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SOLUBILIZATION OF DOPAMINE D_2 RECEPTORS WITH A ZWITTERIONIC DETERGENT DCHAPS

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Abstract—Dopamine D_2 receptors were solubilized from synaptosomal membranes of the bovine caudate nucleus using a novel zwitterionic detergent 3-[(3-deoxycholamidopropyl)-dimethylammonio]-1-propane sulfonate (DCHAPS) supplemented with 1,2-propylene glycol. Optimal conditions for solubilization were: 0.12% DCHAPS, 5% 1,2-propylene glycol, 8 mg/ml membrane protein, 30 min, 4° and the yield of the D_2 receptors was 36.1%. The soluble extract retained the ability to bind [^{3}H]spiperone. This binding was of high affinity ($K_d = 2.28 \pm 0.16$ nM), reversible and saturable ($B_{max} = 1.68 \pm 0.06$ pmol/mg protein). The order of potencies of dopamine agonists and antagonists for inhibition of binding, paralleled that observed on membrane-bound D_2 receptors (correlation factor $r = 0.96$). The stereospecificity of solubilized receptors toward the pairs (+)-[(−)butaclamol, *cis*(Z)-]*trans*(E)flupenthixol and dihydroergosine/dihydroergosinine was pronounced.

Neuroleptic drugs interact with DA–D_2‡ receptors in the brain and they were successfully used as radioligands for the identification and characterization of these receptors by *in vitro* binding assays. However, this type of study does not provide a complete understanding of the mechanism of action, function and regulation of D_2 receptors, it does not elucidate molecular basis of neuroleptic action and does not explain possible mechanisms involved in the disorders related to disfunction of the dopaminergic system. To obtain a better insight into all these problems, it is necessary to separate the receptors from the membranes, preserving their integrity and affinity for DA agonists and antagonists. To achieve this goal, D_2 receptors should be solubilized and purified. DA–D_2 receptors were solubilized by detergents from different chemical categories and several chaotropic agents [1, 2]. Steroid detergents appeared to be the best solubilizers of these receptors [3–15] and the yield of solubilization was increased by high NaCl concentrations [7, 8, 11–13, 16]. However, addition of NaCl makes direct application of some biochemical methods (e.g. ion-exchange chromatography) impossible. Besides, the use of Na-cholate, which solubilizes DA receptors with high efficiency, is limited in the presence of NaCl to the pH range in which this detergent is soluble.

In this work solubilization of DA–D_2 receptors by a new detergent DCHAPS is described. The influence of several compounds improving solubilization was also examined. A high yield of solubilized D_2 receptors was achieved in a low ionic strength buffer supplemented by 5% (v/v) 1,2-propylene glycol, and the solubilized preparation can be directly purified by several biochemical methods. The pharmacological properties of the solubilized DA–D_2 receptors and the membrane-bound DA-receptors were also compared.

MATERIALS AND METHODS

Chemicals. [^{3}H]SP (spec. act. 21 Ci/mmol) was a product of the Radiochemical Centre Amersham, U.K.

The following drugs were gifts: (+)- and (−)butaclamol (Ayerst, Canada); haloperidol, benperidol and spiperone (Jansses Pharmaceutica, Beerse, Belgium); *cis*(Z)- and *trans*(E)flupenthixol (H. L. Lundbeck and Co., Copenhagen, Denmark); chlorpromazine and fluphenazine (E. R. Squibb and Sons, Princeton, NJ); metoclopramide, and sulpiride (Delagrange, Paris, France); mianserin ("Zorka" Chemical Works, Šabac, Yugoslavia); apomorphine (Sandoz Ltd., Basel, Switzerland); dihydroergosine and dihydroergosinine sulphonates ("Lek" Pharmaceutical and Chemical Works, Ljubljana, Yugoslavia).

DCHAPS was prepared starting with deoxycholic acid, analogously to the procedure of Hjelmeland [17] for CHAPS synthesis. On the basis of the data obtained by NMR analysis, i.r. spectroscopy and thin layer chromatography the purity of the preparation was approximately 94%. The detergent can be easily removed by a simple dialysis, demonstrating its high critical micellar concentration.

Membrane preparation. Calf brains were obtained from a local slaughterhouse within 1 hr of death. Caudate nuclei were dissected immediately from the fresh tissue. Synaptosomal membranes were obtained as described by Nishikori *et al.* [18] for the preparation of the M_1 fraction. The final pellet was

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‡ Abbreviations used are: DA, dopamine; DCHAPS, 3-[(3-deoxycholamidopropyl)-dimethylammonio]-1-propane sulfonate; SP, spiperone; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate.

resuspended in 50 mM Tris–HCl, 5 mM Na_4 EDTA, pH 7.8, to protein concentration of 17 mg/ml. Membrane suspension was divided into 4.0 ml aliquots which were frozen in liquid nitrogen and kept at −20°.

Solubilization. Thawed synaptosomal membrane suspensions were diluted with the above Tris–HCl–Na_4 EDTA solution pH 7.8 to protein concentration of 12.0 mg/ml and mixed with half of their volume with varying DCHAPS concentrations with or without addition of several compounds tested for their ability to improve the yield of solubilization, dissolved in the same buffer supplied with 0.06% ascorbic acid and 0.03% NaN_3. Solubilization was performed for 30 min at 4° with gentle stirring and followed by centrifugation (180,000 *g*, 30 min Ti-50 rotor, Beckman $L_{3\text{-}50}$ ultracentrifuge).

Binding assays. [^{3}H]SP binding to the native synaptosomal membranes was assayed according to Clement-Cormier and George [19] and to solubilized membrane proteins by the slightly modified method of Chan *et al.* [20]. Incubation mixtures contained 50 μl of the buffer, or the drug examined in the same buffer, and 400 μl of solubilized membranes (0.2–0.6 mg protein/ml). After 16 hr of incubation at 4°, 100 μl of human gamma-globulin (0.45%) followed by 300 μl of 30% polyethylene glycol were added. Aliquots of these mixtures were vacuum filtered 15 min later through Whatman GF/B filters, rinsed twice with 5 ml of polyethylene glycol solution, dried and transferred into a toluene-based scintillation mixture. Radioactivities were measured in a Packard Tri-Carb scintillation spectrometer at an efficiency of 45%. Incubations were performed at least in triplicate. Non-specific binding was defined as the binding in the presence of 1 μM (+)butaclamol or 100 μM apomorphine, and specific binding was the difference between total and non-specific binding.

Molecular sieving chromatography. Sepharose 4B (Pharmacia, Uppsala, Sweden) column (16.5 × 320 mm) equilibrated with 50 mM Tris–HCl, 5 mM Na_4 EDTA, 0.01% NaN_3, 0.06% DCHAPS, 5% 1,2-propylene glycol solution, pH 7.8, was used. Calf caudate membranes were solubilized under optimal conditions and 3.5 ml of solubilized preparation (0.7 mg protein/ml) were subjected to molecular sieving chromatography. Two millilitre fractions were collected at a flow rate of 25 ml/hr, checked for protein content, total and non-specific binding of [^{3}H]SP in duplicate. Standard proteins of known Stokes radii (Collection MS-II, Serva, Heidelberg, F.R.G.) and blue dextran were chromatographed separately.

Ion-exchange chromatography. DEAE-BioGel A (Bio-Rad, Richmond, CA) column (9 × 40 mm) equilibrated with 7.5 mM Tris–HCl, 0.06% DCHAPS, 2.5% 1,2-propylene glycol solution, pH 8.3, was used. One volume of membrane suspension in 50 mM Tris–HCl, 5 mM Na_4EDTA, pH 7.8 was solubilized with 2 vol. of 0.12% DCHAPS solution supplemented with 3.75% 1,2-propylene glycol. After solubilization, the soluble preparation was diluted with three times its volume of cold distilled water and the pH was adjusted to 8.3, using 0.2 M Tris solution. After the application of 20 ml of soluble preparation (0.21 mg protein/ml), the column was washed with 9.0 ml of equilibrating buffer and eluted with linear NaCl gradient (0–0.3 M NaCl in equilibrating buffer, total volume 54 ml) at a flow rate of 22 ml/hr. 1.5 ml fractions were collected and checked for protein content, specific and non-specific [^{3}H]SP binding.

Protein. Protein content was determined by the method of Lowry *et al.* [21], or Spector [22], using bovine serum albumin as a standard.

Analysis of data. Saturation curves were analyzed by the Eadie–Hofstee technique as recommended by Zivin and Waud [23]. Kinetic binding parameters were determined according to Weiland and Molinoff [24]. Hill coefficients (n_H), representing the slope of Hill plots for the corresponding competition, i.e. saturation curves, were calculated by least square regression analysis.

RESULTS

Solubilization

The effects of DCHAPS concentration on the yield of solubilized [^{3}H]SP-binding sites and synaptosomal membrane proteins is depicted in Fig. 1 A. It can be seen that the best yield of solubilization was achieved by a 0.12% DCHAPS solution. The extent of solubilization strongly depends on the synaptosomal membrane protein concentration in the incubation mixture, as shown in Fig. 1B.

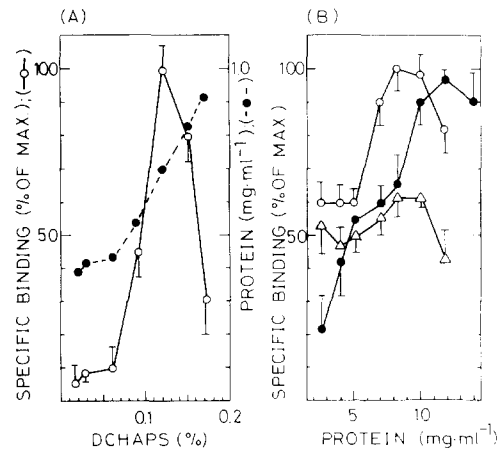

Fig. 1. Yield of solubilization of DA–D_2 receptors obtained by various concentrations of DCHAPS, or as a function of membrane protein concentration. (A) Membrane proteins (11.5 mg/ml) were incubated with different DCHAPS concentrations (30 min, 4°, gentle stirring). After centrifugation (180,000 *g*, 30 min) specfic [^{3}H]SP binding was determined in 400 μl aliquots of supernates at 0.4 nM of the radioligand. Non-specific binding was determined in the presence of 1 μM (+)butaclamol. Specific binding represents the difference between total and non-specific binding. The results are means of three experiments done in triplicate. Maximal specific binding was 310 ± 22 c.p.m./sample. (B) Solubilization was performed as given for (A). Various concentrations of membrane proteins were incubated with 0.09% (△), 0.12% (○) and 0.15% (●) DCHAPS in 50 mM Tris–HCl, 5 mM Na_4 EDTA, 0.06% ascorbic acid and 0.03% NaN_3, pH 7.8. The results are means of three experiments performed in triplicate. Maximal specific binding was 334 ± 24 c.p.m./sample. SEM is shown by vertical bars.

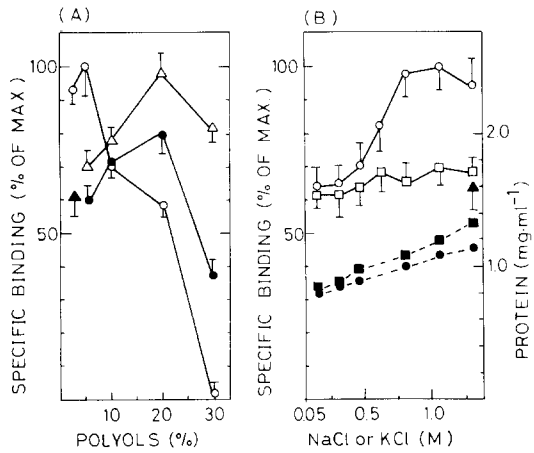

Fig. 2. Effect of addition of different compounds on the yield of solubilization of DA–D_2 receptors achieved by DCHAPS. (A) Membranes were solubilized under optimal conditions (0.12% DCHAPS, 8 mg protein/ml, 30 min, 4°). Different amounts of polyols were added to incubation mixtures. 1,2-Propylene glycol (○); ethylene glycol (●); glycerol (△). Maximal specific binding was 498 ± 47 c.p.m./sample. The data are means of three experiments done in triplicate. Control values were obtained when the membranes were solubilized with DCHAPS alone at optimal conditions. (B) Membranes were solubilized as described for (A) at 5.2 mg protein/ml. Different NaCl and KCl concentrations were added to incubation mixtures. Salts were not added (▲); protein concentration in the presence of NaCl (●); protein concentration obtained with KCl (■); yield of solubilization with KCl (□). SEM shown by vertical bars.

The best results were obtained with 0.12% DCHAPS at a synaptosomal membrane protein concentration of 8.0 mg/ml (Fig. 1).

The influence of the addition of several polyols (glycerol, ethylene glycol, 1,2-porpylene glycol) to the incubation mixture on the yield of solubilization is presented in Fig. 2.

As seen from Fig. 2A, the best yield was achieved with a 0.12% DCHAPS solution supplemented by 5% 1,2-propylene glycol, or 20% glycerol, while ethylene glycol was much less efficient. A significant increase in the yield of solubilized D_2 receptors was obtained when NaCl in concentrations over 0.75 M were used, while KCl was rather inefficient (Fig. 2B). When the samples were solubilized with higher glycerol concentrations (20% and 30%), or NaCl and KCl in the concentrations over 0.75 M, membrane suspensions were diluted with solubilizing buffer (1:1) in order to reduce the density of the mixture and enable complete partitioning of the species into soluble and insoluble fraction based on their buoyant densities.

When combinations of optimal NaCl (1.0 M) and 1,2-propylene glycol (5%) or glycerol (20%) concentrations were used, no additive effects were observed and the yield of solubilized DA receptors was about the same, as when individual compounds were added to the medium containing 0.12% DCHAPS. Soybean trypsin inhibitor (0.5 m/ml)-increased the yield for 22% in comparison with that obtained when 0.12% DCHAPS without 1,2-propylene glycol or NaCl was used, but if 0.12% DCHAPS solution was supplemented with 5% 1,2-propylene glycol, or 1.0 M NaCl, no additional enhance of the extent of was observed. No improvement of solubilization was achieved when different detergents, frequently used in membrane protein solubilization procedures, e.g. 3[(3-laurylamidopropyl)-dimethylammonio]-1-propropane sulfonate, Na-deoxycholate, or Lubrol PX, were added to DCHAPS solution.

Prolonged centrifugation (3 hr, 180,000 *g*) or filtration through small pore size filters (Millipore, 0.45 μ) did not influence either protein concentration in the clear supernate, i.e. filtrate, or the extent of specific [^{3}H]SP binding in the preparations solubilized by DCHAPS.

[^{3}H]*Spiperone binding.*

Saturation curves for [^{3}H]SP binding to membrane-bound and solubilized SP-binding sites (0.12% DCHAPS, 5% propylene glycol) are presented in Fig. 3.

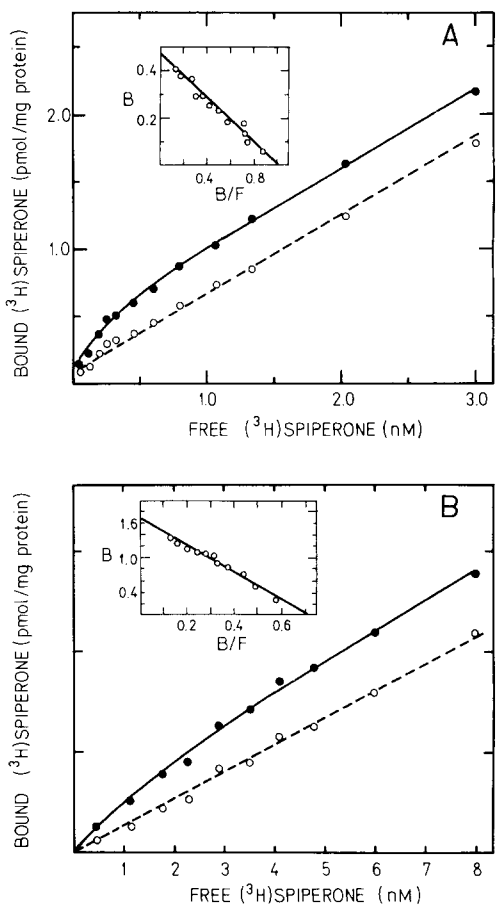

Fig. 3. Saturation curves of membrane-bound and DCHAPS solubilized DA–D_2 receptors. (A) Saturation curve of membrane-bound receptors (see Materials and Methods section). Non-specific binding was determined in the presence of 100 μM apomorphine. (B) Saturation curve of [^{3}H]SP binding to DCHAPS solubilized receptors. Insets represent Eadie–Hofstee analysis of the same data. The variation of triplicate samples for a single experiment was less than 10%. Each point is mean of three experiments done in triplicate: ●, Total binding; ○, non-specific binding.

Table 1. Equilibrium binding of [^{3}H]SP to membrane and solubilized preparations from bovine caudate nucleus and recovery of receptor and protein

	Specific [^{3}H]SP binding			Solubilized	
	K_d (nM)	B_{max} pmol/mg prot.)	n_H	Protein (% starting membrane)	Receptor
Membrane	0.47 ± 0.04	0.46 ± 0.02	1.05 ± 0.02	—	—
DCHAPS extract	2.28 ± 0.16	1.68 ± 0.06	1.05 ± 0.02	10.0 ± 0.52	36.1 ± 4.8

The data were calculated on the basis of the saturation curves presented in Fig. 3.

In both cases, a straight line was obtained by Eadie–Hofstee analysis (insets to Fig. 3 A and B), while Hill coefficients were 1.05 ± 0.02, indicating a single class of binding sites. Table 1 summarizes K_d, B_{max} and n_H values for soluble and corresponding membrane preparations. B_{max} values were used to calculate solubilization yields. As seen (Table 1) the yield of solubilization achieved by DCHAPS was twofold higher than when DCHAPS was used, as previously reported [10].

Specific [^{3}H]SP binding was reversible and association and dissociation kinetic studies (Fig. 4) gave the values for association (k_1) and dissociation (k_{-1}) constants of $(7.48 \pm 0.6)\times10^{-3}\,min^{-1}\,nM^{-1}$ and $(5.55 \pm 0.7)\times10^{-3}\,min^{-1}$, respectively. The ratio of these two constants gave the value of $K_d = k_{-1}/k_1 = 0.74 \pm 0.15$ nM, which was three times lower than the K_d value calculated by Eadie–Hofstee analysis of the saturation curve. This disaccordance could be attributed to the complex nature of the binding reaction [24].

Examinations of thermal stability of solubilized receptors (Fig. 5) at 20° and 37° showed that they were much more heat labile than the membrane-bound receptors. The half-life of the solubilized receptor was 10.8 ± 1.5 min and about 100 min at 37° and 20°, respectively.

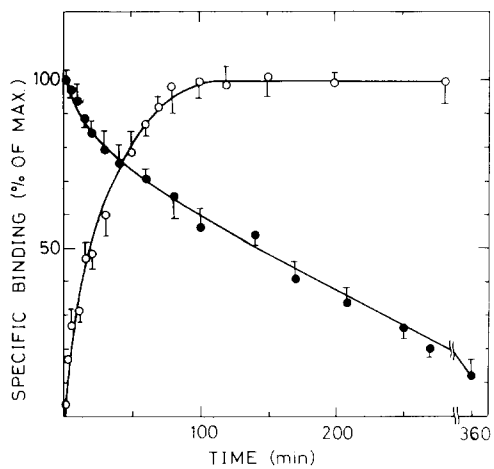

Fig. 4. Association (○) and dissociation (●) curves for specific [^{3}H]SP binding to solubilized receptors. Association kinetics: after solubilization under optimal conditions (0.12% DCHAPS, 5% 1,2-propylene glycol, 50 mM Tris–HCl, 5 mM Na_4EDTA, 0.02% ascorbic acid, 0.01% NaN_3, 8 mg membrane protein/ml, 30 min, 40°) solubilized proteins were separated by centrifugation (30 min, 180,000 *g*) and 20 ml were incubated with 2 nM of [^{3}H]SP in the presence and in the absence of (+)butaclamol at 4°. At indicated time intervals 400 μl aliquots were taken and specific binding was determined as the difference between the total binding and binding in the presence of (+)butaclamol. Points are means of three experiments done in triplicate. SEM shown by vertical lines. Dissociation kinetics: 24 ml of solubilized receptor preparation were incubated with 2 nM [^{3}H]SP for 18 hr at 4° after which at zero point, 1000-fold excess of unlabelled SP were added. Aliquots of incubation mixture were taken at different time intervals and specific binding was determined as described above. Each point is mean of three experiments done in triplicate. SEM shown by vertical lines.

Pharmacological characterization

Competition curves of [^{3}H]SP bound to solubilized D_2 receptors of the bovine caudate nucleus and different DA agonists and antagonists are presented in Fig. 6.

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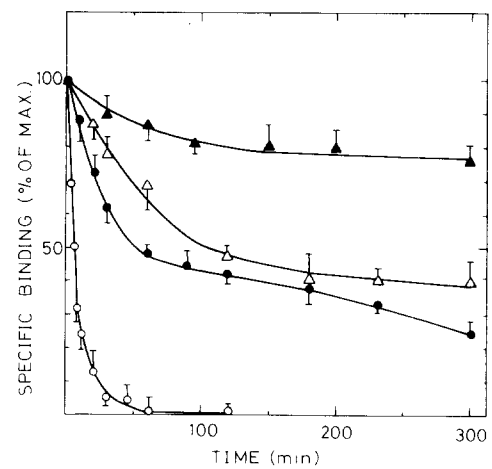

Fig. 5. Thermal stability of DCHAPS-solubilized and membrane-bound DA–D_2 receptors. Solubilized membranes and crude membrane preparation were incubated at 20° and 37° at 0.4 nM of [^{3}H]SP. Aliquots of incubation mixtures were taken at varying time intervals and specific [^{3}H]SP binding was determined as the difference between total and binding in the presence of 1 μM (+)butaclamol. Each point is mean of three experiments done in triplicate. ●, solubilized receptors at 20°; ○, solubilized receptors at 37°; ▲, membrane-bound receptors at 20°; membrane-bound receptors at 37° (△). SEM shown by vertical lines.

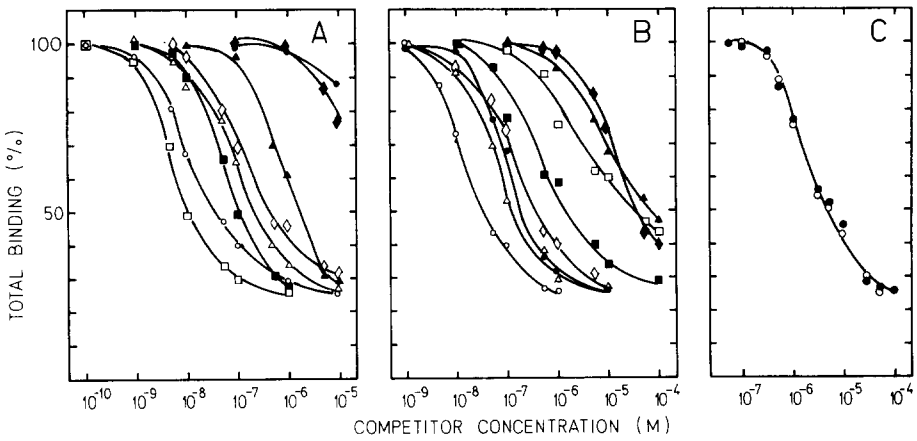

Fig. 6. Inhibition of [^{3}H]SP binding to soluble preparations of calf caudate nuclei synaptosomal membranes by DA agonists and antagonists. Solubilization was done under optimal conditions. 400 μl aliquots of soluble membrane preparations were incubated with various concentrations of the drugs and 0.4 nM [^{3}H]SP for 16 hr at 4°. Non-specific binding was determined in the presence of (+)butaclamol. In a typical experiment specific binding was 490 ± 42 c.p.m./sample, and non-specific 35 ± 12 c.p.m./sample. The amount bound to blank filters was 132 ± 10 c.p.m. Points are mean of at least three experiments done in triplicate. Standard error within a single experiment was less than 10%. (A) ○, (+)butaclamol; ●, (−)butaclamol; △, *cis*(Z)flupenthixol ; ▲, *trans*(E)-flupenthixol; □, spiperone; ■, haloperidol; ◇, dihydroergosine; ◆, dihydroergosinine. (B) ○, benperidol; ●, fluphenazine; △, chlorpromazine; ▲, dopamine; □, sulpiride; ■, metoclopramide; ◆, mianserin; ◇, CH-29 717. (C) ○, apomorphine+ 120 mM NaCl; ●, apomorphine+GTP+120 mM NaCl.

of different affinity for DA receptors were used. As seen from Fig. 6, all competition curves were monophasic. Numerical values for n_H and $-\log IC_{50}$ calculated on the basis of displacement experiments, together with the corresponding data for membrane-bound receptors, obtained with the same membrane preparation used for solubilization, are listed in Table 2.

It is obvious that stereospecificity of solubilized receptors toward the pairs (+)- and (−)butaclamol, *cis*(Z)- and *trans*(E)flupenthixol and dihydroergosine and dihydroergosinine was pronounced. Addition of 100 μM GTP and 120 mM NaCl did not significantly influence the shape of the apomorphine [^{3}H]-SP displacement curve and the -logIC_{50} value (Fig. 6C; Table 2) obtained with solubilized membrane preparation, contrary to the results obtained with membrane-bound receptors (Table 2).

Figure 7 shows a good correlation between -logIC_{50} values in soluble preparations and membrane-bound [^{3}H]SP binding sites and a correlation coefficient r of 0.96 was obtained.

Table 2. $-\log IC_{50}$ values and Hill coefficients (n_H) for various DA agonists and antagonists on the membrane-bound and solubilized receptors

Competitor	Solubilized receptor $-\log IC_{50}$	Solubilized receptor n_H	Membrane-bound receptor $-\log IC_{50}$	Membrane-bound receptor n_H
Spiperone	8.15 ± 0.08	1.05 ± 0.11	8.40 ± 0.05	0.88 ± 0.04
Benperidol	7.70 ± 0.16	1.00 ± 0.12	8.22 ± 0.12	0.69 ± 0.07
(+)Butaclamol	7.63 ± 0.05	0.91 ± 0.07	7.66 ± 0.09	0.73 ± 0.07
Haloperidol	7.20 ± 0.12	0.99 ± 0.09	7.46 ± 0.11	0.62 ± 0.04
Chlorpromazine	7.07 ± 0.36	0.97 ± 0.20	7.26 ± 0.04	0.77 ± 0.02
cis(Z)Flupenthixol	6.91 ± 0.10	0.82 ± 0.08	7.60 ± 0.10	0.71 ± 0.05
Fluphenazine	6.90 ± 0.23	1.03 ± 0.19	7.40 ± 0.08	0.70 ± 0.07
Dihydroergosine	6.56 ± 0.17	0.82 ± 0.14	7.49 ± 0.09	0.79 ± 0.02
Metoclopramide	5.70 ± 0.15	0.66 ± 0.12	5.42 ± 0.10	0.50 ± 0.03
Apomorphine	5.47 ± 0.05	0.92 ± 0.05	6.13 ± 0.04	0.76 ± 0.03
Apomorphine+GTP	5.51 ± 0.04	0.90 ± 0.05	5.82 ± 0.03	0.99 ± 0.03
CH-29 717	6.45 ± 0.07	0.80 ± 0.08	6.77 ± 0.02	0.81 ± 0.02
trans(E)Flupenthixol	6.00 ± 0.13	1.10 ± 0.09	6.10 ± 0.14	0.80 ± 0.10
Mianserin	5.40 ± 0.20	0.85 ± 0.10	5.25 ± 0.24	0.70 ± 0.11
Sulpiride	5.40 ± 0.12	0.54 ± 0.08	5.23 ± 0.18	0.54 ± 0.08
Dopamine	4.80 ± 0.06	0.66 ± 0.05	5.14 ± 0.09	0.80 ± 0.04
(−)Butaclamol	<5	—	5.02 ± 0.18	1.02 ± 0.08
Dihydroergosinine	<5	—	6.24 ± 0.11	0.90 ± 0.07

IC_{50} values were calculated from the competition curves obtained at 0.15 nM and 0.4 nM of [^{3}H]SP for membrane-bound and solubilized receptors, respectively and varying concentrations of different drugs. Shown are values ±SEM from N = 3 independent experiments done in triplicate.

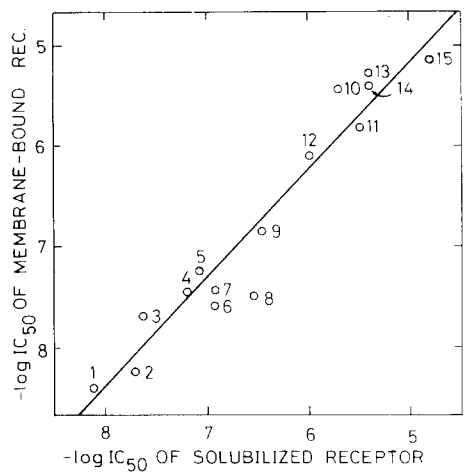

Fig. 7. Correlation between -logIC_{50} values of DCHAPS solubilized and membrane-bound receptors. 1 = Spiperone; 2 = beneperidol; 3 = (+)butaclamol; 4 = haloperidol; 5 = chlorpromazine; 6 = *cis*(Z)-flupenthixol; 7 = fluphenazine; 8 = dihydroergosine; 9 = CH-29 717; 10 = metoclopramide; 11 = apomorphine; 12 = *trans*(E)flupenthixol; 13 = mianserin; 14 = sulpiride; 15 = dopamine. IC_{50} values were determined as the concentrations of unlabelled drugs which protected 50% of DA-binding sites against the binding of [^{3}H]SP (correlation coefficient 0.96). -logIC_{50} values were taken from Table 2.

Molecular sieving chromatography

Elution profiles obtained by molecular sieving chromatography of the DCHAPS solubilized membrane preparation on a calibrated Sepharose 4B column is depicted in Fig. 8.

The elution volume of the fraction with the highest specific [3H]SP binding was equal to the elution volume of aldolase used for Sepharose 4B column calibration. The Stokes radius calculated on the basis of the these experiments of the solubilized D_2 receptor–phospholipid-detergent complex was 4.8 nm, M_r of 140 kD.

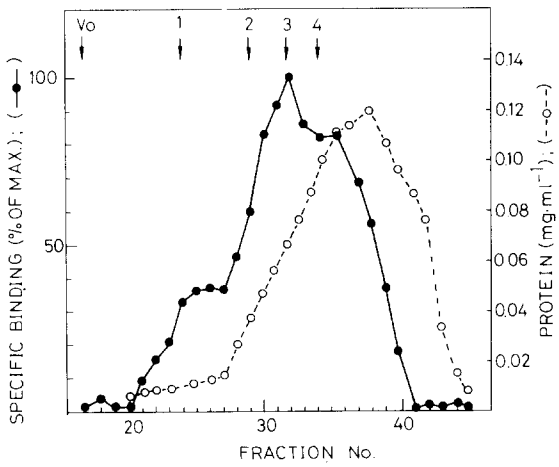

Fig. 8. Elution profile of [^{3}H]SP binding sites solubilized by DCHAPS obtained by molecular sieving chromatography on Sepharose 4B column. 3.5 ml of solubilized membrane preparation (0.7 mg protein/ml) were applied onto Sepharose 4B column (16.5 × 320 mm). 2.0 ml fractions were collected at flow rate of 25 ml/hr. Ferritin (1), catalase (2), aldolase (3) and bovine serum albumin (4) were used as the marker proteins. Specific binding was determined in the presence of 1 μM (+)butaclamol in each fraction. Protein in individual fractions was determined (Spéctor, 1978). Maximal specific binding was obtained in 32nd fraction (198 c.p.m./sample).

Ion-exchange chromatography

The elution profile obtained by ion-exchange chromatography of the DCHAPS solubilized preparation on DEAE-Bio Gel column is presented in Fig. 9.

Maximal specific [^{3}H]SP binding was obtained in the fraction eluted with 0.15 M NaCl solution. Purification of the receptor achieved in this fraction was 4.7-fold in relation to the crude solubilized preparation. Recovery of specific [^{3}H]SP binding was 35%.

DISCUSSION

The results presented in this paper demonstrate that [^{3}H]SP binding sites of synaptosomal membranes of the bovine caudate nucleus solubilized by DCHAPS represent $DA–D_2$, receptors, fulfilling most of the criteria proposed by Laduron [2] for solubilized receptors. The high yield of solubilization achieved by 0.12% DCHAPS supplemented with 5% 1,2-propylene glycol solution indicates that this detergent could be compared to other solublizing agents which are widely applied for the solubilization of DA and other plasma membrane receptors, such as CHAPS [8, 9], CHAPSO [12], Na-cholate [7, 11, 13, 16] and digitonin [3–6, 10, 14, 15].

DCHAPS provides a high yield of solubilized preparation which is increased after addition of polyols, higher NaCl concentrations and protease inhibitors. The exact mechanism by which polyols enhance the extent of solubilization is not well understood, but it could be ascribed to their unspecific action as pro-

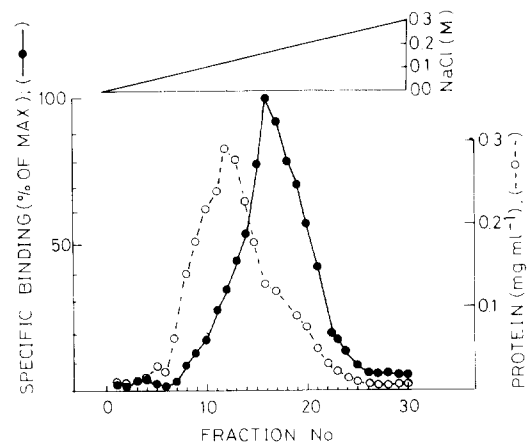

Fig. 9. Elution profile of DCHAPS-solubilized $DA-D_2$ receptors obtained by ion-exchange chromatography. DEAE-Bio Gel column (9 × 40 mm) was used. Elution was done by linear (0–0.3 M) NaCl gradient after the application of 4.2 mg of solubilized preparation, at flow rate of 22 ml/hr. 1.5 ml fractions were collected, tested for protein content (Spector, 1978) and specific [^{3}H]SP binding. Maximal specific binding was obtained in the 16th fraction (420 c.p.m./fraction). The results are from one representative experiment.

tease inhibitors [25]. This is supported by the results demonstrating an increase of the solubilization yield after addition of soybean trypsin inhibitor, because it is known that DA–D_2 receptors are sensitive to protease action [26]. High concentrations of NaCl (over 0.75 M) also enhanced the yield of D_2 receptor solubilization by DCHAPS. Similar results were obtained when CHAPS was used as a solubilizer [8], while Na-cholate did not act as solubilizer if NaCl was not added [7, 11, 13, 16]. It is interesting that the use of DCHAPS and a combination of the agents improving solubilization did not affect the yield of solubilization to a higher degree than when one of these compounds was added to DCHAPS. The most suitable mixture tested in our experiments was 0.12% DCHAPS–5% 1,2-propylene glycol. It was very efficient in solubilizing D_2 receptors from synaptosomal membrane preparations and had a lower density and viscosity in comparison with the mixture containing 20% glycerol, while the absence of NaCl enabled further direct biochemical characterization of solubilized receptors.

Eadie–Hofstee analysis of the saturation curves gave straight lines, while Hill coefficients were close to unity for both solubilized and membrane-bound receptors, pointing out to the homogeneous population of the binding sites. K_d value obtained with solubilized receptors was much higher than that of the membrane-bound receptors. Such a phenomenon was observed earlier by several authors [8, 9, 27, 28] and it could be explained in terms of the changes in chemical environment of the receptors after solubilization.

[^{3}H]SP binding to solubilized membrane preparations was reversible and analysis of association and dissociation curves demonstrated a single class of binding sites. However, the K_d value calculated on the basis of association and dissociation constants was about three times lower than that calculated from the saturation curve. According to Weiland and Molinoff [24], this discrepancy "implies that a simple second order reaction is an inadequate description of the reaction and more detailed kinetic analysis is required".

Solubilized receptors were much more heat sensitive than the membrane-bound ones, which is one of the general characteristics of the solubilized receptors [1, 2, 8, 9]. The denaturation curve of solubilized DA–D_2 receptors was monophasic at 37°, while biphasic at 20°. A similar biphasic shape of the denaturation curve was obtained with membrane-bound receptors at 37°, indicating a certain heterogeneity of the receptors which are impossible to discriminate between except on the basis of saturation isotherms and competition curves.

The shape of the competition curves, and the slopes of these curves obtained when DA antagonists of butyrophenone and phenothiyzine type and solubilized preparation were used, demonstrate a single class of binding sites. DCHAPS-solubilized preparations expressed prominent stereospecificity toward the pairs: (+)-/(−)butaclamol, *cis*(Z)-/*trans*(E)flupenthixol and dihydroergosine/dihydroergosinine, characteristic for DA–D_2 receptors. The competition curve obtained in the presence of mianserin was monophasic and the -logIC_{50} value was 5.40, demonstrating the absence of serotonergic S_2 receptors [29] in soluble preparation. The shape of the apomorphine [^{3}H]SP displacement curve for soluble preparation was not changed after addition of 100 μM GTP in the presence of 120 mM NaCl, while the corresponding curve for membrane-bound receptors was changed under these conditions. A high correlation degree of -logIC_{50} values for solubilized and membrane-bound receptors ($r = 0.96$) obtained with different DA agonists and antagonists may be taken as strong evidence that we were dealing with solubilized DA receptors of D_2 subtype.

Molecular sieving chromatography of the solubilized membrane preparation on a Sepharose 4B column, gave a Stokes radius of 4.8 nm, corresponding to M_r of 140 kD, if globular protein is concerned. This value is somewhat lower than that referred to by the others [8, 11, 12] and could mean that the receptor–phospholipid–detergent complex behaves in this manner. Similar results were obtained by Kidrić *et al.* [9] who solubilized DA–D_2 receptors of the bovine caudate nucleus using CHAPS.

Solubilized D_2 receptors did not sediment during prolonged centrifugation (180,000 *g*, 3 hr), they were not retained at small pore size filters (Millipore, 0.45 μ), and they showed decreased thermostability in comparison with the membrane-bound receptors. These results confirm that true solubilized DA–D_2 receptors are being studied [1, 2].

Ion-exchange chromatography gave 4.7-fold purified receptor in comparison with the crude solubilized membrane preparation and recovery of 35%. Solubilization of DA–D_2 receptors using DCHAPS supplemented with 1,2-propylene glycol, could serve as a method, which would in combination with some more specific methods, e.g. affinity chromatography, provide a high degree of DA–D_2 receptor purification, which can further be used for more detailed characterization.

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