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SOLUBILIZATION AND CHARACTERIZATION OF [^{3}H]SPIROPERIDOL BINDING SITES FROM SUBCELLULAR FRACTIONS OF THE CALF STRIATUM*

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Abstract—Specific [^{3}H]spiroperidol binding sites were obtained from subcellular fractions of the calf striatum in a solubilized form using salt extraction with potassium chloride. Binding activity was assayed by adsorption on Norit SGX to separate unbound radioactive ligand from the macromolecular ligand complex. The solubilized membrane preparation exhibited stereoselectivity for butaclamol with the (+)-enantiomer being the active agent. Neuroleptic drugs were found to have an affinity for the soluble preparation which was identical to that of the native membrane-bound receptor. The effects of various parameters on the binding of [^{3}H]spiroperidol to the soluble extract, including temperature, salt concentration, ionic strength and stabilizing agents, suggest that the binding site is biochemically stable. Electron micrographs of the soluble material reveal the absence of any recognizable membrane structures. Overall, these data demonstrate that a macromolecular component with high affinity and stereospecificity for [^{3}H]spiroperidol can be isolated from calf striatal synaptosomes and microsomes, and suggest that at least one of the receptors for dopamine may be an extrinsic protein.

Dopamine receptors have been shown to play an important role in brain function [1]. Several radiolabeled compounds have been demonstrated to be specific markers for the striatal dopamine receptor, including dopamine, apomorphine, haloperidol, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthalene (ADTN), and spiroperidol [2–10]. It has been suggested that the sites labeled by [^{3}H]apomorphine and [^{3}H]dopamine differ somewhat from those labeled by dopamine antagonists [3]. For example, the dopamine antagonist, spiroperidol, has been shown to bind to sites other than those associated with dopamine and its receptors in the frontal cortex [5]. However, in the striatum, [^{3}H]spiroperidol labels dopamine sites almost exclusively [5, 11].

The heterogeneity of the dopamine receptor has been reported by several investigators [5, 12–15]. Evidence suggests that dopamine binding sites exist in both the coupled and uncoupled state to adenylate cyclase (for review, see Ref. 16). The biochemical characterization and identification of the molecular nature of these receptors have been hindered because of an inability to obtain the receptors in a solubilized state. Recently, digitonin has been reported to solubilize dopamine receptors in the dog striatum [17]. The present study describes the use of salt to extract a soluble dopamine binding site from subcellular fractions of the calf striatum.

EXPERIMENTAL PROCEDURE

3-Hydroxytyramine was purchased from Cal-Biochem (San Diego, CA), [^{3}H]spiroperidol (19.6–26.4 Ci/mmole) from New England Nuclear (Boston, MA), unlabeled haloperidol from Janssen Pharmaceutical (Beerse, Belgium), (+)- and (−)-butaclamol from Ayerst Laboratories (Toronto, Canada), chlorpromazine from Smith, Kline & French (Philadelphia, PA), clozapine from Sandoz (Hanover, NJ), fluphenazine from Squibb (Princeton, NJ), and Norit SG extra from Sigma (St. Louis, MO).

For solubilization and binding studies, frozen calf brains were obtained from Pel Freeze (Rogers, AR). The caudate nuclei were dissected and frozen overnight at −20°. The tissue was homogenized in 10 vol. (w/v) of 0.32 M sucrose–2 mM Tris (hydroxymethyl) aminomethane maleate (pH 7.4). The crude synaptosomal (P_2) and microsomal pellets (P_3) were obtained as follows. The homogenate was centrifuged at 900 *g* for 10 min yielding a pellet, P_1 which was discarded, and a supernatant fraction S_1. The S_1 was centrifuged at 11,500 *g* for 20 min to yield the crude synaptosomal fraction, P_2 and a supernatant fraction S_2. The P_2 was washed with half of the original volume of the homogenizing buffer and recentrifuged for 20 min to yield the washed pellet, P_2', and a supernatant fraction S_2'. The S_2 and S_2' were combined and centrifuged at 100,000 *g* for 75 min to yield the microsomal pellet, P_3, and the supernatant fraction S_3.

For salt extraction, the P_2' and P_3 pellets were each resuspended in 0.25 M sucrose–15 mM Na_2HPO_4, pH 7.2, in a volume representing 20 per cent of the original starting volume plus 50% KCl (w/w), and were homogenized. Unless otherwise stated, the salt-treated homogenate was agitated at 4° by stirring for 16 hr. In some studies, the KCl-treated extract was allowed to stand on ice for 15 min. At the end of the indicated time period, the salt-treated homogenates of both the P_2 and P_3 pellets were centrifuged at 11,500 *g* for 10 min to remove excess salt. The supernatant fraction was then cen-

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trifuged at 100,000 *g* for 60 min. The resultant supernatant fraction was taken as the source for the soluble preparation. The binding site in the supernatant fraction was not retained by filtration through a 0.22 μm millipore filter. Routinely, KCl was not removed prior to assay for binding activity.

The standard assay mixture for binding studies (final volume 1.0 ml) contained in mmoles/liter: Tris–HCl (pH 7.4), 15; Na_4EDTA, 5; ascorbate, 1.1; pargyline, 0.1; [^{3}H]spiroperidol (0.2 nM); 800 μl of the soluble extract (400–600 μg protein); plus test substances as indicated. The reaction was routinely initiated by the addition of the soluble extract, and carried out for 16 hr at 4°. Initiation of the reaction with the addition of radioligand made no significant difference in binding activity compared to that initiated by the addition of the soluble extract.

For the termination of the binding assay, the separation of bound from free ligand was routinely accomplished by the addition of 100 μl of a charcoal slurry containing 2% bovine serum albumin (BSA) and 10% Norit SGX charcoal in a buffer of 50 mM Tris–8 mM theophylline–6 mM mercaptoethanol, pH 7.4. The tubes were allowed to remain in the presence of charcoal for 5 min at 0–4° and were then immediately centrifuged in a microfuge for 4 min. An aliquot of 400 μl was removed from each sample and placed in a scintillation vial in the presence of biofluor plus 1 ml ethanol and analyzed by liquid scintillation spectrometry, along with appropriate quenching standards. Results similar to those reported using the charcoal adsorption method were obtained by layering 0.2 ml of the incubation mixture onto a Sephadex G-50 (13 × 0.5 cm) column maintained at 4°. Fractions of four drops each were collected by eluting the column with 15 mM Na_2HPO_4 buffer, pH 7.2. The radioactivity was then measured by liquid scintillation spectrometry in a Searle Mark III.

Protein determinations were made using the assay of Lowry *et al.* [18] or Bradford [19].

For electron microscopy, drops of the salt extract were placed onto formvar films, dried, stained with lead citrate, and examined.

RESULTS

Treatment of the P_3 fraction with KCl released a membrane bound [^{3}H]spiroperidol binding site in a solubilized form. Solubilization with KCl was used routinely throughout the studies reported here. The solubilization of the [^{3}H]spiroperidol binding site appeared to be quite specific for this treatment, since a number of other detergents were ineffective in solubilizing the binding sites in an active form, including sodium dodecylsulphate (SDS), Triton X-100, 305, CF 21, Lubrol WX, Brij 35 and Brij W-1, all of which were tested at 0.1, 1 and 10% concentrations. These data do not rule out the possibility that variations in the concentration of detergent used along with other biochemical parameters, such as pH, ionic strength and stabilizing agents, may yield a preparation exhibiting specific binding activity. Digitonin at a concentration of 0.2% was tested and found to be effective in solubilizing a [^{3}H]spiroperidol binding site (data not shown). Similar results have been reported by others [20].

To verify the soluble state of the [^{3}H]spiroperidol binding site released by treatment with KCl, the solubilized preparation was subjected to a number of experimental tests, including ultracentrifugation, protein precipitation, dialysis, gel filtration, millipore filtration and electron microscopy. When the KCl extract was ultracentrifuged at 100,000 *g* for 2 hr, no sedimented material was observable, and after passage of the supernatant fraction through a 0.45 μm or 0.22 μm millipore filter, no decrease in binding activity was observed. Extensive dialysis of the KCl extract, followed by centrifugation at 100,000 *g* for 2 hr, did not alter binding activity, nor did filtration through BioGel P_2. In addition, electron microscopy indicated the total absence of any recognizable membrane vesicles or fragments in the solubilized preparation. Electron dense particles were observed, some of which aggregated during evaporation on formvar film to form an amorphous substance with an internal micro-lamellar-like pattern (data not shown). Similar structures have been observed with preparations of purified proteolipid, which have been

Table 1. Effect of KCl treatment of the striatal microsomal fraction*

Fraction	Total protein	fmoles bound	Specific activity	Increase
Homogenate	3500	17,500	4	1
P_3	400	16,000	40	10
KCl extract	70	4750	68	17
(1) Ammonium sulfate	0.5	85	170	43
(2) Polyethylene glycol	0.2	28	140	35

* Specific binding was defined as the difference between total and 10^{-7} M (+)-butaclamol. The concentration of [^{3}H]spiroperidol was 0.2 nM. The assay for binding activity was performed at 0°. (1) Precipitation of KCl extract with 10% ammonium sulfate. The pellet was resuspended in 15 mM KH_2PO_4 buffer and centrifuged at 100,000 *g*; the resultant supernatant fraction was used for measurement of binding activity. The data represent the mean from three separate experiments. (2) KCl extract was precipitated with 10% polyethylene glycol, pH 7.2. The pellet was resuspended and centrifuged as described for (1).

Table 2. Effects of various concentrations of KCl on [^{3}H]spiroperidol binding*

Condition	Total binding (fmoles/mg protein)	(+)-Butaclamol (fmoles/mg protein)	Specific binding (fmoles/mg protein)
50% KCl	28	25	3
100% KCl	31	26	5
250% KCl	35	30	5
500% KCl	118	76	42

* P_3 pellets were exposed to the indicated concentration of KCl for 15 min. The concentration of butaclamol used was 10^{-7} M. The assay for binding activity was carried out, as described in the Experimental Procedure, at 0° for 16 hr, using 0.2 nM [^{3}H]spiroperidol.

exposed to salt solutions [21] and have been reported for the soluble acetylcholine receptor [22].

From the data presented in Table 1, it is apparent that the use of KCl to extract a membrane-bound binding site did not greatly enhance the specific binding activity for [^{3}H]spiroperidol over that present in the P_3 fraction. Precipitation of the KCl extract with 10% ammonium sulfate, resuspension of the supernatant fraction in salt-free buffer, and centrifugation of the supernatant at 100,000 *g* resulted in a preparation exhibiting a 43-fold increase in the specific activity for [^{3}H]spiroperidol binding, compared to the crude homogenate. Exposure of the KCl extract to polyethylene glycol also resulted in an increase in specific [^{3}H]spiroperidol binding activity (Table 1).

The results of a study to determine the effective concentration of KCl for the extraction of [^{3}H]spiroperidol binding activity is shown in Table 2. In this experiment the microsomal pellet (P_3) of the calf striatum was resuspended in 15 mM Na_2HPO_4 buffer and divided into four aliquots, each of which was treated with KCl at a concentration of either 50% (w/w), 100% (w/w), 250% (w/w) or 500% (w/v) of the original starting material. The data demonstrate that a 15-min exposure of the P_3 to 500% KCl provided the highest number of total and specific [^{3}H]spiroperidol binding sites. However, the lowest concentration of KCl (50%) was determined to be almost as effective as the 500% KCl concentration, provided the exposure time was increased from 15 min to 16 hr. The results shown in Table 3 indicate that, in the presence of low KCl concentrations, the total binding sites for [^{3}H]spiroperidol increased by about 250 per cent when the exposure time was increased from 1 hr to 16 hr, with little change in the nonspecific binding. Whereas specific [^{3}H]spiroperidol binding was not detectable following a 1-hr exposure to 50% KCl, 55 fmoles/mg protein specific [^{3}H]spiroperidol binding sites were determined after overnight exposure to KCl. The substitution of NaCl for KCl in the solubilization procedure resulted in a larger increase in the absolute amount of membrane proteins solubilized compared to the KCl extract. However, the NaCl extract did not exhibit stereospecific displacement of sites labeled with [^{3}H]spiroperidol (Table 2). Specific binding activity was still not detected following overnight dialysis of the NaCl extract, prior to assaying for [^{3}H]spiroperidol binding sites (data not shown).

Time course experiments indicate that [^{3}H]spiroperidol binding reaches equilibrium in 10 min at 36°, in 30 min at 23° and in 16 hr at 0° (data not shown). Once equilibrium was reached, it was maintained for 1.5 hr at 37°, 3 hr at 23° and up to 5 hr (the longest time period tested) at 4°. The results shown in Fig. 1 indicate that, for a given concentration of [^{3}H]spiroperidol (0.2 nM), total specific binding obtained in the P_3 fraction was two and one-half times greater than that in the P_2 extract when assayed at 23° and 37°. Specific binding, the difference between total sites and those labeled in the presence of 10^{-7} butaclamol, was calculated to be five times higher in the P_3 compared to the P_2 extract at 23°,

Table 3. Effects of exposure time and salt concentration on [^{3}H]spiroperidol binding*

Salt	Conc.	[^{3}H]spiroperidol binding			
		Time (hr)	Total	(+)-Butaclamol	Specific binding
KCl	50% (w/w)	1	30	25	NS
		16	80	25	55
	500% (w/w)	1	118	76	42
		16	138	78	50
NaCl	0.2 M	1	143	146	NS
		16	268	282	NS
	2.0 M	1	240	261	NS
		16	498	514	NS

* Binding is expressed as fmoles/mg protein. The data represent the mean of two separate experiments assayed in triplicate. The error was less than 10 per cent. NS = not significant.

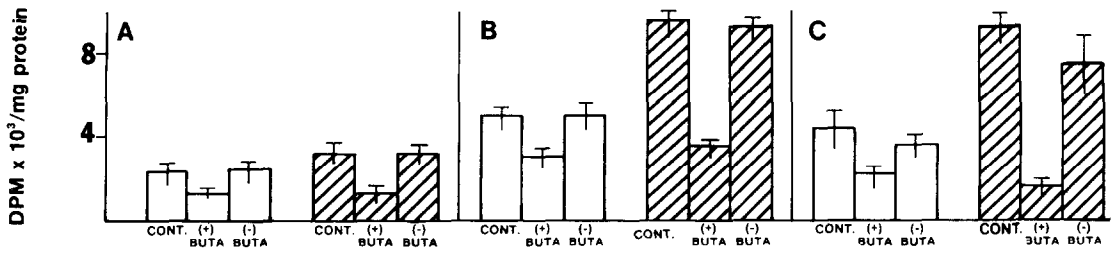

Fig. 1. Effect of incubation temperature on total and nonspecific binding on salt extracts of the striatal microsomal (P_3) and mitochondrial (P_2) fractions. Data for total binding, control, binding in the presence of 10^{-7} M (+)-butaclamol, [(+)-BUTA] and binding in the presence of 10^{-7} M (−)-butaclamol [(−)-BUTA] are shown. The results represent the average of three separate experiments; S.E.M. was less than 10 per cent. Clear bars (□) show the data from the P_2 extracts and striped bars (▨) show the data from the P_3 extracts. Panel A indicates the binding observed after incubation at 0° for 16 hr. Panel B reveals binding activity after incubation for 30 min at 23°, and panel C after incubation for 10 min at 37°. The binding assay on each fraction was performed as described in Results, and the solubilized extracts were obtained by overnight exposure to KCl.

and represented 130 fmoles/mg protein. It is noteworthy that the number of total and specific binding sites detected at 0° for the P_3 extract was substantially less than that observed at 23° or 36°. Thus, 7842 d.p.m./mg protein or 130 fmoles/mg protein were calculated for specific [^{3}H]spiroperidol sites assayable at 36° in the P_3 extract, and 2808 d.p.m./mg protein or 50 fmoles/mg protein were determined for the P_3 extract assayed at 0° for a 16 hr incubation period. Additionally, the total of binding sites determined from the 0° assay for the P_3 extract was only slightly higher than from the P_2 extract assayed at 0°. However, the ratio of specific P_3- to P_2-extracted sites was equal to that assayed at the other temperatures and represented both the high and low affinity sites. At 0°, the specific binding sites in the P_2 came to 10 fmoles/mg protein, compared to 50 fmoles/mg protein observed in the P_3 extract. All components, regardless of the assay temperature, showed stereoselectivity, with (+)-butaclamol being the active enantiomer. Because some displacement with (−)-butaclamol was detectable at 23° and 36° but not at 0°, the latter temperature was chosen for routine assays.

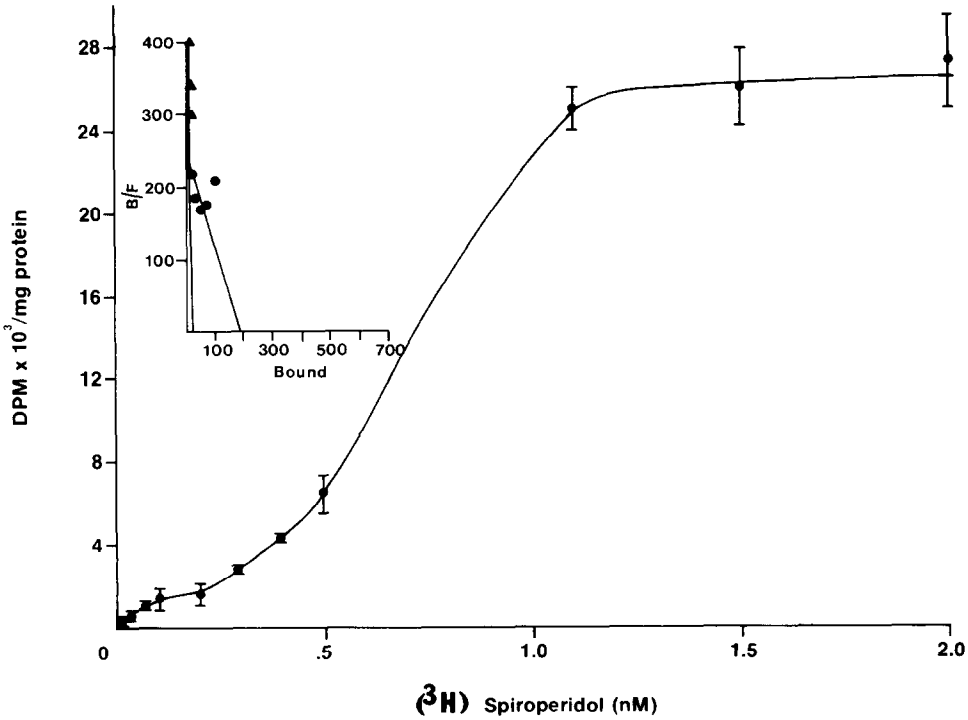

Fig. 2. Saturation curve for [^{3}H]spiroperidol binding in the calf P_3 potassium chloride extract. Specific binding is defined as the difference between total binding and that observed in the presence of 10^{-7} M (+)-butaclamol. The binding assay was performed as described in the Experimental Procedure for 16 hr at 0°. The data represent the average of three separate experiments; S.E.M. was less than 10 per cent. The Scatchard plot is depicted in the inset.

The specific binding of [^{3}H]spiroperidol to calf striatal P_2 and P_3 extracts was determined to be linear, with protein concentrations up to 4 mg/ml, the highest concentrations tested. The results shown in Fig. 2 reveal that the binding of spiroperidol to the mitochondrial preparation P_2 was saturable. Scatchard analysis of the saturation curve indicates the presence of at least two sites with respective K_d values of 0.01 and 0.7 nM.

The displacement of [^{3}H]spiroperidol binding to the P_3 extract by various neuroleptic drugs is shown in Table 4. The butyrophenone, haloperidol, was shown to have an IC_{50} of 6×10^{-9} M, which was approximately equal to that determined for (+)-butaclamol in displacing binding from membranes. The rank order of potency for the phenothiazines tested was: fluphenazine > chlorpromazine > promazine, with respective IC_{50} values of 5×10^{-9}M, 4.0×10^{-8} M and 2.0×10^{-7} M. In addition, α-fluphenthixol was found to be 100 times more potent than dopamine or *n*-methyl dopamine. Isoproterenol, norepinephrine and serotonin were virtually ineffective as displacers of [^{3}H]spiroperidol binding. The IC_{50} values shown in Table 4 for the P_3 extract were very similar to those obtained with the P_2 component (data not shown). The data in Table 4 also show that the IC_{50} displacement values for all agents tested in the soluble component are identical to those obtained in membrane preparations of the P_3 fraction.

The results of a study to compare the effect of long-term storage of both the membrane-bound and solubilized receptor at 4° are shown in Table 5. The data indicate that the number of specific binding sites is higher in potassium buffer than in Tris buffer for both the particulate and KCl extracts. Specific binding in the KCl extract was determined to be 18.8 fmoles/mg protein in Tris buffer and 131.6 fmoles/mg protein in potassium phosphate buffer. It was of interest that exposure to KCl for 15 min did not remove all of the binding activity available in the normal pellet. In fact, the data show that the KCl-extracted sites, plus the sites remaining in the pellet, exceed those of the starting material. Moreover, storage of the normal tissue of the KCl extract for 24 hr at 4° almost doubled the number of binding sites available for [^{3}H]spiroperidol when phosphate buffer was used. In the Tris buffer system, such increases were only observed in the normal tissue. After storage of the normal preparation, the KCl extract, and the pellet at 4° for 48 hr prior to assay, the number of specific binding sites was similar to those observed at 24 hr. The increase in binding sites was an equal increase in both high and low affinity sites (data not shown). Storage of the soluble binding component for up to 2 weeks at −20° did not result in any loss of binding activity compared to the 24 hr, 4° component.

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 KH_2PO_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 β-mercaptoethanol (1%) alone provided no advantage to the solubilized binding site for protection against 5-min thermal denaturation at 60°. The presence of 10% glycerol or 5 mM EDTA did

Table 4. Displacement of specific [^{3}H]spiroperidol binding from calf caudate membranes and solubilized extract*

Drug	Inhibition of membranes	[^{3}H]Spiroperidol binding extract
	(IC_{50}, nM)	
Haloperidol	3	6
(+)-Butaclamol	2	5
(−)-Butaclamol	> 1000	> 1000
α-Flupenthixol	1	4
β-Flupenthixol	150	390
Fluphenazine	3	5
Chlorpromazine	25	38
Promazine	150	280
Dopamine	3500	2000
N-Methyldopamine	3375	2100
Apomorphine	250	300
Serotonin	18,000	20,000
Norepinephrine	15,000	30,000
Isoproterenol	> 100,000	> 100,000

* IC_{50} values shown are the concentration of each test substance required to inhibit the binding of [^{3}H]spiroperidol in a calf striatum microsomal preparation, P_3, or in a P_3 fraction which has been treated with KCl as described in the Experimental Procedures by 50 per cent compared to that displaced by 10^{-7} M (+)-butaclamol which is taken to represent the maximum displaceable binding of [^{3}H]spiroperidol. The results represent the average of three separate determinations. The IC_{50} values were determined by log-probit analysis, using five separate concentrations of test substance, each assayed in triplicate.

Table 5. Effect of buffer system and storage time on [^{3}H]spiroperidol binding*

Condition	Protein mg/ml	Immediate assay		Post 24 hr storage		Post 48 hr storage	
		Bindings (fmoles/mg protein)	Specific binding (fmoles/mg protein)	Bindings (fmoles/mg protein	Specific binding (fmoles/mg protein)	Bindings (fmoles/mg protein)	Specific binding (fmoles/mg protein)
Tris (normal)	2.75	72.3		126.0		137.6	
+ BUTA		25.7	46.6	37.6	88.4	35.2	102.0
Tris 400% KCl extract	0.24	42.7		85.0		80.2	
+ BUTA		23.9	18.8	77.5	7.5	65.5	14.7
Tris pellet	2.50	79.7		132.0		128.0	
+ BUTA		21.2	58.5	39.7	92.3	36.2	91.8
KH_2PO_4 (normal)	2.65	95.4		161.0		147.0	
+ BUTA		30.0	65.4	48.2	112.8	47.0	100.0
KH_2PO_4 400% KCl	0.24	158.0		292.0		255.0	
+ BUTA		26.4	131.6	58.7	233.0	95.8	159.2
KH_2PO_4 pellet	2.47	86.7		177.4		167.0	
+ BUTA		23.6	63.1	69.8	107.6	71.3	95.7

* The data represent [^{3}H]spiroperidol (0.2 nM) binding to 50,000 *g* crude pellets of the striatum (normal) resuspended in either 50 mM Tris–HCl (pH 7.2) buffer or 15 mM KH_2PO_4 (pH 7.2). Aliquots of both pellets were saturated with KCl (400%) and allowed to stand on ice for 30 min. The undissolved KCl was removed by low speed 2000 *g* centrifugation. The supernatant fraction was centrifuged at 100,000 *g* and the pellet and supernatant fraction were assayed for binding activity.

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° (data not shown).

DISCUSSION

Ligand binding is a very useful tool in characterizing receptor recognition and in describing receptor topography. A study of the biophysical process of receptor recognition for various ligands reveals the existence of multiple binding sites for the dopamine receptor [4–8, 10, 12, 13, 16, 17]. These results support *in vivo* studies which also suggest the multiple receptors for dopamine receptors [23–25]. However, because the receptors are in their native membrane-bound state, it is difficult to characterize biochemically the receptor and distinguish whether such receptors exist as multiple forms of the same macromolecule or as completely different macromolecules. The results presented here on the solubilization of the 'dopamine receptor' provide some basic information about the biochemical nature of a soluble component that binds ligands which recognize the dopamine receptor and that shows potential for the purification of dopamine binding sites.

Recent studies comparing the subcellular distribution of dopamine-sensitive adenylate cyclase activity and radioligand binding to dopamine recognition sites in the rat striatum reveal a distinct difference in the localization of the dopamine-stimulated enzyme activity and receptor binding [5, 13, 26, 27]. The highest specific activity of dopamine-sensitive adenylate cyclase was observed in the P_2 fraction, which showed some binding activity for the [^{3}H]spiroperidol, [^{3}H]haloperidol and [^{3}H]apomorphine. However, the P_3 fraction showed the highest specific binding activity for all three ligands to the native bound receptor. The results communicated in this report show that the P_3 fraction exhibited the highest amount of salt-extractable [^{3}H]spiroperidol binding activity. Recently, Gorissen and Laduron [20] revealed that the highest specific activity of a digitonin-solubilized extract from the dog striatum was also in the P_3 fraction.

The isolation and purification of membrane proteins in a native form and in aqueous buffer have mostly employed non-denaturing detergents. It is generally believed that these molecules are incorporated into the lipid bilayer of membranes as monomers. By increasing the concentration of detergent in the membrane, various components within it become solubilized by forming mixed micelles, some containing only lipid and detergent and others containing protein, lipid and detergent. For example, some membrane proteins are released from membranes under conditions which do not disrupt the lipid bilayer. Extrinsic membrane proteins can be released from the membrane with salt or metal chelating agents. Such is the case for at least one binding site for dopamine.

The salt-extracted [^{3}H]spiroperidol binding site, as described here, was determined to have a high affinity for spiroperidol, and exhibited stereospecific characteristics. In addition, neuroleptic drugs, dopamine and dopamine agonists were determined to have the same affinity for the salt-extracted site as for the membrane preparation. The binding protein was much more stable in potassium phosphate buffer than in Tris buffer and was stable to storage at 4° for 48 hr. However, for protection of the binding site against thermal denaturation, the sodium phosphate buffer was much more effective than the

potassium phosphate buffer. It is noteworthy that exposure of the membranes to salt resulted in the unmasking of new binding sites in the membranes. Studies are in progress to characterize these binding sites in the particulate fraction that have been exposed by salt treatment.

In summary, the extraction of striatal membranes with potassium chloride yields a soluble macromolecule which bears a close resemblance to the particulate binding site for [^{3}H]spiroperidol and shows potential for purification as a recognition site of the dopamine receptor. Although no functional activity can be attributed to the binding site at present, the biochemical characterization of this macromolecule should provide information on the molecular topography of such sites.

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