 *g* for 60 min. The pellet was first fixed in 2% glutaraldehyde, post-fixed in 2% Osmium tetroxide and en bloc stained with saturated uranyl acetate. The pellet was dehydrated, embedded and sectioned; the sections then stained with lead citrate and examined under electron microscope.

vi) Protein Estimation. Protein in solubilized and reconstituted preparations was estimated by the method of Bradford (37) using bovine serum albumin as standard. The striatal membrane protein content was estimated by the method of Lowry et al. (38).

vii) Data Analysis. The binding data were analyzed on an IBM-PC by weighted nonlinear curve-fitting programs, BDATA (for Scatchard analysis) and CDATA (for antagonist versus agonist competition curves) of EMF Software, Knoxville, TN, USA. The data were analyzed for either one or multiple populations of binding sites together with statistical analysis comparing "goodness of fit" between one or two affinity state models. A two-site model was selected only if a statistically significant improvement (F-test) of the fit of the data was obtained over a one-site model. The IC_{50} values (concentration of competing ligands that inhibits the binding of the labelled ligand by 50%) obtained from the competition curves were converted to K_i values (inhibitor constant) as described earlier (36).

RESULTS

i) Solubilization of Dopamine D_1 Receptor from Bovine Striatal Membranes. Figure 1 depicts the effect of different concentrations of sodium cholate, along with 1 M sodium chloride, in solubilizing dopamine D_1 receptors from agonist (SKF-82526-J) preincubated and non-preincubated bovine striatal membranes. A concentration of 0.5% (w/v) cholate was found optimal for solubilizing the receptors from agonist preincubated membranes; increasing the cholate concentration did not result in higher yield of solubilization although the amount of protein solubilized continued to increase up to 0.8% sodium cholate. At a cholate concentration of 0.5%, 30% and 10% of the receptors were solubilized with agonist preincubated and non-preincubated membranes respectively. The receptor binding in the insoluble pellet in both cases was only about 10% of the total, suggesting that a substantial amount of receptor is inactivated during solubilization.

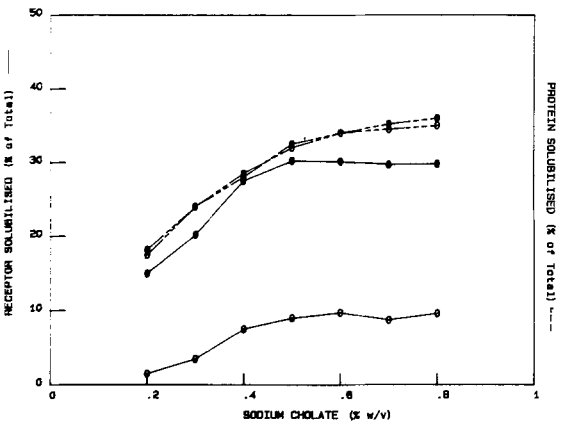

Fig. 1. Solubilization of bovine striatal dopamine D_1 receptor by sodium cholate-sodium chloride.
The bovine striatal membrane preparation (12–14 mg/ml) was solubilized either as such (○) or preincubated with 10 μM SKF-82526-J (●) as described in Experimental Procedures. The membranes were solubilized with indicated concentrations of sodium cholate in the presence of 1M NaCl, 0.1 mM DTT, 0.1 mM PMSF, 0.1 mM benzamidine, and 1 mg/ml of soybean phospholipid as described in Materials and Methods. The mixture was gently stirred in an ice bath for 30 min and then centrifuged at 130,000 *g* for 60 min. The supernatant was passed through a Sephadex G-50 column and [^{3}H]SCH-23390 (2 nM) binding in the pooled void volume fractions was performed in the presence of 100 mM NaCl as described in Experimental Procedure. The results are mean of three independent experiments.

Table I. Effect of Preincubation of Bovine Striatal Membranes with Dopaminergic Ligands on the Solubilization of Dopamine D_1 Receptor.

Ligand	Concentration (M)	Receptor solubilized (% of total)	[^{3}H]SCH-23390 binding (fmol/mg protein)
SKF-82526-J	10^{-8}	7.9	220
	10^{-7}	18.1	489
	10^{-6}	22.3	621
	10^{-5}	31.2	860
	10^{-4}	31.1	824
SCH-23390	10^{-8}	14.9	402
	10^{-7}	17.8	526
	10^{-6}	21.3	643
	10^{-5}	29.7	884
	10^{-4}	30.0	889
Dopamine	10^{-5}	16.8	500
SKF-38393	10^{-5}	13.3	423
ADTN	10^{-5}	6.3	197

The bovine striatal membranes were preincubated with the indicated concentrations of the ligands for 30 min at 37°C in 50 mM Tris-1 mM EDTA buffer (pH 7.4) containing 5 mM $MgCl_2$ and 0.1% ascorbate. The membranes were pelleted at 12,000 *g*, washed with the same buffer and then used for solubilization as described in Materials and Methods. [^{3}H]SCH-23390 (2 nM) binding in the presence of 100 mM NaCl was performed after passing the solubilized preparation through a Sephadex G-50 column as described in Experimental Procedure. The results are means of three experiments.

In order to test the possibility that active receptors can be solubilized from membranes pretreated with an antagonist, we compared the yield and properties of the solubilized and reconstituted dopamine D_1 receptor after preincubating the striatal membranes with various concentrations of SKF-82526-J (an agonist) and SCH-23390 (a specific antagonist). The results (Table I) demonstrate that at each concentration tested, preincubation of the membranes with SCH-23390 and SKF-82526-J resulted in similar yield of the receptor binding activity with an optimum concentration of 10 μM for both the ligands. The results also revealed that some other dopamine D_1 agonists were not as efficient as SKF-82526-J in enhancing solubilization of the receptors, when tested at a single fixed concentration of 10 μM. The amount of total protein solubilized remained similar irrespective of the preincubating ligands used.

The procedure described here to solubilize the dopamine D_1 receptor fulfilled the essential criteria of solubilization, viz: (a) no significant loss of specific [^{3}H]SCH-23390 binding upon ultracentrifugation of the solubilized preparation at 130,000 *g* for 3 h; (b) filtration of the soluble preparation through 0.22 μm filters resulted in over 90% recovery of [^{3}H]SCH-23390 binding activity; and (c) absence of lamellar membrane fragments under electron microscope.

ii) Size-Exclusion HPLC and Sucrose Density Gradient Centrifugation of the Solubilized Receptor. Fig. 2 shows the HPLC profile of the cholate solubilized dopamine D_1 receptor on a size-exclusion column. The receptor, solubilized after agonist preincubation of the membranes, consistently eluted in fractions behind the thyroglobulin peak, as measured by maximum [^{3}H]SCH-23390 binding (Figure 2A). This gives the apparent molecular size of the receptor-detergent complex to be 360,000. This molecular size of the receptor differs considerably from the value reported for canine striatal dopamine D_1 receptor solubilized with digitonin, which on size exclusion HPLC column, eluted at Mr 158,000 (34). In order to test the possibility that the apparent higher molecular size of the receptor in our studies was due to the association of a guanine nucleotide binding protein caused by agonist preincubation of the membranes, the solubilized preparation obtained from membranes pretreated with an antagonist (SCH-23390) was also chromatographed on the same column. As is evident from Figure 2B, [^{3}H]SCH-23390 binding activity eluted in the same fractions as those observed with solubilized preparation obtained from the agonist-preoccupied membranes. If guanine nucleotides cause a loss of high-affinity

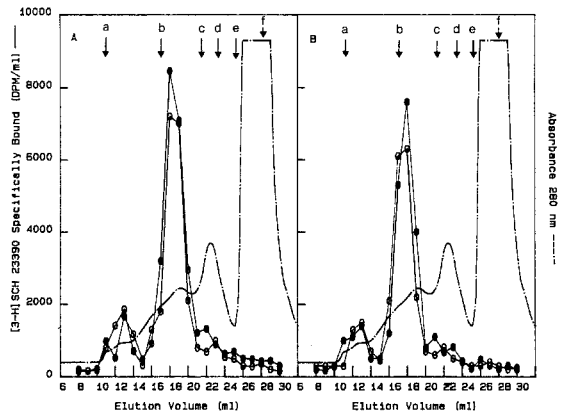

Fig. 2. HPLC of cholate-solubilized dopamine D_1 receptor on size-exclusion column

The bovine striatal membranes were preincubated with either 10 μM SKF-82526-J or 10 μM SCH-23390 for 30 min at 37°C and then solubilized as described in Figure 1. One ml of the 130,000 g supernatant containing soluble receptor, obtained from either SKF-82526-J preincubated membranes (Panel A) or SCH-23390 preincubated membranes (Panel B), was chromatographed at room temperature on Bio-Sil TSK 400 (600 × 7.5 mm) column. The mobile phase was 50 mM Tris-1 mM EDTA buffer (pH 7.1) containing 0.5% sodium cholate and 250 mM NaCl. The samples were chromatographed at a flow rate of 1 ml/min; 1 ml fractions were collected and assayed for [^{3}H]SCH-23390 (1 nM) binding after treating each fraction with Bio-Bead SM-2 (0.5 g/ml for 30 min at 4°C) in the presence of 0.5 mg/ml soybean phosphatidylcholine. The solubilized receptors obtained from membranes pretreated with SKF-82526-J or SCH-23390 were injected into the HPLC column either as such (●) or preincubated with 100 μM Gpp(NH)p for 60 min at 0°C (○) before injection. The elution profile shown here is a representative of three to four independent experiments with similar results. The position of molecular weight markers (Bio-Rad) are shown by arrow—a: void volume, b: thyroglobulin (670 K), c: bovine gamma globulin (158 K), d: chick ovalbumin (44 K), e: horse myoglobin (17 K) and f: vitamin B_{12} (1.35 K).

agonist binding to the dopamine D_1 receptor by dissociating a guanine nucleotide binding protein from the solubilized receptor, preincubation of the solubilized preparation with Gpp(NH)p, a nonhydrolyzable analogue of GTP, should result in lower apparent molecular size of the receptor. However, as shown in Figure 2A and 2B, [^{3}H]SCH-23390 binding activity again elutes in the same fractions of HPLC column, whether or not the two solubilized preparations are exposed to Gpp(NH)p. The results, thus suggest that the molecular size of the cholate solubilized dopamine D_1 receptor remains similar whether the membranes from which it is solubilized is exposed to an agonist or antagonist, and Gpp(NH)p probably does not cause a complete dissociation of the guanine nucleotide binding protein from the receptor. Similar results were obtained from the sucrose-density gradient centrifugation of the cholate-solubilized bovine striatal dopamine D_1 receptor (data not shown). The solubilized receptor obtained from either agonist or antagonist preincubated membranes sedimented at the same sucrose density as determined by peak [^{3}H]SCH-23390 binding of the gradient fractions.

iii) Reconstitution of the Receptor. The cholate/NaCl solubilized dopamine D_1 receptor exhibited little specific [^{3}H]SCH-23390 binding unless it was reconstituted with lipid vesicles. Various experimental protocols were examined to maximize ligand binding to the reconstituted receptor and co-solubilization of the receptor with 1 mg/ml soybean phosphatidylcholine was found to be optimal. When the receptors were solubilized in the absence of lipids, mixed later with sonicated soybean phosphatidylcholine and passed through Sephadex G-50, the binding activity in the void volume fractions represented only about 50% of that observed when receptor was solubilized in the presence of lipids.

Sucrose-density gradient centrifugation of the reconstituted preparation obtained from membranes solubilized with a trace amount of ^{14}C phosphatidylcholine, demonstrated that the receptor is tightly associated with the lipids. The receptor binding activity and the ^{14}C counts co-migrated on the density gradient irrespective of whether the preparation was loaded on the top or bottom of the gradient (results not shown). That the D_1 dopamine receptors were reconstituted into vesicles was also verified by subjecting the reconstituted preparation to high-speed centrifugation. The reconstituted D_1 receptor sediments upon centrifugation at 164,000 *g* for 1 hr whereas the solubilized receptor does not under these conditions. Electron microscopy of the reconstituted preparation revealed predominantly unilamellar vesicles with a mean diameter of 80 nm (results not shown).

iv) [^{3}H]SCH-23390 Binding to Reconstituted Dopamine D_1 Receptor. The Scatchard analysis of [^{3}H]SCH-23390 binding to reconstituted preparation obtained from membranes preincubated with agonist (Figure 3) revealed a maximum receptor density (B_{max}) of 918 fmol/mg protein with a K_d of 0.95 nM. In a typical experiment using 100 μg protein, the total and nonspecific binding of [^{3}H]SCH-23390 at saturation represented about 25,000 and 6,000 dpms, respectively. Antagonist pretreatment of the membranes resulted in no appreciable change in the binding parameters of the reconstituted preparation (K_d, 1.1 nM; B_{max}, 885 fmol/mg protein). Assayed in the presence of 120 mM NaCl, the reconstituted receptor obtained either from agonist- or antagonist-pretreated membranes showed an increase in affinity (K_d = 0.4 – 0.5 nM) for [^{3}H]SCH-23390 with little change in the Bmax (Figure not shown). These [^{3}H]SCH-23390 binding parameters match closely to those obtained with native membrane-bound receptors (B_{max} = 1.1 pmol/mg

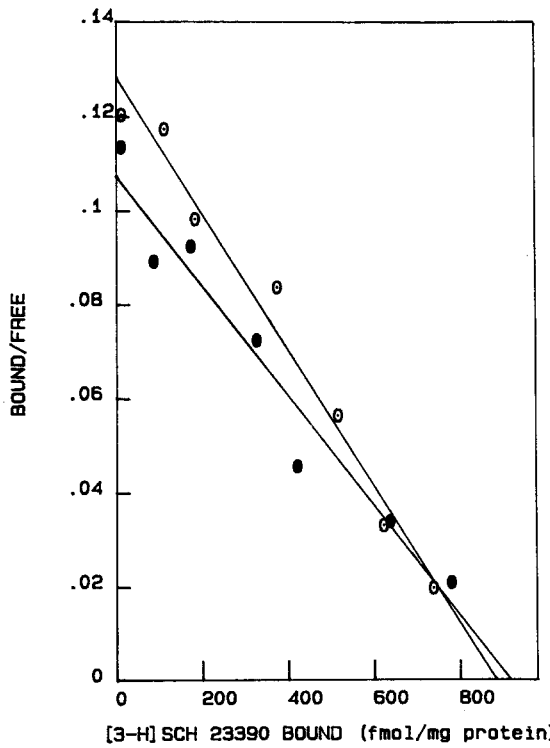

Fig. 3. Scatchard plot of [^{3}H]SCH-23390 binding to solubilized and reconstituted striatal dopamine D_1 receptor.
The receptor was solubilized and reconstituted from striatal membranes preincubated with SKF-82526-J (●) or SCH-23390 (○) as described in Materials and Methods. The binding of [^{3}H]SCH-23390 (0.01–4.0 nM) was done on 250 μl of the reconstituted preparation (~100 μg protein) in a final volume of 1 ml with 50 mM Tris-1 mM EDTA buffer (pH 7.4). The incubation and filtration conditions are as described in Experimental Procedure. Nonspecific binding was determined in parallel assays containing 1 μM *cis*-flupentixol additionally. The results are representative of three to four independent experiments carried out in triplicate.

protein; K_d = 0.42 nM) suggesting that the reconstituted receptor completely retains its affinity for the ligand. Since [^{3}H]SCH-23390 has been reported to also label serotonergic binding sites (39), the effect of 50 nM ketanserin (a specific serotonin receptor antagonist) was tested on the [^{3}H]SCH-23390 binding; however, no significant difference in either K_d or B_{max} was obseved (data not shown). With regard to the effect of guanine nucleotides, Gpp(NH)p, at 100 μM, did not have any significant effect on the binding of [^{3}H]SCH-23390 as shown by the Scatchard plot (results not shown).

v) Pharmacological Specificity of [^{3}H]SCH-23390 Binding to the Solubilized and Reconstituted Dopamine D_1 Receptor. In order to investigate the pharmacological specificity of the solubilized and reconstituted dopamine D_1 receptor, a variety of dopaminergic and nondopaminergic drugs were tested for their ability to compete with [^{3}H]SCH-23390 binding (Table II). The competition data for various antagonists shows that most potent compound inhibiting [^{3}H]SCH-23390 binding was SCH-23390 itself with a K_i of 0.49 and 0.41 nM for reconstituted preparation from agonist- or antagonist-pretreated membranes, respectively. The value is similar to that obtained with native membranes. The competition curves of antagonists for [^{3}H]SCH-23390 binding were monophasic with Hill number close to unity. The antagonists demonstrated the following rank order of potency for inhibiting [^{3}H]SCH-23390 binding in the reconstituted receptor obtained from agonist-pretreated membrane: SCH-23390 > (+)butaclamol > *cis*-flupentixol > fluphenazine > haloperidol > ketanserin > spiroperidol > YM-09151-2 > *trans*-flupentixol > sulpiride > phentolamine > propranolol. The selective dopamine D_2 receptor antagonists, viz., sulpiride and YM-09151-2 have much less affinity for [^{3}H]SCH-23390 binding sites, suggesting D_1 dopaminergic characteristic of [^{3}H]SCH-23390 binding to the reconstituted preparation. An almost 5000-fold difference in the potency of *cis*- and *trans*-isomers of flupentixol demonstrates the stereoselective nature of the [^{3}H]SCH-23390 binding. The rank order of potency of various dopaminergic drugs for the reconstituted receptor obtained from antagonist-pretreated membranes, though largely similar to that obseved with the membrane-bound or agonist-pretreated receptor, shows a notable difference. Whereas (+)butaclamol is about four-fold more potent than *cis*-flupentixol in agonist-pretreated preparation, both these antagonists display almost equal potency in antagonist-pretreated preparation.

In contrast to monophasic curves obtained with [^{3}H]SCH-23390/antagonist competition experiments, the competition curves of agonists for [^{3}H]SCH-23390 binding to reconstituted preparation obtained from either agonist or antagonist-preincubated membranes (Figure 4 and 5) were shallow and best fit to two different affinity states of the receptor. Computer analysis of the agonist competition curves for both membrane-bound and reconstituted receptors (Table III) indicates that the high affinity state (R_H) represents 20-40% of the total number of specific binding sites, whereas 60-80% of the sites are in low affinity states (R_L). Compared to the membrane-bound receptor, the number of high affinity agonist sites is slightly lower in reconstituted receptor from agonist-pretreated membrane. In contrast, the number of high-affinity sites in reconstituted receptor obtained from antagonist-pretreated membrane is more than 20% higher than the native membranes and about 40-80% higher than agonist-pretreated preparation, indicating a potentiation of agonist high-affinity state by antagonist treat-

Table II. Competition by Dopaminergic and Nondopaminergic Antagonists for [^{3}H]SCH-23390 Binding to Bovine Striatal Membranes and Solubilized and Reconstituted Dopamine D_1 Receptor from Agonist- or Antagonist-pretreated Membranes.

	K_i (nM)		
		Reconstituted Receptor	
Antagonist	Membrane	Agonist-Pretreated	Antagonist-Pretreated
SCH-23390	0.33 ± 0.02	0.49 ± 0.03	0.41 ± 0.02
(+)Butaclamol	0.55 ± 0.04	0.65 ± 0.06	1.30 ± 0.10
Cis-flupentixol	1.0 ± 0.08	2.8 ± 0.15	1.20 ± 0.10
Fluphenazine	44.5 ± 4.0	67.5 ± 4.0	124.0 ± 9.3
Haloperidol	150.0 ± 11.5	230.0 ± 20.0	190.0 ± 21.5
Ketanserin	255.0 ± 20.7	266.0 ± 17.5	455.0 ± 50.0
Spiroperidol	257.0 ± 30.4	488.0 ± 29.9	405.0 ± 21.1
YM-09151-2	2,160.0 ± 301	3,490.0 ± 474	3,310.0 ± 202
Trans-flupentixol	5,500.0 ± 700	14,500.0 ± 1100	7,600.0 ± 605
Sulpiride	27,500.0 ± 3900	34,850.0 ± 2700	42,760.0 ± 3,070
Phentolamine	≥50,000.0	38,000.0 ± 5500	≥50,000.0
Propranolol	≥50,000.0	39,000.0 ± 6700	47,800.0 ± 4,700

Increasing concentration of the competing ligands were incubated with 1 nM [^{3}H]SCH-23390 and 250 μl of either striatal membrane preparation or solubilized and reconstituted preparation in a total volume of 1 ml in 50 mM Tris-1 mM EDTA buffer, pH 7.4. Nonspecific binding was determined in parallel assay in the presence of 1 μM *cis*-flupentixol. The membranes were pretreated with agonist (SKF-82526-J) or antagonist (SCH-23390) and then solubilized and reconstituted as described in Materials and Methods. The values are means ± SEM of three experiments performed in triplicate.

ment. Interestingly, the affinity of the high-affinity agonist sites in reconstituted receptor obtained from agonist-treated membrane is considerably high (as reflected in decreased K_i) compared to the native membrane, using both dopamine and SKF-38393 as the competing agonist. The reconstituted receptor from antagonist-treated membrane also shows a decrease in K_i for only dopamine high-affinity state.

In the presence of 100 μM Gpp(NH)p, the agonist competition curves shifted to the right with no detectable high affinity component in both the reconstituted receptors obtained from agonist- and antagonist-pretreated membranes (Figure 4 and 5). This observation of complete conversion of the high affinity states into the low-affinity ones in the reconstituted preparation is in sharp contrast to that observed for the native membranes. In the membranes, under identical conditions, Gpp(NH)p caused only partial conversion of the high affinity state of the dopamine D_1 receptor as indicated by 8-12% receptors still remaining in the high-affinity state (Table III).

DISCUSSION

In the present report, we demonstrate that bovine striatal dopamine D_1 receptor can be solubilized efficiently by a combination of sodium cholate and sodium chloride, and that the solubilized receptors can be reconstituted in phospholipid vesicles in a form that displays all the pharmacological characteristics of the membrane-bound dopamine D_1 receptor. The efficiency of solubilization depended on preoccupation of the membrane receptor with a dopaminergic ligand, agonist or antagonist, since preincubation of the membrane with both SKF-82526-J, a D_1 agonist (40) or SCH-23390 an antagonist, gave similar yields of the solubilized receptors. These results differ from those reported for solubilization of rat brain dopamine D_1 receptor (35) in which the author shows a much lower yield of the solubilized and reconstituted receptors when the membranes are pretreated with SCH-23390 compared to the yield obtained from an agonist (SKF-38393) treated membranes. This discrepancy could possibly be attributed to the different washing procedures of membranes following ligand-pretreatment. SCH-23390 has very high-affinity towards dopamine D_1 receptor, and requires thorough washing of the membranes to remove it. The other possiblity could be the difference in our reconstitution procedure. In the report on rat brain (35), Bio-Bead SM-2 resin was used to reconstitute the receptors, whereas we reconstituted on a Sephadex G-50 column to ensure that any

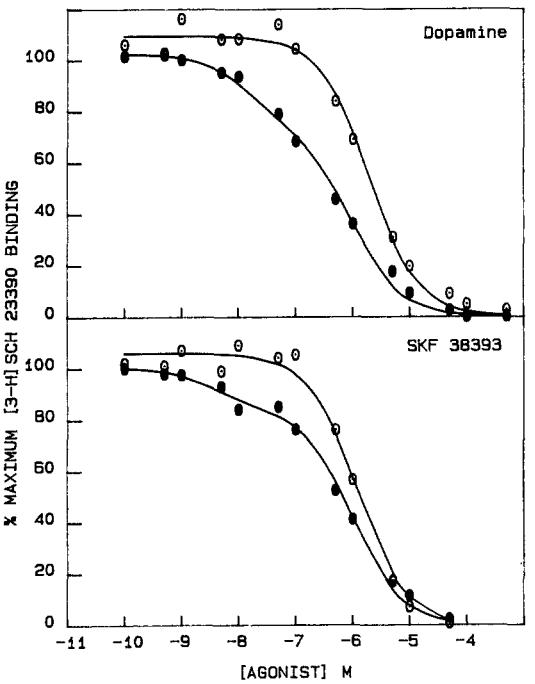

Fig. 4. Competition by dopamine and SKF-38393 for [^{3}H]SCH-23390 binding to reconstituted dopamine D_1 receptor from agonist preincubated striatal membranes.
Striatal membrane preparations were treated with agonist (10 μM SKF-82526-J), solubilized and reconstituted as described in Materials and Methods. Increasing concentrations of agonists were incubated with 1 nM [^{3}H]SCH-23390 and 250 μl of the reconstituted preparation in a total volume of 1 ml with 50 mM Tris-1 mM EDTA buffer (pH 7.4) containing 5 mM $MgCl_2$ and 0.1% ascorbate. Nonspecific binding was determined in parallel in the presence of 1 μM *cis*-flupentixol. The incubation and filtration conditions were as described in Experimental Procedure. Symbols (●) and (○) represent the competition curve obtained in the absence and presence of 100 μM Gpp(NH)p respectively. The curves were computer analyzed for one or two-site fit and the binding parameters are shown in Table III. The data points are means of triplicate determinations in a typical experiment representative of three such experiments.

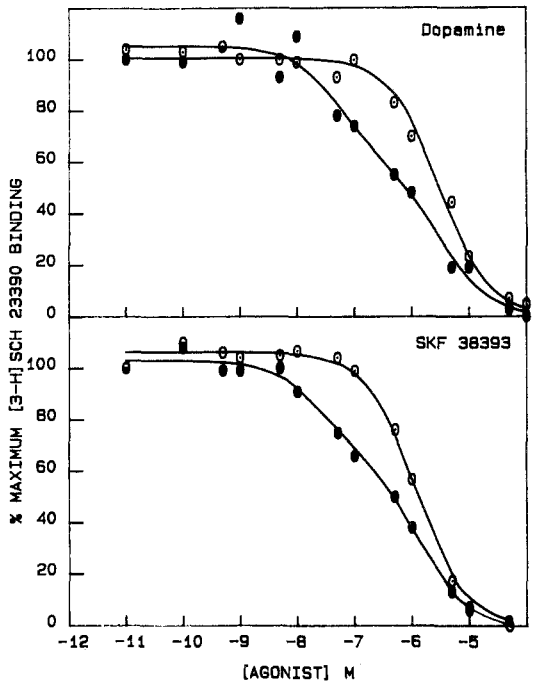

Fig. 5. Competition by dopamine and SKF-38393 for [^{3}H]SCH-23390 binding to reconstituted dopamine D_1 receptor from antagonist preincubated membranes.
The striatal membranes were incubated with 10 μM SCH-23390 for 30 min at 37°C and then solubilized and reconstituted as described in Experimental Procedure. The competition assays were carried out as described in Figure 4 in the absence (●) or presence (○) of 100 μM Gpp(NH)p. The data were analyzed for one or two-site fit and the binding parameters are shown in Table III. The data are mean of triplicate determinations and are representative of three independent experiments.

residual ligand is maximally removed. Apparently, the affinity of the preincubating ligand for the dopamine D_1 receptor appears to be important, as preincubation of the membrane with dopamine, SKF-38393, and ADTN, ligands that have lower affinity than SKF-82526-J and SCH-23390, resulted in lower yields of solubilization. It is, however, not clear why pretreatment of bovine striatal membranes with SKF-38393 in our experiment resulted in lower yield of solubilized and reconstituted receptors (13%) compared to 37% yield obtained with rat brain (35). Possibly, the difference could be attributed to the use of racemic mixture of SKF-38393 in our studies as opposed to the R-isomer of the compound used in the study of Sidhu (35).

The solubilized bovine striatal D_1 receptors reconstituted into phospholipid vesicles display all the ligand-binding characteristics of the native membrane-bound receptor. Both the reconstituted receptors bind [^{3}H]SCH-23390 to a homogeneous site with saturability, stereospecificity and reversibility, and the affinity and density of binding sites resemble closely to that obtained with the membrane-bound receptor either by us or other investigators (16,21). For canine dopamine D_1 receptor solubilized with digitonin (34), a K_d of 5 nM for [^{3}H]SCH-23390 is reported which reflects about 10-fold lower affinity of the ligand compared to the membrane-bound receptor. In the report of solubilization of rat brain dopamine D_1 receptor (33), the author has also reported a 2-fold decrease in the affinity of [^{125}I]SCH-23982 for the solubilized receptor. The similar binding parameters of [^{3}H]SCH-23390 to reconstituted dopamine D_1 receptor obtained from membranes preincubated with either SKF-82526-J or SCH-23390 emphasizes the point that

Table III. Competition by Dopaminergic Agonists for [^{3}H]SCH-23390 Binding and the Effect of Gpp(NH)p on Striatal Membrane, and Solubilized and Reconstituted Dopamine D_1 Receptor

Agonist	Receptor Preparation and Condition	Ki (nM)		% of sites	
		High Affinity	Low Affinity	High Affinity	Low Affinity
Dopamine	Membrane				
	−Gpp(NH)p	58 ± 3.3	3900 ± 170	35 + 2.0	65 ± 3.7
	+Gpp(NH)p	71 ± 7.2	5500 ± 222	12 ± 0.6	88 ± 4.1
	Reconstituted I				
	−Gpp(NH)p	16 ± 1.0	950 ± 45	30 ± 1.1	70 ± 2.5
	+Gpp(NH)p	—	400 ± 44	0	100
	Reconstituted II				
	−Gpp(NH)p	30 ± 2.3	1650 ± 92	43 ± 2.2	57 ± 2.8
	+Gpp(NH)p	—	1600 ± 124	0	100
SKF-38393	Membrane				
	−Gpp(NH)p	8.7 ± 0.4	550 ± 20	28 ± 0.8	72 ± 2.0
	+Gpp(NH)p	11.2 ± 1.2	840 ± 29	8 ± 0.6	92 ± 6.8
	Reconstituted I				
	−Gpp(NH)p	4.8 ± 0.3	489 ± 33	18 ± 0.6	82 ± 2.7
	+Gpp(NH)p	—	500 ± 23	0	100
	Reconstituted II				
	−Gpp(NH)p	12.6 ± 0.9	600 ± 60	34 ± 3.0	66 ± 5.8
	+Gpp(NH)p	—	569 ± 61	0	100

Increasing concentrations of the competing agonists in the absence or presence of 100 μM Gpp(NH)p were incubated with 1 nM [^{3}H]SCH-23390 and 250 μl of either the membrane preparation or solubilized and reconstituted preparation in a total volume of 1 ml in 50 mM Tris-1 mM EDTA buffer, pH 7.4 containing 5 mM $MgCl_2$ and 0.1% ascorbate. The non-specific binding was determined in parallel assays containing 1 μM *cis*-flupentixol. The competition data were computer-analyzed for one or two-site fit to get the IC_{50} of the agonist for high- and low-affinity component of [^{3}H]SCH-23390 binding. The IC_{50}'s were converted to inhibition constant (K_i) as described in Materials and Methods. Reconstituted I and II refer to the solubilized and reconstituted preparation obtained from agonist (SKF-82526-J) or antagonist (SCH-23390) pretreated membranes respectively. The pretreatment and solubilization conditions were as described in Materials and Methods. The results are means ±SEM of three experiments performed in triplicate.

the characteristics of the receptor are maintained irrespective of whether the membranes are pretreated with an agonist or an antagonist.

The agonist/[^{3}H]SCH-23390 competition reveals the presence of a high-affinity component of agonist binding in both the reconstituted dopamine D_1 receptor, irrespective of whether the membranes were pretreated with agonists or antagonists. An interesting feature is that in the reconstituted preparation obtained from antagonist-pretreated membranes, the number of high-affinity agonist sites is about 20% more than the native membranes and about 40–80% higher than agonist-pretreated reconstituted preparation. This is similar to the phenomenon of 'potentiation' of high-affinity agonist sites reported for rat brain dopamine D_1 receptor solubilized and reconstituted following agonist-pretreatment of membranes (35). However, in this report, the author has not shown whether potentiation of high-affinity sites is also seen in reconstituted receptors obtained from antagonist-pretreated membranes. In contrast to the report ðn rat brain (35), we did not see a potentiation of high-affinity sites in the reconstituted preparation from agonist-pretreated membranes; rather our report shows a 2-3 fold increase in the affinity of high-affinity agonist binding sites, similar to that observed for solubilized muscarinic cholinergic receptors (41). The mechanism of increase in high-affinity sites is not clear. Because of the differences observed in agonist- and antagonist-preincubated membranes, it does not appear to be entirely the result of reconstitution procedure which may favour increased coupling between the receptor and G-protein. It may be possible that preincubation of the membranes with different ligands results in different conformational state of the receptor in so far as agonist binding is concerned, and when these receptors are solubilized and reconstituted in conditions favoring their enhanced coupling to G-proteins, they show increased high-affinity binding sites or increased affinity of these sites.

The coupling of the G-proteins with the reconstituted receptors is also indicated by their modulation with guanyl nucleotides. In both the reconstituted receptors, 100 μM Gpp(NH)P completely abolished the high-affinity component of agonist binding, in contrast to the membranes where only partial abolition of high-affinity sites is observed. This guanine nucleotide modulation in both the reconstituted preparations is in contrast to reports on the solubilization of dopamine D_2, and α-adrenergic receptors (31,32,23) where the guanine nucleotide sensitivity was noted in the solubilized preparations from the membranes pretreated with an agonist. Again, a complete conversion of high-affinity agonist sites by Gpp(NH)p in our reconstituted receptors is quite distinct from digitonin solubilized canine striatal dopamine D_1 receptor (34) and membrane-bound dopamine D_1 and D_2 receptors where Gpp(NH)p causes only partial conversion of the agonist high affinity sites (20,21,42). This result is, nevertheless, similar to the report on cholate solubilization of the rat brain dopamine D_1 receptor from agonist-pretreated membranes where Gpp(NH)p was observed to cause complete conversion of agonist high affinity sites (35).

The size exclusion HPLC profile of the solubilized receptor was similar when either SKF-82526-J or SCH-23390 was used for preincubating the membrane, suggesting similar molecular mass of receptor-detergent complex. This is in contrast to results obtained with reticulocyte β-adrenergic (43) α-adrenergic (23) and pituitary dopamine D_2 receptor (32), wherein an increase in apparent molecular size of the receptors was noted when they were solubilized from membranes preoccupied with an agonist compared to unoccupied or antagonist-occupied membranes. It is believed that increase in the molecular size of the receptor following agonist pretreatment is a reflection of the receptor-guanine nucleotide protein coupling promoted by agonist preoccupancy of the receptor, an effect that is not elicited by antagonists. Thus, the guanine nucleotide sensitive high-affinity agonist binding is preserved in the receptors solubilized following agonist preincubation of the membranes. In this context, our results would appear to suggest that dopamine D_1 receptors solubilized from membranes preincubated with an agonist or an antagonist are coupled to a G-protein under both the conditions probably due to a close coupling between the receptor and G-protein. The latter possibility appears plausible and is borne out by the sedimentation profile and pharmacological properties of the solubilized and reconstituted receptors including retention of guanine nucleotide-sensitive high affinity agonist binding. Interestingly, similar results have been obtained with A-1 adenosine (44) and canine D_1 dopamine receptor (34) solubilized with digitonin. In these studies, the solubilized receptors displayed similar molecular size and high-affinity guanine nucleotide sensitive agonist binding, whether the receptors were solubilized following preincubation of the membrane with agonists or antagonists or no ligand at all. The lack of effect of Gpp(NH)p on apparent molecular size of the solubilized D_1 receptor indicates that guanine nucleotides in this system probably do not cause a complete dissociation of receptor-G-protein complex. As suggested by Niznik *et al.* (34), it is possible that effect of Gpp(NH)p on high-affinity agonist binding and on dissociation of receptor-G-protein complex are two different processes; and, at least in dopamine D_1 receptor system, these processes may not occur together. Taken together, these results suggest a fundamental difference in receptor G-protein coupling for dopamine D_1 receptors compared to the α- and β-adrenergic and dopamine D_2 receptors.

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REFERENCES

1. Kebabian, J. W., and Calne, D. B. 1979. Multiple receptors for dopamine. Nature. 277:93–96.
2. Creese, I., Sibley, D. R., Hamblin, M. W., and Leff, S. E. 1983. The classification of dopamine receptors: relationship to radioligand binding. Annu. Rev. Neurosci. 6:43–71.
3. Mishra, R. K. 1986. Central nervous system dopamine receptors. In: Neuromethods, Vol. 4 Boulton A., Baker, G., and Hrdina, P.; eds., pp. 23–54, Humana Press, Clifton, N.J.
4. Seeman, P. 1980. Brain dopamine receptors. Pharmacol. Rev. 32:229–313.
5. Lew, J. Y., and Goldstein, M. 1984. Solubilization and characterization of striatal dopamine receptors. J. Neurochem. 42:1298–1305.
6. Kazmi, S. M. I., Ramwani, J., Srivastava, L. K., Rajakumar, G., Ross, G. M., Cullen, M., and Mishra, R. K. 1986. Characterization of high-affinity dopamine D_2 receptors and modulation of affinity states by guanine nucleotide in cholate-solubilized bovine striatal preparations. J. Neurochem. 47:1493–1502.
7. Ramwani, J., and Mishra, R. K. 1986. Purification of bovine striatal dopamine D-2 receptor by affinity chromatography. J. Biol. Chem. 261:8894–8898.
8. Srivastava, L. K., Kazmi, S. M. I., Blume, A. J., and Mishra, R. K. 1987. Reconstitution of affinity-purified dopamine D_2 receptor binding activities by specific lipids. Biochim. Biophys. Acta. 900:175–182.

9. Senogles, S. E., Amlaiky, N., Falardeau, P., and Caron, M. G. 1988. Purification and characterization of the D_2-dopamine receptor from bovine anterior pituitary. J. Biol. Chem. 263:18996–19002.
10. Elazar, Z., Kanety, H., David, C., and Fuchs, S. 1988. Purification of the D-2 dopamine receptor from bovine striatum. Biochem. Biophys. Res. Commun. 156:602–609.
11. Bunzow, J. R., Van Tol, H. H. M., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K. A., and Civelli, O. 1988. Cloning and expression of a rat D_2 dopamine receptor cDNA. Nature 336:783–787.
12. Barone, P., Davis, T. A., Braun, A. R., and Chase, T. N. 1986. Dopaminergic mechanisms and motor function: characterization of D-1 and D-2 dopamine receptor interactions. Eur J. Pharmacol. 123:109–114.
13. Hess, E. J., Bracha, H. S., Kleinman, J. E., and Creese, I. 1987. Dopamine receptor subtype imbalance in schizophrenia. Life Sci. 40:1487–1497.
14. Cash, R., Raisman, R., Ploska, A., and Agid, Y. 1987. Dopamine D-1 receptor and cyclic AMP-dependent phosphorylation in Parkinson's disease. J. Neurochem. 49:1075–1083.
15. Iorio, L. C., Barnett, A., Leitz, F. H., Houser, V. P., and Korduba, C. A. 1983. SCH-23390, A potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J. Pharmacol. Exp. Ther. 226:462–468.
16. Billard, W., Ruperto, V., Crosby, G., Iorio, L. C., and Barnett, A. 1984. Characterization of the binding of ^{3}H-SCH-23390, a selective D-1 receptor antagonist ligand, in rat striatum. Life Sci. 35:1885–1893.
17. Reader, T. A., Briere, R., Gottberg, E., Diop, L., and Grondin, L. 1988. Specific [^{3}H]SCH-23390 binding to dopamine D_1 receptors in cerebral cortex and neostriatum; evidence for heterogeneities in affinity states and cortical distribution. J. Neurochem. 50:451–463.
18. Gingrich, J. A., Amlaiky, N., Senogles, S. E., Chang, W. K., McQuade, R. D., Berger, J. G., and Caron, M. G. 1988. Affinity chromatography of the D_1 dopamine receptor from rat corpus striatum. Biochemistry 27:3907–3912.
19. Huff, R. M., and Molinoff, P. B. 1985. Assay of dopamine receptors with [α-^{3}H]flupentixol. J. Pharmacol. Exp. Ther. 232:57–61.
20. Seeman, P., Ulpian, C., Grigoriadis, D., Pri-Bar, I., and Buchman, O. 1985. Conversion of dopamine D_1 receptors from high to low affinity for dopamine. Biochem. Pharmacol. 34:151–154.
21. Hess, E. J., Battaglia, G., Norman, A. B., Iorio, L. C., and Creese, I. 1986. Guanine nucleotide regulation of agonist interactions at [^{3}H]SCH23390-labeled D_1 dopamine receptors in rat striatum. Eur. J. Pharmacol. 121:31–38.
22. DeLean, A., Kilpatrick, B. F., and Caron, M. G. 1982. Dopamine receptor of the porcine anterior pituitary gland — evidence for two affinity states discriminated by both agonists and antagonists. Mol. Pharmacol. 22:290–297.
23. Smith, S. K., and Limbird, L. E. 1981. Solubilization of human platelet α-adrenergic receptors: evidence that agonist occupancy of the receptor stabilizes receptor-effector interactions. Proc. Natl. Acad. Sci. U.S.A., 78:4026–4030.
24. DeLean, A., Stadel, J. M., and Lefkowitz, R. J. 1980. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. J. Biol. Chem. 255:7108–7117.
25. Gilman, A. G. 1987. G proteins: Transducers of receptor-generated signals. Ann. Rev. Biochem. 56:615–649.
26. Niznik, H. B. 1987. Dopamine receptors: molecular structure and function. Mol. Cell. Endocrinol. 54:1–22.
27. Leff, S. E., Hamblin, M. W., and Creese, I. 1985. Interactions of dopamine agonists with brain D_1 receptors labeled by ^{3}H-antagonists. Mol. Pharmacol. 27:171–183.
28. Grigoridias, D., and Seeman, P. 1985. Complete conversion of brain D_2-dopamine receptors from high- to low-affinity state for dopamine agonists, using sodium ions and guanine nucleotide. J. Neurochem. 44:1925–1935.
29. Heidenrich, K. A., Weiland, G. A., and Molinoff, P. B. 1980. Characterization of radiolabeled agonist binding to β-adrenergic receptors in mammalian tissues. J. Cycl. Nucl. Res. 6:217–230.
30. Hoffman, B. B., Michel, T., Brennan, T. B., and Lefkowitz, R. J. 1982. Interactions of agonists with platelet α_2-adrenergic receptors. Endocrinology 110:926–932.
31. Leff, S. E., and Creese, I. 1982. Solubilization of D-2 dopamine receptors from canine caudate: agonist-occupation stabilizes guanine nucleotide sensitive receptor complexes. Biochem. Biophys. Res. Commun. 108:1150–1157.
32. Kilpatrick, B. F., and Caron, M. G. 1983. Agonist binding promotes a guanine nucleotide reversible increase in the apparent size of the bovine anterior pituitary dopamine receptors. J. Biol. Chem. 258:13528–13534.
33. Sidhu, A., and Fishman, P. H. 1986. Solubilization of the D-1 dopamine receptor from rat striatum. Biochem. Biophys. Res. Commun. 137:943–949.
34. Niznik, H. B., Otsuka, N. Y., Ross, A. D., Grigoriadis, D., Tirpak, A., and Seeman, P. 1986. Dopamine D_1 receptors characterized with [^{3}H]SCH-23390. Solubilization of a guanine nucleotide-sensitive form of the receptor. J. Biol. Chem. 261:8397–8406.
35. Sidhu, A. 1988. Solubilization and reconstitution of the D_1 dopamine receptor: potentiation of the agonist high-affinity state of the receptor. Biochemistry 27:8768–8776.
36. Srivastava, L. K., Bajwa, S. B., Johnson, R. L., and Mishra, R. K. 1988. Interaction of L-prolyl-L-leucyl glycinamide with dopamine D_2 receptor: Evidence for modulation of agonist affinity states in bovine striatal membranes. J. Neurochem 50:960–968.
37. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.
38. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
39. Bischoff, S., Heinrich, M., Sonntag, J. M., and Krauss, J. 1986. The D-1 dopamine receptor antagonist SCH23390 also interacts potently with brain serotonin 5-HT_2 receptors. Eur. J. Pharmacol. 129:367–370.
40. Flaim, K. E., Gessner, G. M., Crooke, S. T., Sarau, H. M., and Weinstock, J. 1985. Binding of a novel dopaminergic agonist radioligand [^{3}H] fenoldopam (SKF 82526) to D-1 receptors in rat striatum. Life Sci. 36:1427–1436.
41. Florio, V. A., and Sternweis, P. C. 1985. Reconstitution of resolved muscarinic cholinergic receptors with purified GTP-binding proteins. J. Biol. Chem. 260:3477–3483.
42. Wreggett, K. A., and Seeman, P. 1984. Agonist high- and low-affinity states of the D_2 dopamine receptor in calf brain: partial conversion by guanine nucleotide. Mol. Pharmacol. 25:10–17.
43. Limbird, L. E., Gill, D. M., and Lefkowitz, R. J. 1980. Agonist-promoted coupling of the β-adrenergic receptor with the guanine nucleotide regulatory protein of the adenylate cyclase system. Proc. Natl. Acad. Sci., U.S.A. 77:775–779.
44. Stiles, G. L. 1985. The A_1 adenosine receptor. Solubilization and characterization of a guanine nucleotide-sensitive form of the receptor. J. Biol. Chem. 260:6728–6732.