Biochemical Pharmacology, Vol. 29, pp. 2009–2016

0006-2952/80/0715-2009 $02.00/0

SOLUBILIZATION OF MULTIPLE BINDING SITES FOR THE DOPAMINE RECEPTOR FROM CALF STRIATAL MEMBRANES*

YVONNE C. CLEMENT-CORMIER,† LAURENCE R. MEYERSON‡ and ANDREW MCISAAC†

†Departments of Pharmacology and Neurobiology, The University of Texas Medical School at Houston, Houston, TX 77025 and ‡Department of C.N.S. Research, Medical Research Division, American Cyanamid Company, Pearl River, NY 10965, U.S.A.

(*Received* 17 *November* 1979; *accepted* 23 *January* 1980)

Abstract—Specific dopamine binding sites from calf striatum were solubilized using potassium chloride. The solubilized salt extract was found to have the same properties as the native membrane preparation, including binding affinity and stereoselectivity. The binding site required the presence of potassium chloride for solubilization, but not for the maintenance of binding. Multiple binding sites were detected in the potassium chloride extract. Hill coefficients for selected agonists and antagonists using three different ligands which interact with the dopamine receptor, [^{3}H]spiroperidol, [^{3}H]-2-amino-6,7-dihydroxy 1,2,3,4-tetrahydronapthalene (ADTN) and [^{3}H]-*N*-propylnorapomorphine, revealed the presence of more than one population of agonist binding sites. In addition, gel filtration chromatography on Sephacryl S-200 demonstrated three peaks of stereospecific [^{3}H]spiroperidol binding. Similar results were obtained following gel filtration chromatography of the potassium chloride extract on Sephadex G-100. The predominant specific site which was labeled by [^{3}H]apomorphine, [^{3}H]ADTN and [^{3}H]spiroperidol on this column had a calculated molecular weight of about 50,000, as calibrated for globular proteins. These data confirm the existence of multiple binding sites for the dopamine receptor in the central nervous system.

Recently, the multiplicity and heterogeneity of the dopamine receptor have been reported by several investigators using *in vitro* binding assays. Multiple binding sites for the dopamine receptor have been identified using radiolabeled ligands such as apomorphine [1], 2-amino-6,7-dihydroxy 1,2,3,4-tetrahydronapthalene (ADTN) [2, 3], haloperidol [4, 5] and spiroperidol [6, 7]. Kinetic analysis of the binding sites using these ligands revealed biphasic saturation curves and non-linear Scatchard plots. These data, which demonstrate multiple binding sites for a given ligand [5, 8], lend support to a variety of *in vivo* evidence suggesting the existence of multiple dopamine receptors [9–12].

The ability to obtain specific [^{3}H]spiroperidol binding sites from rat and calf striatum in a soluble form [13, 14] has made it possible to isolate and study these multiple receptor sites, using established chromatographic techniques. The data which appear here present the results of such studies on the solubilization and isolation of multiple binding sites for the dopamine receptor.

EXPERIMENTAL PROCEDURE

Materials. Frozen bovine brains were obtained from Pel-Freeze and stored at −20° until use. [^{3}H]Spiroperidol (26.4 Ci/mmole), [^{3}H-*N*-propylnorapomorphine (80 Ci/mmole) and [^{3}H]ADTN (18 Ci/mmole) were purchased from New England Nuclear (Boston, MA). Unlabeled haloperidol was obtained from Janssen Pharmaceuticals (Beerse, Belgium). (+)- and (−)-Butaclamol were gifts from Ayerst Laboratories (Toronto, Ontario, Canada), and Norit SGX was purchased from the Sigma Chemical Co. (St. Louis, MO). All other drugs and chemicals were obtained from their respective commercial sources in the highest purity available.

Subcellular fractionation. Calf caudate nuclei were dissected, frozen and stored overnight at −20°. All subsequent fractionation procedures were conducted at 0–4°. The tissue was homogenized in 10 vol. of 0.32 M sucrose–2 mM Tris (hydroxymethylaminomethane maleate, pH 7.4). The crude synaptosomal fraction (P_2) and the microsomal (P_3) fractions were obtained as follows. The homogenate was centrifuged at 900 *g* for 10 min, yielding a pellet (P_1) and a supernatant fraction (S_1). The S_1 was further centrifuged at 11,500 *g* for 20 min to yield a crude synaptosomal pellet (P_2) and a supernatant fraction (S_2). The P_2 was suspended in half the original volume of the homogenizing buffer and recentrifuged for 20 min to yield a washed pellet (P_2') and supernatant fraction (S_2'). S_2 and S_2' were combined and centrifuged at 100,000 *g* for 75 min to yield a microsomal pellet (P_3) and the cytosolic fraction (S_3).

Solubilization. The P_3 microsomal pellet was resuspended in chilled 0.25 M sucrose, 15 mM Na_2HPO_4 (pH 7.2) at a volume equivalent to 20 per cent of that originally used for homogenization. KCl (50%, w/w) was added to the P_3 suspension and was immediately homogenized. Unless indicated otherwise, the salt-treated homogenate was allowed to

* This work was supported by grants from the Pharmaceutical Manufacturers Association, Merck, Sharp & Dohme, PHS-MH 32431 and BNS 7816003.

stand on ice for 15 min and was then centrifuged at 20,000 *g* to remove the excess undissolved salt. The supernatant fraction from the 20,000 *g* spin was then centrifuged at 100,000 *g* for 60 min. The resultant supernatant fraction was taken as the soluble extract.

Binding assay. The standard assay constituents for binding studies (final volume, 1.0 ml) contained 15 mM Tris–HCl (pH 7.4), 5 mM Na_4EDTA, 1.1 mM ascorbate, ^{3}H-ligand, 800 μl of the solubilized tissue preparation (400 μg protein, plus test substance as indicated. The reaction was routinely initiated by the addition of the soluble extract and was incubated for 16 hr at 0°. Similar results were obtained with incubation for 10 min at 37°. The binding reaction was terminated as described previously [14]. The method essentially involves the addition of 100 μl of a Norit SGX slurry to bind free ligand. The Norit SGX slurry contained 50 mM Tris–HCl, 8 mM theophylline, 6 mM β-mercaptoethanol containing 2% BSA, and 10% Norit SGX. The tubes were allowed to stand on ice in the presence of the slurry for 5 min and were then centrifuged for 4 min in a Brinkmann microfuge. Aliquots of the supernatant fraction (400–600 μl) were withdrawn and added to scintillation vials containing 7 ml of Biofluor (NEN). Radioactivity was measured in a Searle Mark III liquid scintillation spectrometer.

Gel filtration chromatography. Gel filtration of the soluble extract was performed on 2.4 × 50 cm columns of Sephacryl S-200 and on Sephadex G-100, equilibrated with 15 mM sodium phosphate containing 0.25 M sucrose (pH 7.0). The column jacket and effluent were maintained at 4° throughout the procedure. A 2-ml aliquot of the soluble extract (2 mg protein) was applied to the column via an automatic sample injector and was eluted at a constant pressure flow rate of 2.5 ml/min. Five-ml fractions were collected, utilizing an automatic refrigerated fraction collector (Buchler Instruments). An ultraviolet detector set at 280 nm was used to monitor the effluent peaks. Both columns were calibrated with Aldolase (158,000), BSA monomer (66,000), ovalbumin (48,000), chymotrypsinogen A (25,000), and ribonuclease A, (13,500), for molecular weight estimations.

Gel electrophoresis. The SDS system was essentially that described by Laemmli [15], with gel dimensions of 11 × 16 × 4 cm and staining for protein in 0.25% coomassie blue–10% acetic acid for 1 hr, followed by destaining overnight in 7.5% acetic acid.

Protein determination. Protein concentrations were determined by the method of Lowry *et al.* [16] or Bradford [17].

RESULTS

[^{3}H]Spiroperidol binding sites from calf striatum were solubilized using salt extraction with KCl. The results in Table 1 show the effect of various solubilizing agents on the extraction of a macromolecular component from the striatum which exhibits specific [^{3}H]spiroperidol binding. Detergents such as Triton and Lubrol WX did not produce a significant increase in specific binding activity, as defined by displacement in the presence of 10^{-7} M (+)-butaclamol. The largest number of binding sites was observed when KCl was used as the solubilizing agent. The recovery of the soluble receptor, compared to the membrane bound site, averaged between 20 and 30 per cent. Specific [^{3}H]spiroperidol binding in the KCl-treated extract represented a 2-fold increase in binding over that observed in the crude striatal homogenate. Concentrations of detergent greater than 1%, up to 10%, did not alter the number of specific [^{3}H]spiroperidol binding sites (data not shown) from that reported in Table 1. These data do not rule out the possibility that variations in the concentration of detergent as well as other biochemical variables such as pH, ionic strength and stabilizing agents, may yield a preparation exhibiting specific binding activity. In addition, the use of frozen tissue, which seems to be required for KCl extraction, is probably not optimal for detergent extraction, since most procedures using detergents utilize fresh tissue.

Table 1. Effect of various solubilizing agents on [^{3}H]spiroperidol binding*

Test substance	Specific [^{3}H]spiroperidol binding (fmoles/mg protein)
KCl	11.0
Deoxycholate	0
Octyl-8-D-glucopyranoside	0
Brij-35	0.4
Brij-W-1	0.8
Triton X-100	0.6
Triton X-207	0
Triton X-305	1.4
Triton X-405	0.8
Triton X-15	0
Triton CF-21	0.2
Triton B-1956	0.4
Triton QS-15	0
Lubrol WX	0
Lubrol DX	0
NP 40	0.1
Digitonin	0.4

* The data are taken from a representative experiment testing the effects of various solubilizing agents on a microsomal pellet of the calf striatum. All agents were suspended in 15 mM K_2HPO_4, pH 7.4, at the indicated concentrations. The error range for the values shown is approximately 10 per cent from samples assayed in triplicate. The concentration of all detergents used was 0.1% except for digitonin which was 0.2%. The concentration of KCl was 400% (w/w). The concentration of [^{3}H]spiroperidol was 0.2 nM.

The displacement of [^{3}H]spiroperidol binding to the sites in the KCl-P_3 extract by various neuroleptic drugs is shown in Table 2. The concentration of (+)-butaclamol which displaced 50 per cent of the total counts (5×10^{-9} M) was equal to that of the particulate site. Other neuroleptic drugs and agonists of dopamine had IC_{50} values for [^{3}H]spiroperidol binding in the soluble fraction that were equivalent to those observed in the particulate fraction. Removal of the KCl present in the extract by overnight dialysis neither altered the binding activity for

Table 2. Displacement of specific [^{3}H]spiroperidol binding sites from calf caudate microsomes solubilized with potassium chloride*

Drug	IC_{50} (nM)
Chlorpromazine	4
Fluphenazine	4
Promazine	250
α-Fluphenthixol	4
(+)-Butaclamol	5
Dopamine	2000
Apomorphine	280

* The IC_{50} values shown are the concentrations of test substances required to inhibit the binding of 0.2 nM [^{3}H]spiroperidol in a calf striatal microsomal preparation (P_3, which has been treated with KCl as described in the Experimental Procedure); (−)-butaclamol, serotonin, and norepinephrine were inactive in this preparation, with an IC_{50} value > 10,000 nM. Each result represents the average of four experiments.

[^{3}H]spiroperidol (Table 3) nor changed the binding affinity for displacement by various neuroleptic drugs (data not shown).

The soluble state of the [^{3}H]spiroperidol binding sites that were released by treatment with KCl was verified by subjecting the extract to a number of experimental manipulations, including ultracentrifugation, protein precipitation, dialysis, gel filtration, millipore filtration and electron microscopy. When the KCl extract was ultracentrifuged at 100,000 *g* for 4 hr, no sedimented material was detected and, after passage of the supernatant fraction through a 0.45 or 0.22 μm millipore filter, no decrease in binding activity was observed. Extensive dialysis of the KCl extract or filtration through BioGel P_2, followed by centrifugation at 100,000 *g* for 2 hr, did not alter binding activity. In addition, electron microscopy indicated a total absence of any recognizable membrane vesicles or fragments in the solubilized preparation. This solubilization procedure was also specific for a dopaminergic brain region. When the cerebellum was used as the tissue source for treatment with KCl, specific [^{3}H]spiroperidol binding was not detected in the 100,000 *g* supernatant fraction.

The soluble [^{3}H]spiroperidol binding site was found to be relatively thermostabile. Preincubation of the solubilized P_3 extract at 60° for various time periods in the presence of either Na_2HPO_4 or K_2HPO_4 for 10 min at 60° reduced the total binding capacity. However, 25 mM Na_2HPO_4 at pH 7.4 was more effective in protecting the soluble binding site from the long-term exposure to 60°. When K_2HPO_4 was substituted for Na_2HPO_4 at equal molar concentrations, protection against thermal denaturation was not observed. The presence of ascorbate (0.1%), sucrose (0.25%), albumin (1%), dithiothreitol (5 mM) or mercaptoethanol (1%), alone, did not protect the solubilized binding site against 5-min thermal denaturation at 60°. The presence of 10% glycerol or 5 mM EDTA did offer some protection against thermal denaturation, but the amount of specific binding observed with either of these agents alone was 50 per cent of that observed with 25 mM Na_2HPO_4 after a 5-min exposure to 60° (Fig. 1).

The nature of the specific binding of such dopamine agonists and antagonists was assessed by analysis of their competitive behaviors with ^{3}H-antagonists and ^{3}H-agonists. Theoretically, if the ligand has a relatively low binding constant and a sufficiently high specific activity, so that only a small fraction of receptors are occupied, the depression of specific binding can be viewed as a direct measure of receptor site occupancy. The ligand selected for this purpose was [^{3}H]-*N*-propylnorapomorphine ([^{3}H]NPNA), with a specific activity of 80 Ci/mmole. Receptor occupancy of less than 10 per cent can be obtained with 10^{-10}–10^{-9} M [^{3}H]NPNA. As shown in Fig. 2, the displacement in the P_3-KCl extract of [^{3}H]NPNA binding by apomorphine did not follow the simple mass action law. The experimental curve is flatter than the theoretical curve for a single binding site, suggesting the presence of multiple binding sites.

Additional support for multiple binding sites for [^{3}H]NPNA can be derived from the analysis of the

Table 3. [^{3}H]spiroperidol binding in the presence and absence of KCl*

Condition	Addition	Binding activity (fmoles/10 mg protein)	% Specifically bound
KCl extract	None	112	
	(+)-Butaclamol (10^{-7} M)	33	71
	(−)-Butaclamol (10^{-7} M)	110	
Dialyzed KCl extract	None	101	
	(+)-Butaclamol (10^{-7} M)	32	68
	(−)-Butaclamol (10^{-7} M)	95	
KCl extract/Biogel P_2	None	75	
	(+)-Butaclamol (10^{-7} M)	25	67
	(−)-Butaclamol (10^{-7} M)	76	

* The data represent the mean from three separate experiments with each point assayed in triplicate; the S.E.M. was less than 15 per cent. The procedure for the assay of binding activity is described in the text. Dialysis was carried out overnight at 4° and the dialysate was centrifuged at 100,000 *g* for 2 hr before assaying for binding activity.

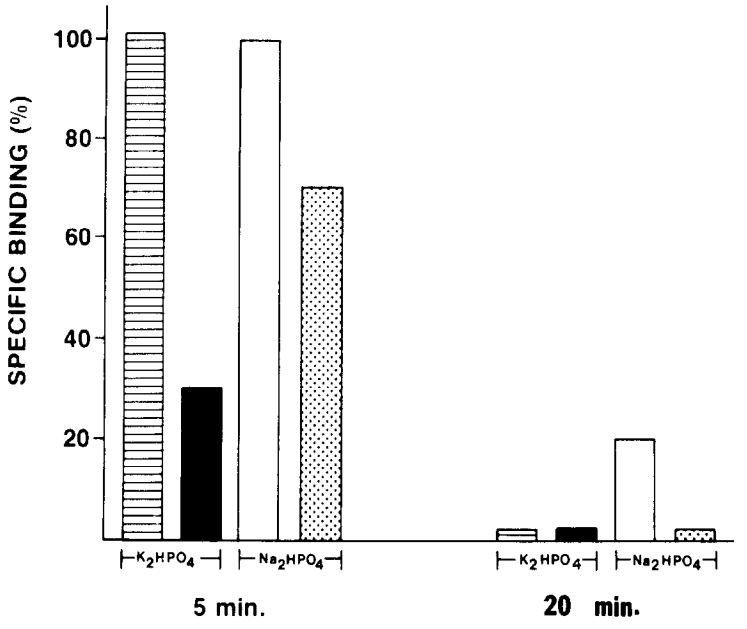

Fig. 1. Heat denaturation of [^{3}H]spiroperidol binding sites. Specific [^{3}H]spiroperidol binding was defined as the difference between total binding and that determined in the presence of 10^{-7} M (+)-butaclamol using one of the following buffers, all at pH 7.4: 100 mM K_2HPO_4 (▤), 50 mM K_2HPO_4 (■), 25 mM Na_2HPO_4 (□), and 50 mM Na_2HPO_4 (▨). The potasium chloride extract in each buffer system was exposed to 60° for 5 min or 20 min prior to assay for binding activity. The level of specific binding shown is calculated on the basis of binding observed in each buffer system without any preincubation. The data represent the average of three separate experiments.

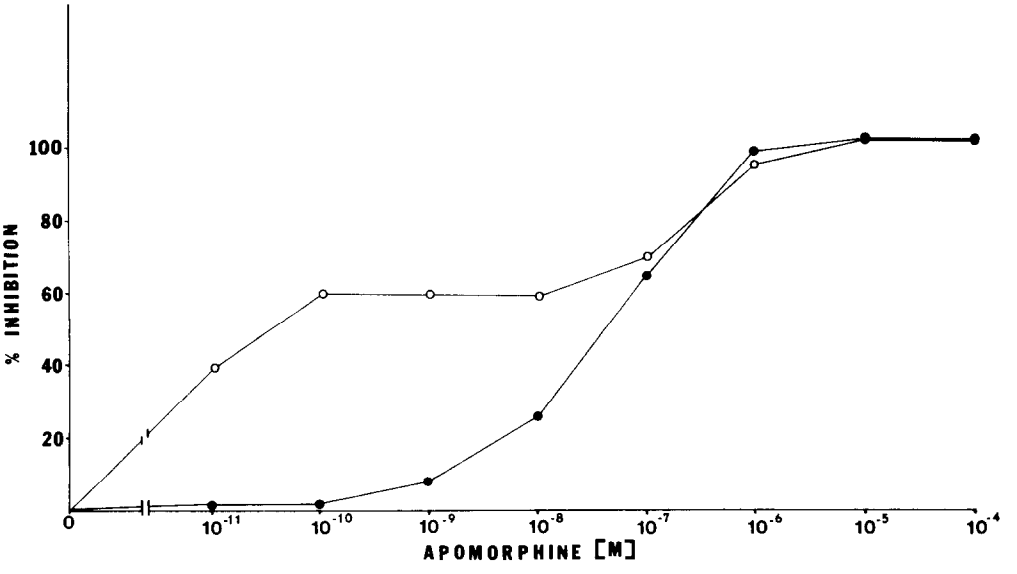

Fig. 2. Competitive binding of sites labeled with [^{3}H]NPNA by apomorphine. Occupancy concentration curve for apomorphine derived by inhibition of the specific binding of [^{3}H]NPNA. The closed circles (●—●) represent a theoretical profile for the simplest type of receptor binding, only one binding site. In this system, the ligand–receptor complex behaves as a homogenous system obeying mass action law where B_{max} equals that of the actual data generated. The open circles (○—○) represent the displacement of 3 nM [^{3}H]NPNA by the indicated concentrations of apomorphine, using the salt-treated extract of the striatal microsomes. The extract was prepared and the binding assay was performed as described in the Experimental Procedure. The data represent the mean from three separate experiments; the S.E.M. was less than 10 per cent.

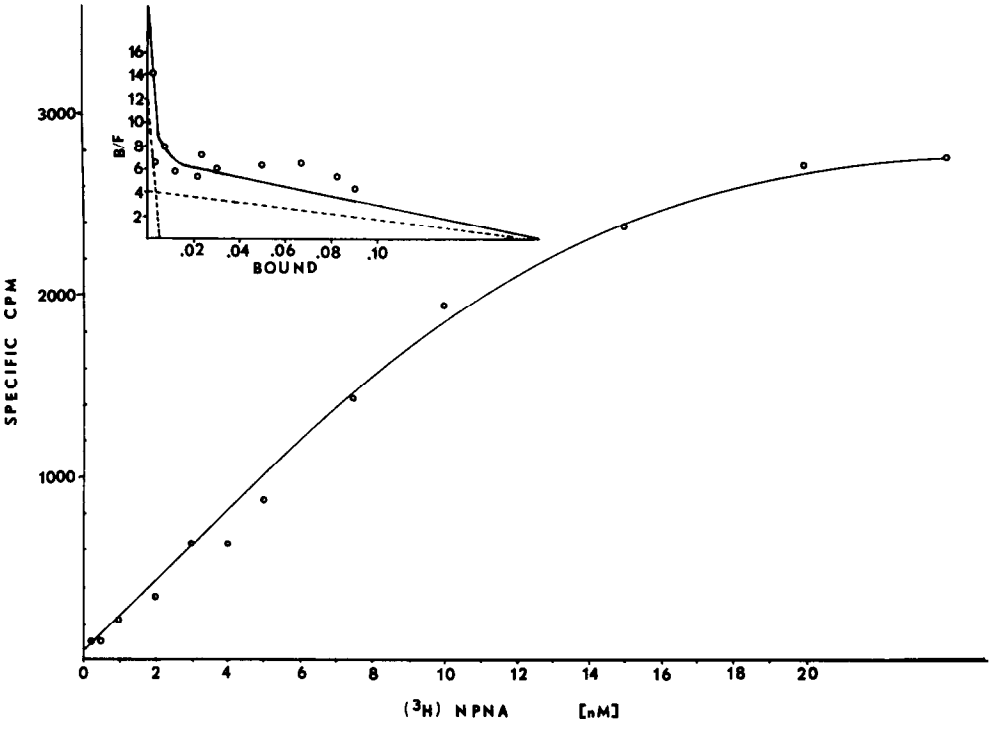

Fig. 3. Saturation curve for [^{3}H]NPNA binding to the salt extract of striatal microsomes. Non-specific binding was determined in the presence of 10^{-6} M apomorphine. Each concentration of ligand was assayed in triplicate. The data represent the mean from three experiments; the S.E.M. was less than 10 per cent. The inset is the Scatchard plot of the saturation curve.

Table 4. Calculated Hill coefficients for selected agonists and antagonists of the dopamine receptor

Test substance	[^{3}H]Spiroperidol	[^{3}H]ADTN	[^{3}H]NPNA
Apomorphine	0.65	0.60	1.08
ADTN	0.48	0.90	0.21
Epinine	0.50	0.87	0.30
Dopamine	0.50	0.72	0.25
(+)-Butaclamol	0.70	0.30	0.16
(−)-Butaclamol	1.00	0.60	0.50
Spiroperidol	0.98	0.45	0.38
(+)-Sulpiride	0.75	0.17	0.60
(−)-Sulpiride	1.00	0.80	0.88
(α)-Fluphenthixol	0.85	2.00	1.60
(β)-Fluphenthixol	1.00	2.20	2.00
Chlorpromazine	1.02	0.50	0.60
Fluphenazine	1.00		2.00
Domperidone	1.00		1.12
Clozapine	1.00		0.70

* Hill plots were generated from the results of displacement experiments, in which a fixed concentration of radiolabeled ligand was incubated with increasing concentrations of unlabeled ligands. Seven different concentrations of each unlabeled ligand were used to generate a displacement curve. The specific radioligand binding (total minus blank) occurring in the absence of any displacing agent was taken as the B_{max}. Binding occurring in the presence of displacing agents was expressed as a percentage of B_{max}. The data were plotted as

$$\log \frac{\%B_{max}}{100\% - \%B_{max}}$$

vs log (displacer), to yield a Hill plot. The Hill coefficient which appears in the table represents the slope of such a line. The S.E.M. for all members replotted was 10 per cent or less. The blanks used for [^{3}H]spiroperidol, [^{3}H]ADTN and [^{3}H]NPNA were (+)-butaclamol, ADTN and apomorphine, respectively, at concentrations of 10^{-6} M.

saturation curve for specific binding, as shown in Fig. 3. Scatchard analysis of the data reveals the presence of at least two binding sites, a high affinity site with a K_d of 0.01 nM and a low affinity site with a K_d of 20 nM.

The use of Hill plots is another means of detecting deviations from mass action. As seen in Table 4, the slopes for the competitive displacement of [^{3}H]NPNA by selected agonists are less than one. Similar results were obtained with agonist displacement of the antagonist ligand, [^{3}H]spiroperidol. The values are close to unity for antagonist displacement of [^{3}H]spiroperidol which suggests that these agents were displacing the labeled compound from only one site. The Hill coefficients for the displacements by agonists of sites labeled with [^{3}H]ADTN are about one and are less than one for all the antagonists except for flupenthixol. The calculated value for flupenthixol is greater than one for both [^{3}H]ADTN and [^{3}H]NPNA competitive binding. One explanation for such flat Hill plots is that there may be some heterogeneity in the population of agonist binding sites.

Following gel filtration chromatography of the KCl-P_3 extract on Sephacryl S-200, three zones of specific [^{3}H]spiroperidol binding were observed, as determined by stereoselective ligand displacement using (+)- and (−)-butaclamol (Fig. 4B). The eluted fractions possessing specific spiroperidol binding activity had molecular weights of approximately 96,000 and 50,000 (zone I), 25,000 (zone II) and 13,000 (zone III), as calibrated for globular proteins (Fig. 4D). The rank order of potency for displacement of specific [^{3}H]spiroperidol binding for the zone I protein was (+)-butaclamol > promazine > apomorphine > dopamine >>norepinephrine >> serotonin, and the respective IC_{50} values for each compound were similar to those reported in Table 2. A less extensive series of experiments using a fixed concentration of antagonist (10^{-6} M) showed that the rank order of antagonist potency for zones II and III was (+)-butaclamol = fluphenazine = α-flupenthixol > promazine >> (−)-butaclamol. Multiple binding peaks for [^{3}H]apomorphine were also observed on the eluates chromatographed from the Sephacryl S-200 column (data not shown).

Chromatography of the dialyzed P_3-KCl extract on Sephadex G-100 revealed multiple peaks of specific binding for [^{3}H]spiroperidol and [^{3}H]apomorphine (Fig. 5). The multiple peaks shown

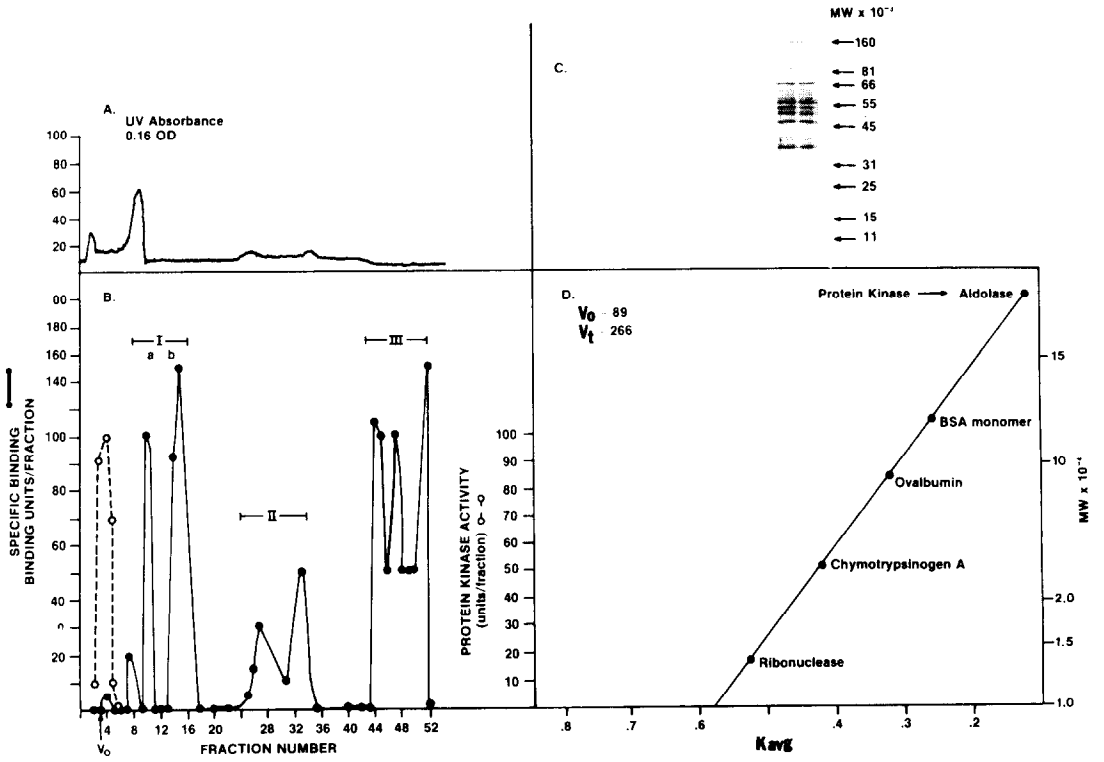

Fig. 4. Specific [^{3}H]spiroperidol binding. (A) Ultraviolet absorbance of the Sephacryl S-200 column effluent at a flow rate of 2.5 ml/min. The u.v. sensitivity was 0.160 AuFs. The chart speed was 6 inches/hr and the recorder setting was 5 mV. (B) Binding of 0.2 nM [^{3}H]spiroperidol detected in the presence of 10^{-7} M butaclamol. The column was run at 4° using 15 mM K_2HPO_4 (pH 7.4) containing 0.25 M sucrose as the mobile phase. The column length was 50 cm and the diameter was 2.6 cm (i.d.). Five-milliliter fractions were collected following the collection of 73 ml. A total of fifty-two 5-ml fractions were collected. The data for protein kinase are expressed as a percentage of maximum activity where 100 is equivalent to 1 unit/mg protein. (C) The SDS-PAGE of the soluble extract. The gel was run as described in the Experimental Procedure and represents a 10–15% gradient SDS gel. Molecular weight markers for the gel are indicated. (D) Molecular weight calibration of the Sephacryl S-200 column using standard molecular weight markers.

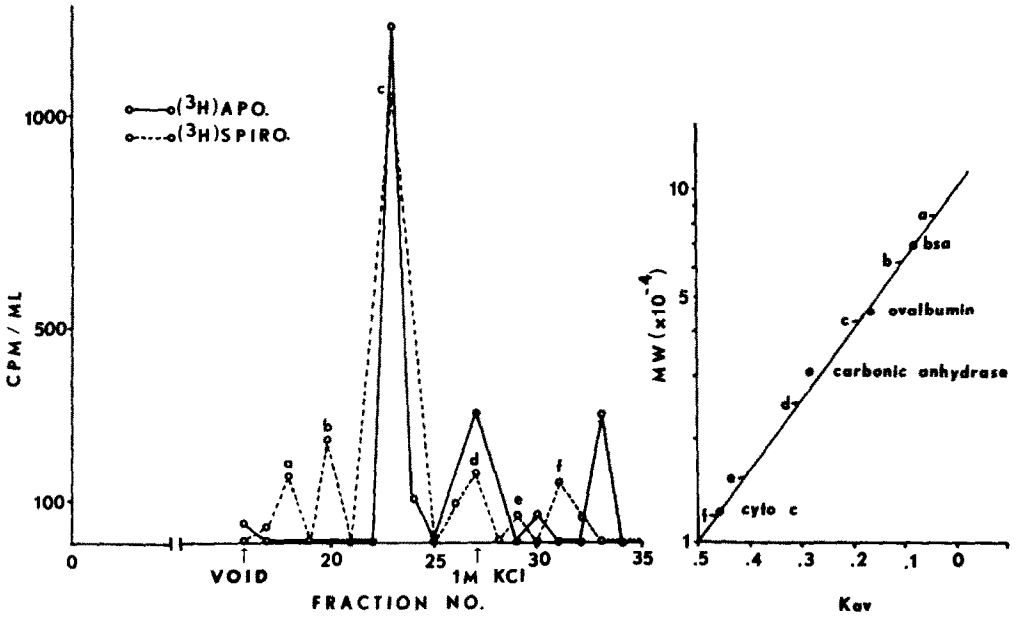

Fig. 5. Specific binding of [^{3}H]spiroperidol and [^{3}H]apomorphine. A microsomal KCl-treated extract, prepared as described in the Experimental Procedure was applied to a Sephadex G-100 column: 2-6 cm, bed length 38.7 cm. The void volume of the column was 80 ml, the bed volume was 265 ml, and the flow rate was 6 ml/hr. The column was equilibrated with 15 mM K_2HPO_4 and twenty-seven 4-ml fractions were collected using this buffer for elution. A solution of 1 M KCl, 15 mM K_2HPO_4 (pH 7.2) was then added to the column, and eight additional 4-ml fractions were collected. The eluates were assayed for [^{3}H]apomorphine binding (3 nM) and [^{3}H]spiroperidol binding (2 nM). Apomorphine 10^{-6} M was used to define specific binding for [^{3}H]apomorphine and (+)-butaclamol (10^{-6} M) was used for [^{3}H]spiroperidol. The data shown represent the specific binding of both of these ligands from a representative column run repeated three times. The molecular weight equilibration of this column using known molecular weight standards is shown on the right.

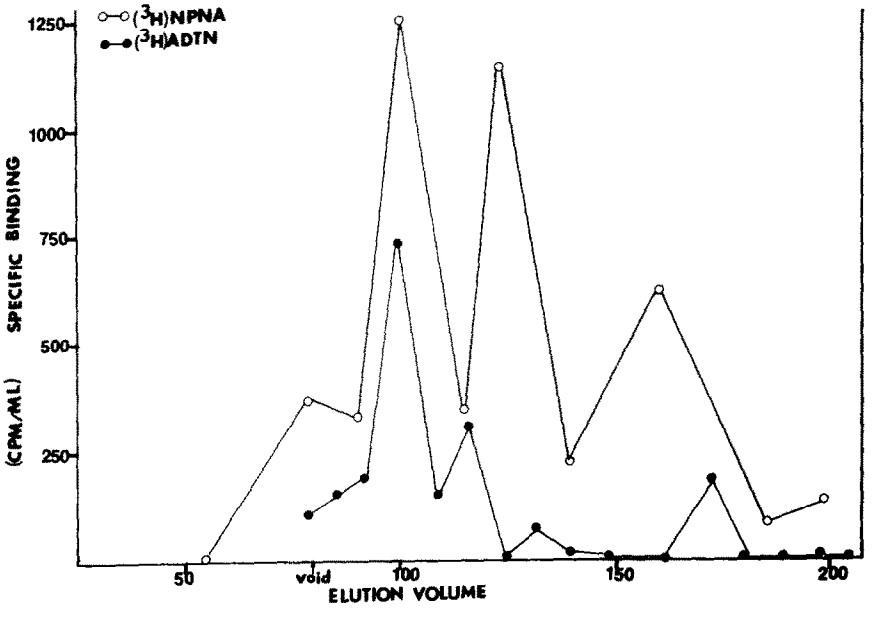

Fig. 6. Specific binding of [^{3}H]ADTN and [^{3}H]NPNA. A microsomal KCl-treated extract, prepared as described in the Experimental Procedure, was applied to a Sephadex G-100 column as described in the legend to Fig. 5. Fractions of 6 ml each were collected and assayed for specific binding, using [^{3}H]NPNA (0.3 nM) and [^{3}H]ADTN (1 nM). Apomorphine (10^{-6} M) and ADTN (10^{-6} M) respectively, were used to define the non-specific binding. The data shown represent specific binding from a representative column run repeated three times.

for [^{3}H]spiroperidol, a–f, were reproducibly observed. It is noteworthy that three of the column eluates showed exclusively antagonist binding. The profile for [^{3}H]apomorphine was similar to that obtained with [^{3}H]NPNA (Fig. 6). Three major peaks of binding sites which recognized both ligands are shown. There are some minor peaks for which only one ligand preferentially labeled a site. The largest peak of specific [^{3}H]apomorphine binding had a calculated molecular weight of approximately 50,000, which corresponds to peak Ib from the Sephacryl S-200 column. Also shown in Fig. 6 is the elution profile for specific binding sites labeled with [^{3}H]ADTN. The calculated molecular weight of the major peak labeled by [^{3}H]ADTN is about 50,000.

DISCUSSION

The biophysical and molecular characteristics of a receptor can be studied by measuring a variety of biochemical variables. Two such variables which have been successfully measured in an effort to characterize the dopamine receptor in the past include dopamine-sensitive adenylate cyclase activity and radioligand binding. However, because the receptor is membrane-bound, such measurements do not identify all of the components associated with the receptor site. For this purpose, a soluble macromolecule that specifically binds ligands that recognize the dopamine receptor would be more appropriate.

Attempts to solubilize and purify membrane proteins in aqueous buffers have successfully employed detergents, salts, chelating agents and organic solvents. Some receptor proteins are solubilized by more than one procedure. For example, the acetylcholine receptor can be extracted from membranes with either sodium chloride or detergents [18]. The data presented here demonstrate that potassium chloride can be used to extract a soluble dopamine receptor from partially purified membranes of the calf striatum. This receptor was found to be relatively thermostabile and to exhibit binding affinities for selected compounds similar to those reported for native membrane sites.

The results also demonstrate that multiple binding sites exist in the potassium chloride microsomal extract of the caudate nucleus. The saturation curve for the binding of the agonist [^{3}H]NPNA demonstrates at least two binding sites for this ligand. These results are corroborated by the finding that the Hill coefficients calculated for selected dopaminergic agonists in competitive binding experiments were less than one. Interestingly, the chromatographic analysis of these agonist binding sites on Sephadex G-100 indicates at least three specific binding sites for this ligand. Preliminary studies show that the rank order of potency for selected agonists and antagonists for each site is similar to that for the membrane-bound dopamine receptor. A more detailed study is in progress to evaluate each peak for the presence of high affinity and low affinity binding.

In summary, the finding of multiple agonist binding sites in a soluble extract of the caudate nucleus is consistent with the hypothesis that the dopamine receptor exists as a subpopulation of sites. The identification of a functional role for each binding site remains to be established.

REFERENCES

1. M. Titeler, P. Weinreich, D. Sinclair and P. Seeman, *Proc. natn. Acad. Sci. U.S.A.* **75**, 1153 (1978).
2. Y. C. Clement-Cormier and M. S. Abel, *Res. Commun. Chem. Path. Pharmac.* **22**, 15 (1978).
3. I. Creese and S. Snyder, *Eur. J. Pharmac.* **50**, 459 (1978).
4. I. Creese, R. Schneider and S. Snyder, *Eur. J. Pharmac.* **46**, 377 (1977).
5. Y. C. Clement-Cormier and R. George, *Life Sci.* **23**, 539 (1978).
6. J. Z. Fields, T. Reisine and H. I. Yamamura, *Brain Res.* **136**, 587 (1977).
7. J. E. Leysen, W. Gommeren and P. M. Laduron, *Biochem. Pharmac.* **27**, 307 (1978).
8. Y. C. Clement-Cormier and R. George, *J. Neurochem.* **32**, 1061 (1978).
9. J. W. Kebabian and D. B. Calne, *Nature, Lond.* **277**, 93 (1979).
10. A. R. Cools, H. A. J. Struyker Boudier and J. M. Van Rossum, *Eur. J. Pharmac.* **37**, 283 (1976).
11. J. R. Walters and R. H. Roth, *Naunyn-Schmiedeberg's Archs Pharmac.* **296**, 5 (1976).
12. B. S. Bunney, G. K. Aghajanian and R. H. Roth, *Nature New Biol.* **245**, 123 (1973).
13. H. Gorissen and P. M. Laduron, *Life Sci.* **23**, 575 (1978).
14. Y. C. Clement-Cormier and P. E. Kendrick, *Biochem. Pharmac.* **29**, 897 (1980).
15. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
18. P. Alberts and T. Bartfai, *J. biol. Chem.* **251**, 1543 (1976).