J. Pharm. Pharmacol. 1985, 37: 320–328
Received December 12, 1984

Properties of rat striatal D-2 dopamine receptors solubilized with the zwitterionic detergent CHAPS

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The specific binding of [^{3}H]spiperone was determined in membrane preparations of rat striatum and following solubilization treatment with the zwitterionic detergent CHAPS. Membrane protein solubilization was confirmed by ultrafiltration, gel filtration and increased heat sensitivity. Specific binding of [^{3}H]spiperone to the solubilized preparation was saturable and of high affinity, although solubilization led to an approximate 10 fold decrease in receptor affinity for [^{3}H]spiperone. The drug displacement profile of binding to the CHAPS solubilized preparation corresponded to that of the dopamine D-2 receptor; binding was stereoselectively displaced by the isomers of butaclamol. The sodium dependence of sulpiride displacement of specific [^{3}H]spiperone binding was retained in the CHAPS solubilized preparation. GTP (100 μM) only altered the ability of dopamine to displace [^{3}H]spiperone binding to the solubilized preparation in the presence of 120 mM sodium chloride. The GTP effect was small compared with that observed in the membranes. Specific [^{3}H]spiperone binding sites in the solubilized preparation were preferentially retained by a wheat germ agglutinin affinity column and subsequently eluted with *N*-acetyl-D-glucosamine. Gel filtration of the solubilized preparation using a Sepharose column resulted in two peaks of specific [^{3}H]spiperone binding, the larger component had a Stokes radius of 7·7 nm. CHAPS treatment of rat striatal membranes results in solubilization of the D-2 receptor in an active form. The D-2 site appears to be a glycoprotein of high molecular weight.

Brain dopamine receptors can be divided into those linked directly to adenylate cyclase (D-1) and those which are either negatively or not directly linked to this enzyme (D-2). A variety of sub-classifications of this latter receptor population have been proposed (agonist and antagonist sites; D-2 and D-4; D-2_H, D-2_L) (see Seeman 1980; Grigoriadis & Seeman 1984) but there is no clear indication of whether such binding sites represent molecular entities which can be defined as true receptors.

The striatal D-2 dopamine receptor may mediate many of the clinical and behavioural effects of neuroleptic drugs (Seeman 1980). This receptor can be identified in-vitro by the specific binding of dopamine antagonist ligands such as [^{3}H]spiperone. The molecular characteristics of the D-2 receptor remain unknown and a necessary prerequisite for appropriate study is the solubilization of the receptor in an active form. Solubilization of the striatal D-2 receptor sites identified using [^{3}H]spiperone and [^{3}H]sulpiride from canine (Gorissen & Laduron 1979; Leff & Creese 1982; Templeton & Woodruff 1983), bovine (Kuno et al 1983; Hall et al 1983; Lew & Goldstein 1984) and human brain (Davis et al 1981; Berrie et al 1983) has been achieved using a variety of detergents.

Initial attempts to solubilize D-2 receptor binding sites from rat striatum using digitonin resulted in the solubilization of a site exhibiting a high affinity for [^{3}H]spiperone (Gorissen & Laduron 1978). However, neither dopamine agonist nor antagonist drugs displaced [^{3}H]spiperone with high affinity; only structural analogues of spiperone were effective suggesting this to be a spirodecanone site (see Leysen & Gommeren 1978). However, when spirodecanone sites were masked using R 5260 a high affinity dopamine receptor binding site was revealed (Gorissen et al 1980). More recently, the zwitterionic detergent CHAPS (Hjelmeland 1980) was shown to solubilize a specific [^{3}H]spiperone binding site from rat striatal membranes that resembled the membrane bound D-2 receptor (Lew et al 1981). Thus the binding of [^{3}H]spiperone to the soluble receptor was displaced stereoselectively by the isomers of butaclamol and by nanomolar concentrations of dopamine antagonist drugs but micromolar concentrations of apomorphine and dopamine.

We now report on the initial characterization of the specific binding site for [^{3}H]spiperone in CHAPS solubilized preparations of rat striatal tissue.

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MATERIALS AND METHODS

The zwitterionic detergent CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonate) was obtained from Calbiochem. [^{3}H]Spiperone (16·7 Ci mmol^{-1}) was supplied by Amersham International, wheat germ agglutin-ultragel by LKB and Sepharose CL-4B by Pharmacia. Dopamine hydrochloride, noradrenaline bitartrate, 5-hydroxytryptamine hydrochloride and guanosine triphosphate (GTP) were supplied by Sigma Chemical Co. The following drugs were employed: 2-amino-6,7-dihydro-1,2,3,4-tetrahydronaphthalene (6,7-ADTN; Wellcome Research Laboratories), (+)- and (−)-butaclamol hydrochloride (Ayerst Laboratories), *cis*-flupenthixol hydrochloride (Lundbeck; Copenhagen), haloperidol and ketanserin (Janssen Pharmaceutica, Belgium) and (±)-sulpiride (Delagrange, France).

Preparation of striatal membranes

Female Wistar rats (150 ± 10 g; Bantin & Kingman Ltd) were stunned and decapitated, the brains removed and the paired corpora striata rapidly dissected into 10 volumes of ice-cold 50 mM Tris (hydroxymethyl)aminoethane hydrochloride (Tris-HCl) at pH 7·4. Paired tissue from 10–20 animals was homogenized using a Polytron homogenizer (setting 5 for 10 s) and centrifuged at 48 000*g* for 10 min in a Sorvall RC5B centrifuge at 4 °C. The resulting pellet was resuspended in 10 volumes of the same buffer and the process repeated. The final pellet was resuspended in 270 volumes of Tris-HCl buffer.

Soluble preparations

Initially striatal membranes were prepared as described above except that tissue was homogenized in 10 volumes of ice-cold 50 mM Tris HCl buffer containing 1 mM ethylenediaminetetraacetic acid (pH 7·4; Tris-EDTA). The final membrane pellet was resuspended in 10 volumes of ice-cold Tris-EDTA buffer containing 5 mM CHAPS and 1 mM dithiothreitol (pH 7·4) and left on ice for 60 min. After this period the resulting mixture was centrifuged at 180 000*g* for 60 min at 4 °C using an MSE SS50 centrifuge. The supernatant was carefully removed and retained on ice for the assay of solubilized receptor sites.

[^{3}H]Spiperone binding to membrane and solubilized preparations

For the determination of specific binding to membrane preparations, aliquots (900 µl) of the final tissue suspension were incubated with 50 µl of a solution of a displacing agent or its vehicle and 50 µl of a solution of [^{3}H]spiperone. For the determination of IC50 values a single final ligand concentration of 0·1 nM was employed. For kinetic analysis six ligand concentrations from 0·01–1·0 nM were used. Specific binding was routinely defined by the incorporation of 3×10^{-5} M (±)-sulpiride. This was justified in control experiments (not presented) where displacement of [^{3}H]spiperone binding by (±)-sulpiride was maximal at 3×10^{-5} M; Eadie-Hofstee analysis of data suggested a single site for specific binding of [^{3}H]spiperone defined at this concentration of sulpiride. All samples were examined in triplicate at each ligand or displacing drug concentration. Samples were incubated for 10 min in a shaking water bath at 37 °C following which the reaction was terminated by rapid vacuum filtration through Whatman GF/C filters over Millipore 3025 manifolds at 50 cm Hg vacuum. Filters were washed immediately with 2 × 5·0 ml of cold buffer. Filters were then placed with 5 ml of Packard ES299 scintillation cocktail and left overnight before counting in a Packard 460C scintillation spectrometer at an efficiency of approximately 45%.

For assay of the solubilized receptor sites, aliquots (100 µl) of the solubilized striatal membrane preparation were added to microcentrifuge tubes (1·5 ml) that contained 300 µl of Tris-EDTA buffer, 50 µl of a solution of [^{3}H]spiperone and 50 µl of a solution of displacing agent or its vehicle. In some experiments the Tris-EDTA buffer also contained 120 mM sodium chloride. For the determination of IC50 values a final ligand concentration of 0·35 nM was employed. For kinetic analysis six ligand concentrations between 0·2 and 8 nM were used. Non-specific binding was routinely determined by the incorporation of 3×10^{-5} M (±)-sulpride; specific binding was defined as total minus non-specific. All samples were examined in triplicate at each ligand or displacing drug concentration. Samples were left on ice for 2 h after which 100 µl of a solution containing 10%(w/v) activated charcoal and 2% (w/v) bovine serum albumin was added to the samples and mixed. Free ligand was adsorbed onto the charcoal, and the tubes were centrifuged at room temperature for 1 min using a Beckman Microfuge B to produce a charcoal pellet. Samples (200 µl) of the resulting supernatant were added to 5·0 ml of Packard ES299 scintillation cocktail before counting as described above.

In some experiments, both soluble and membrane preparations were preincubated at 50 °C for 30 s–10 min and then returned to ice before the assay procedure for [^{3}H]spiperone binding. In other

experiments, soluble preparations of striatal membranes (0·7 ml) were filtered through Millipore HAWP 02400 0·45 μm filters under vacuum. The filters were washed three times with 0·7 ml of Tris-EDTA buffer. Specific [^{3}H]spiperone binding was then determined using the filtered preparations.

Gel filtration of CHAPS solubilized preparation

Samples (7·0 ml) of the CHAPS solubilized striatal membrane preparation were placed in Visking dialysis tubing and dialysed against a 500 fold excess of Tris-EDTA buffer overnight at 4 °C to remove excess detergent. The procedure was adopted to decrease the possibility of the incorporation of soluble receptor sites into micelles. Sample volume was reduced to approximately 1 ml by further dialysis against polyethyleneglycol 8000. The resulting solution was applied to a Sepharose CL-4B column (1·5 × 88 cm) previously equilibrated with Tris-EDTA buffer at 4 °C. Solutions of standard proteins (aldolase, catalase, ferritin and thyroglobulin) of known Stokes radii were applied separately.

The column was perfused with Tris-EDTA buffer at approximately 15 ml h^{-1} at 4 °C and the eluate collected in 100 drop fractions. Fractions from solubilized preparations were examined for the presence of specific [^{3}H]spiperone binding as described above. The protein content of samples obtained using standard protein and solubilized preparations was determined by the Bio-Rad protein assay technique (Bradford 1976).

Adsorption to wheat germ agglutinin affinity columns

Samples (7·0 ml) of the CHAPS-solubilized preparation were placed in Visking dialysis tubing and dialysed against a 500 fold excess of the Tris-EDTA buffer overnight at 4 °C. The resulting solution was applied to a column (0·9 × 16 cm) containing wheat germ agglutinin ultragel previously equilibrated to Tris-EDTA buffer. The column was then washed with Tris-EDTA buffer at approximately 10 ml h^{-1} until no further protein was present in the eluate. Tris-EDTA buffer containing 0·15 M *N*-acetyl-D-glucosamine was then passed through the column to displace bound protein. Samples were examined for specific [^{3}H]spiperone binding and protein as described above.

Data calculation

A computer curve fitting program (minimization of weighted sum of squares by an iterative process) was used for calculation of the kinetics of binding by fitting to the equation: $b = B_{max}/[1 + (K_d/F)]$ where b is the amount bound, K_d the affinity constant, B_{max} the amount bound at infinite ligand concentration and F the free (unbound) ligand.

RESULTS

Exposure of striatal membranes to 2·5 and 5·0 mM CHAPS increased the percentage of membrane proteins solubilized but at higher concentrations only a slight increase was observed (Table 1). The specific binding of [^{3}H]spiperone to the soluble fraction also increased markedly between 2·5 and 5·0 mM CHAPS. Specific binding of [^{3}H]spiperone remained stable between 5·0 and 20 mM CHAPS but at 40 mM specific binding was no longer measurable. The specific binding of [^{3}H]spiperone (as defined using 3 × 10^{-5} sulpiride) was routinely 60–70% of total binding. No specific binding of [^{3}H]spiperone was observed when similarly treated preparations of rat cerebellar tissue were employed.

Table 1. The effect of varying the concentration of CHAPS on protein solubilized and specific [^{3}H]spiperone (0·35 nM) binding. The results are those from a single experiment which was performed in triplicate at each CHAPS concentration as described in methods.

Concn of of CHAPS (mM)	% Starting protein solubilized	Specific [^{3}H]spiperone binding (a) pmol (g tissue)$^{-1}$	(b) fmol (mg protein)$^{-1}$
2·5	8·6	0·526	102
5·0	23·1	1·96	141
10	23·8	1·95	136
20	24·5	1·89	128
40	27·7	0	0

Following filtration of the CHAPS solubilized striatal extract through Millipore HAWP 02400 filters the specific binding of [^{3}H]spiperone was identical to that obtained in the initial preparation. This procedure resulted in a 98% recovery of binding sites.

Preincubation of striatal membranes at 50 °C for up to 10 min resulted in minimal loss of specific [^{3}H]spiperone binding. In contrast, preincubation of a (5·0 mM CHAPS) solubilized preparation led to a complete loss of specific [^{3}H]spiperone binding within 5 min (Fig. 1).

Kinetics of specific [^{3}H]spiperone binding to the soluble and membrane preparation

Specific [^{3}H]spiperone binding to both membrane and soluble preparations appeared saturable and of high affinity (Fig. 2A, B). Computer curve fitting of the data (Table 2) revealed that the apparent dissociation constant (K_d) was increased about

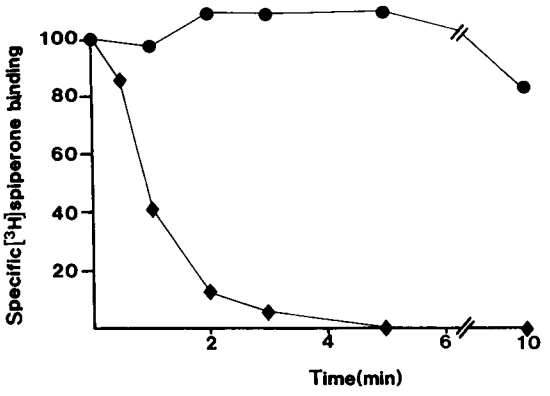

FIG. 1. The effect of preincubation at 50 °C for 30 s–10 min on the specific binding of [^{3}H]spiperone to membrane (0·1 nM) (●) and CHAPS solubilized (0·35 nM) (◆) preparations of rat striatal tissue. Specific binding was defined as total minus non-specific (in the presence of 3×10^{-5} M (±)-sulpiride), each performed in triplicate. The standard is presented as percent of total specific binding. Standard errors are within the size of the points.

ten-fold following solubilization. The density of specific [^{3}H]spiperone binding sites (B_{max}) following solubilization was approximately 28% of that found in the membrane preparation. A similar percentage (23·1 ± 0·5%) of total membrane protein was recovered following solubilization.

Drug displacement profile of the membrane and soluble [^{3}H]spiperone binding site (Fig. 3, Table 3) Specific [^{3}H]spiperone binding to the membrane and CHAPS solubilized site was stereoselectively displaced by the isomers of butaclamol. In the membrane preparation there was an approximately 5000 fold difference between the isomers but this was reduced to a 1250 fold difference in the CHAPS solubilized preparation. Other dopamine antagonist drugs (haloperidol, sulpiride and *cis*-flupenthixol)

Table 2. The kinetics of specific [^{3}H]spiperone binding to the membrane and CHAPS-solubilized preparations of rat striatum. The kinetics of binding were calculated by computer curve fitting (as described in methods). The results are mean ± s.e.m. of at least three independent experiments, all performed in triplicate. Non-specific binding was determined by the incorporation of 3×10^{-5} M (±)-sulpiride in the assay. Experiments were performed with six different concentrations of [^{3}H]spiperone (membrane, 0·01–1 nM, solubilized 0·2–8 nM).

	Membrane preparation	Soluble preparation	% recovery
K_D (nM)	0·04 ± 0·007	0·525 ± 0·02	—
B_{max} (pmol (g tissue)$^{-1}$)	20·62 ± 1·62	5·88 ± 0·51	28·5
B_{max} (fmol (mg protein)$^{-1}$)	362·1 ± 28·4	445·1 ± 38·6	—

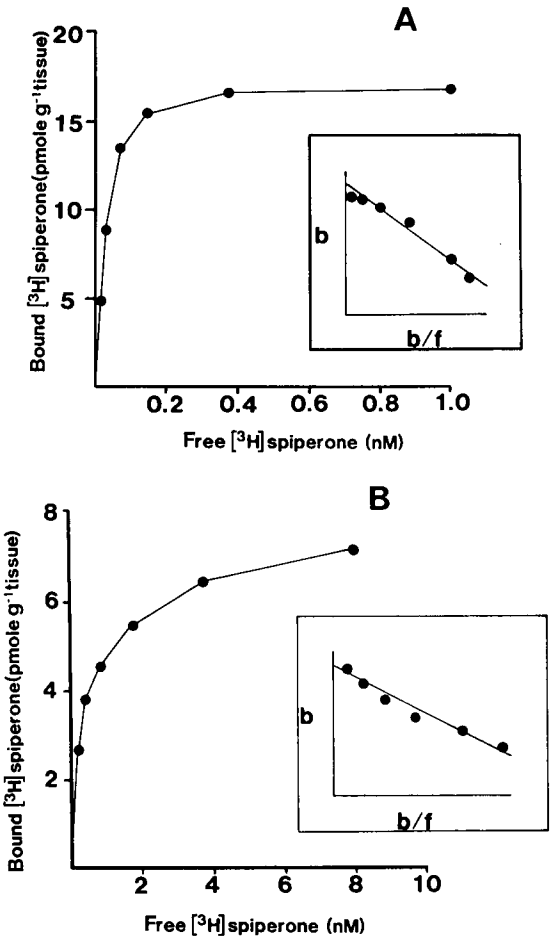

FIG. 2. Saturation analysis of specific [^{3}H]spiperone binding to (A) the membrane and (B) CHAPS solubilized preparations of rat striata. The data shown is from a single experiment; non-specific binding was determined with 3×10^{-5} M (±)-sulpiride, specific binding was defined as total-non-specific, each performed in triplicate. Inset shows Eadie-Hofstee analysis of the data.

also potently displaced [^{3}H]spiperone binding to both preparations. Dopamine and ADTN, but not 5-HT or noradrenaline, displaced [^{3}H]spiperone in both preparations. Ketanserin, a 5-HT_2 antagonist, displaced [^{3}H]spiperone binding in both soluble and membrane preparations in high nanomolar concentrations.

Most drugs showed a lower affinity to displace [^{3}H]spiperone binding following solubilization. This ranged from a 60 fold loss in affinity for (+)-butaclamol to a 5 fold loss for *cis*-flupenthixol. However, sulpiride was more potent in displacing [^{3}H]spiperone binding from the CHAPS solubilized site than from membrane preparations.

The effects of sodium ions and GTP on the displacement of [³H]spiperone binding

The ability of (±)-sulpiride to displace specific [³H]spiperone binding to the membrane preparation was increased approximately 10 fold if NaCl (120 mM) was added to the incubation buffer (Table 3). In the CHAPS solubilized preparation the affinity of (±)-sulpiride for the site labelled by [³H]spiperone also increased by almost 10-fold if NaCl (120 mM) was included in the assay medium.

The ability of dopamine to displace specific [³H]spiperone binding to the CHAPS solubilized preparation was not altered by the inclusion of GTP (100 μM) into the incubation buffer (Table 4). However, in the presence of sodium chloride (120 mM) the inclusion of GTP (100 μM) caused a small but reproducible decrease in the ability of dopamine to displace specific [³H]spiperone binding. Under similar conditions in the membrane preparation the decrease in dopamine affinity was 3–4 fold (Kilpatrick, Jenner & Marsden, unpublished observations).

Sepharose column chromatography

The CHAPS solubilized striatal preparation was applied to a Sepharose CL-4B column following concentration by dialysis. Elution with Tris-EDTA buffer showed specific [³H]spiperone binding to elute in 2 peaks (Fig. 4). The major peak (71%, fractions 29–35) eluted between the peaks obtained with thyroglobulin and ferritin. Comparison of the K_{av} of this peak with the line obtained by least squares linear regression obtained from the K_{av} of proteins of known Stokes radii (thyroglobulin, ferritin, catalase and aldolase) gave a value of 7·7 ± 0·28 nm (mean ± 1 s.e.m., n = 3) for the Stokes radius of the [³H]spiperone binding site. The minor peak (29%, fractions 19–26) eluted near the void volume. When compared with the original non-dialysed CHAPS preparation 66 ± 3% of specific [³H]spiperone binding sites were recovered from the Sepharose column.

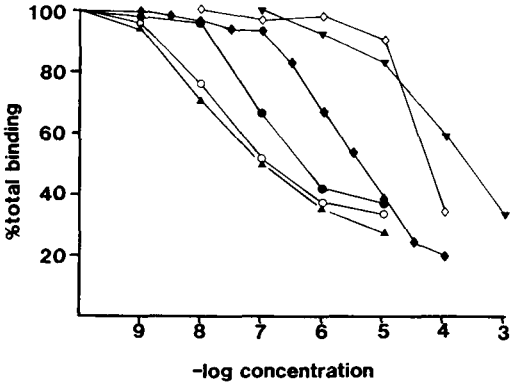

FIG. 3. Drug displacement of [³H]spiperone (0·35 nM) binding to the CHAPS solubilized preparation of rat striatal tissue. The data shown is from one experiment, each point is the mean of triplicate determination carried out at each concentration of displacing drug. Total binding is calculated after subtraction of background counts. (▲), (+)-butaclamol; (○) *cis*-flupenthixol; (●), (±)-sulpiride; (◆), ketanserin; (◇), (−)-butaclamol; (▼), dopamine.

Table 3. Affinity of drugs for displacing [³H]spiperone (0·1–0·35 nM) binding to membrane and CHAPS solubilized preparations of rat striatum. IC50 values were defined as the concentration of drug giving 50% displacement of specific binding. The results are the mean of at least 3 separate experiments each performed in triplicate at each drug concentration. At least 4 different concentrations of each drug were (from 10^{-9}–10^{-4} M for soluble preparation and 10^{-11}–10^{-5} M for membrane preparation). Standard errors of the mean were all within 30%.

Drug	IC50 (nM) Membrane preparation	Soluble preparation
(+)-Butaclamol	0·56	20·0
(−)-Butaclamol	2660	25 500
Haloperidol	0·60	19·7
cis-Flupenthixol	5·1	16·7
(±)-Sulpiride	1750	230
(±)-Sulpiride (+NaCl)	158	25·5
ADTN	125	886
Dopamine	6600	47 000
Noradrenaline	$>10^{-5}$	$>10^{-4}$
5-HT	$>10^{-5}$	$>10^{-4}$
Ketanserin	170	1200

Wheat germ agglutinin affinity chromatography

CHAPS solubilized preparations of rat striatum were applied to a wheat germ agglutinin agarose column (Fig. 5). Following washing with Tris-EDTA buffer a retention of 37·8 ± 2·0% (mean ± s.e.m., n = 4) of specific [³H]spiperone binding sites was achieved, which were subsequently eluted by the addition of buffer containing 0·15 M *N*-acetyl-D-glucosamine. 15·7 ± 0·35% (mean ± s.e.m., n = 4) of protein was retained and subsequently eluted. Therefore the [³H]spiperone binding site was preferentially retained on this affinity column, resulting in a 2·4 fold purification of the receptor.

DISCUSSION

The data reported in this work suggest that solubilization of the dopamine D-2 receptor identified by

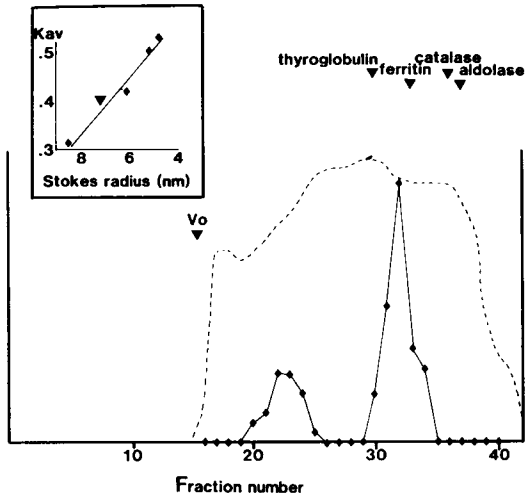

FIG. 4. Sepharose column chromatography of the CHAPS solubilized preparation from rat striata. 100 Drop fractions were collected and assayed for [^{3}H]spiperone binding (——) and protein content (– – –) as described in the text. Marker proteins were chromatographed separately under the same conditions. The inset shows the K_{av} of the marker proteins plotted against the Stokes radius (nm). The K_{av} was calculated as $(V_e - V_o)/(V_t - V_o)$ where V_e = elution volume of peak, V_o = void volume of column and V_t = total volume of gel bed. In this experiment a Stokes radius of 7·2 nm was found for the specific [^{3}H]spiperone binding site.

the specific binding of the [^{3}H]spiperone from rat striatum may be successfully achieved using the zwitterionic detergent CHAPS.

Clearly it is necessary to confirm that the detergent treatment results in the production of a soluble receptor preparation rather than a suspension of small membrane particles. Solubilization was confirmed using criteria described by Laduron & Ilien (1982): (a) the specific [^{3}H]spiperone binding sites did not sediment after centrifugation of the detergent treated preparation for 1 h at 180 000*g* in a low density medium (Tris-EDTA buffer), (b) specific [^{3}H]spiperone binding sites were not retained on small pore size filters, (c) the greater thermolability of the [^{3}H]spiperone binding site following solubilization, and (d) elution of the majority of specific [^{3}H]spiperone binding sites between two soluble proteins (thyroglobulin and ferritin) following application of the CHAPS-treated preparation to a Sepharose column.

The specific binding of [^{3}H]spiperone to CHAPS solubilized preparations was saturable and of high affinity, accounting for some 60–70% of total binding. The use of CHAPS resulted in a 30% recovery of membrane bound binding sites for [^{3}H]spiperone. The results compare well with the use of CHAPS to solubilize dopamine receptors in other species (Templeton & Woodruff 1983: Kuno et al 1983), and with the ability of CHAPS to solubilize [^{3}H]spiperone binding sites from rat striatum (Lew & Goldstein 1984). Other detergent treatments, such as cholate plus sodium chloride may result in higher recoveries of [^{3}H]spiperone binding sites of approximately 50–60% from bovine tissue but we have not found such treatments effective in the rat (unpublished data). Compared with other detergents, the high ratio of specific to non-specific binding in the CHAPS solubilized preparation is an advantage.

Table 4. The effect of inclusion of GTP (100 μM) on the ability of dopamine to displace [^{3}H]spiperone binding to the CHAPS solubilized preparation of rat striata. IC50 values and Hill coefficients were calculated from Hill plots of the data. The final concentration of GTP used was 100 μM in the presence and absence of NaCl (120 mM). Each value is the mean ± 1 s.e.m. of at least 3 independent experiments each carried out in triplicate with at least 10 different concentrations of dopamine (10^{-3}–10^{-7} M).

		IC50 μM	Hill coefficient
−NaCl	−GTP	47·0 ± 3·0	0·91 ± 0·03
	+GTP	47·6 ± 4·0	0·85 ± 0·08
+NaCl	−GTP	51·8 ± 3·3	0·97 ± 0·05
	+GTP	69·2 ± 3·0*	1·05 ± 0·05

* $P < 0·01$ compared to absence of GTP, Student's *t*-test.

Previous attempts to solubilize the rat D-2 striatal receptor using digitonin resulted in the solubilization of both D-2 sites and a high concentration of spirodecanone sites which masked the dopamine receptor. In contrast CHAPS treatment does not appear to result in a similar degree of solubilization of spirodecanone sites. Thus, a variety of structurally unrelated dopamine active drugs, including the specific D-2 antagonist sulpiride, displaced 60–70% of the total binding of [^{3}H]spiperone to the CHAPS-solubilized preparation. The involvement of a pharmacologically select site was shown by the stereoselectivity of displacement by the isomers of butaclamol and the close similarity of the rank order of displacing potency for a range of drug compounds to that observed in the membrane preparation. Dopamine agonists showed a lower ability to displace [^{3}H]spiperone binding than antagonists. These characteristics are compatible with the solubilization

of the striatal D-2 receptor identified by the specific binding of [^{3}H]spiperone. In addition, no specific binding of [^{3}H]spiperone could be observed to solubilized proteins from cerebellar tissue, a region that is known to be almost devoid of dopamine receptors (Seeman 1980). We have recently demonstrated (unpublished data) the interaction of [^{3}H]spiperone with its specific binding site on the CHAPS-solubilized preparation to be reversible.

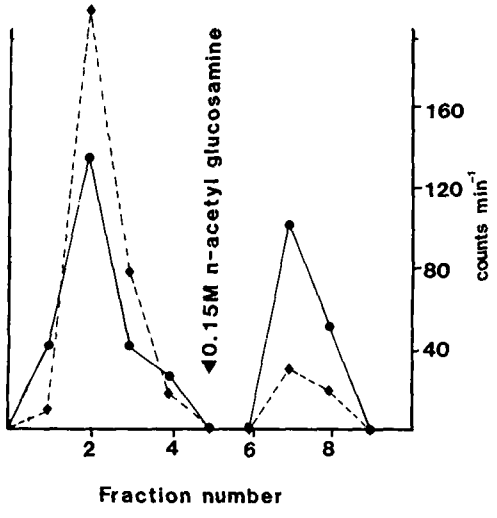

FIG. 5. Chromatography of the CHAPS solubilized striatal preparation on a wheat germ agglutinin-agarose affinity column (0·9 × 16 cm). 300 drop fractions were collected. Specific [^{3}H]spiperone binding (●—●) was determined on 400 µl samples of each fraction. The volume in the assay was corrected accordingly to 0·5 ml. Specific binding was defined as total-non-specific binding (in the presence of 3×10^{-5} M (±)-sulpiride) each performed in triplicate. Protein determinations (◆—◆) were performed as described in the methods section. After no further protein was present in the eluate (fraction 5) 0·15 M *N*-acetyl-D-glucosamine was added to the eluting buffer (Tris-EDTA) to elute protein which was bound to the column (fraction 6–9). Control experiments revealed that *N*-acetyl-D-glucosamine had no effect on the [^{3}H]spiperone binding or protein assay. The points are from a single experiment which was repeated 3 more times with similar results.

Most drugs showed a decreased ability to displace [^{3}H]spiperone from its specific binding site following CHAPS solubilization. The alteration of drug affinity was reflected by an increase in the K_D for [^{3}H]spiperone binding to the soluble preparation. The decrease in receptor affinity was observed previously for the D-2 receptor solubilized from other species using cholate plus sodium chloride (Hall et al 1983; Berrie et al 1983). Presumably this reflects the perturbation of the receptor environment incurred during detergent solubilization. An alternative explanation for the decrease in receptor affinity might be that CHAPS treatment leads to a partial denaturation. However, in recent experiments we have utilized [^{3}H]piflutixol to label CHAPS solubilized D-2 receptors and found no change in affinity following solubilization (unpublished data). So it appears more likely that the loss of affinity observed using [^{3}H]spiperone relates to alterations in receptor membrane environment.

An apparent exception to the decrease in drug affinity was sulpiride which showed an increased ability to displace [^{3}H]spiperone binding following CHAPS solubilization. There would appear to be two likely explanations for this change. Firstly, sulpiride is poorly lipid soluble, so some lipid barrier may prevent the access of sulpiride to the membrane bound receptors which may not be present in the soluble preparation. However, it may be that the different temperatures at which the assay for soluble and membrane bound receptors are carried out is responsible. Thus sulpiride displaces specific [^{3}H]spiperone with greater potency at 4 °C than at 37 °C in the membrane preparation (Kilpatrick, Jenner & Marsden, unpublished observation). The affinity of typical neuroleptics such as haloperidol and *cis*-flupenthixol is less dependent on temperature. Some changes in affinity do occur between 4 and 37 °C but the order of potency remains largely unaltered. It is necessary to assay the solubilized receptor at 4 °C rather than 37 °C because of its greater thermolability compared to membrane bound receptors.

Ketanserin, a 5-HT_2 antagonist (Leysen et al 1981) displaced [^{3}H]spiperone binding to both the membrane and soluble preparations only at high concentrations (10^{-7}–10^{-4} M). Recently, CHAPS solubilization of bovine striatal tissue was shown to result in binding of [^{3}H]spiperone to a 5-HT_2 site (Wheatley et al 1984; Kuno et al 1983). Indeed, in the present study a small component of [^{3}H]spiperone binding (<10%) was displaced at low concentrations of ketanserin (10^{-9}–10^{-7} M) (see Fig. 3). This may be [^{3}H]spiperone binding to 5-HT_2 receptors but a similar small decrease was also observed with sulpiride. Clearly no major 5-HT_2 component is revealed by ketanserin displacement. Indeed, 5-HT itself and noradrenaline did not displace 50% of specific binding to the solubilized preparation even at 10^{-4} M. This suggests that [^{3}H]spiperone binding is mainly to a dopamine receptor and not to 5-HT_2 or

α_1 sites with which [^{3}H]spiperone also will interact (Seeman 1980).

The routine use of sulpiride to define the specific binding of [^{3}H]spiperone to both membrane and soluble preparations ensured only dopamine receptor binding sites were investigated. Indeed, Eadie-Hofstee plots of specific [^{3}H]spiperone binding to membrane and soluble preparations were linear, suggesting a homogeneous population of binding sites.

In membrane preparations of rat striatum, sodium and GTP are implicated in changes in the affinity state of the D-2 receptor population (Grigoriadis & Seeman 1984). The interaction of sulpiride with its receptor is highly sodium-dependent. The effect of sodium ions to increase the ability of sulpiride to displace [^{3}H]spiperone occurs both in the membrane and CHAPS solubilized preparation. In membrane preparations both sodium and GTP are required to maximize the conversion of D-2_H to D-2_L states (Grigoriadis & Seeman 1984). It would seem that in the soluble preparation sodium chloride may allow the reassociation of receptors with the *N*-protein (Rodbell 1980). However, whilst the sodium shift of sulpiride displacement was maintained, GTP only altered dopamine displacement of specific [^{3}H]spiperone binding in the presence of sodium ions, and the effect was small. The explanation for the relative lack of effect of GTP in the soluble preparation may be in the extent to which coupling to the *N*-protein may occur following solubilization. Alternatively the affinity state of the receptor as controlled by sodium ions may be a critical factor. The ability of GTP to shift agonist displacement curves was reported previously for dopamine receptors labelled with [^{3}H]spiperone solubilized from bovine striatum using CHAPS (Kuno et al 1983). However, other groups using different detergents and striata from a variety of species have not reported this effect (Gorissen & Laduron 1979; Lerner et al 1981; Hall et al 1983). Why GTP shifts are evident following CHAPS solubilization is not clear but might suggest a functionally intact form of the receptor in such preparations.

It is possible to assess the Stokes radius of a protein by examining its retention on a calibrated gel filtration column. Gel filtration of the CHAPS solubilized preparation showed the eluate to contain two peaks of specific [^{3}H]spiperone binding. The major component was of a lower molecular weight (Stokes radius 7·7 nm); the minor component of higher molecular weight eluted near the void volume. What these different forms represent is not clear but the higher molecular weight peak might represent an association of receptor with other proteins. It is conceivable that the aggregation of receptor sites may have occurred during the column procedure. So interpretation of this data must be guarded. Indeed, while our findings are in agreement with previous estimates of the molecular size of the D-2 receptor (Kuno et al 1983; Hall et al 1983), others have reported much lower values (see Gorissen et al 1979; Lerner et al 1981).

A wheat germ agglutinin affinity column will bind carbohydrates containing an *N*-acetylglucosamine moiety. The ability of *N*-acetyl-D-glucosamine to elute fractions containing specific binding sites for [^{3}H]spiperone from a wheat germ agglutinin affinity column suggests that the rat D-2 receptor is probably a glycoprotein with *N*-acetylglucosamine in its carbohydrate moiety. This is in agreement with findings for the D-2 receptor from bovine striatum (Abbot & Strange 1984) and in a recent report by Lew & Goldstein (1984) for rat D-2 receptors.

Acknowledgements

This study was supported by the National Fund for Research into Crippling Diseases, the Wellcome Trust, the Medical Research Council and the Research Funds of the Bethlem Royal, Maudsley and King's College Hospitals.

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