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Solubilization of dopamine-D_2receptors from synaptosomal membranes of the bovine caudate nucleus

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1 Dopamine D_2-receptors were solubilized from synaptosomal membranes of the bovine caudate nucleus using different detergents. They were labelled with [^{3}H]-spiperone and assayed by polyethylene glycol precipitation.

2 CHAPS was found to be the best solubilizing agent among all detergents used. Optimal conditions for solubilization were: 0.25% CHAPS, 3.5 mg ml^{-1} protein, 25 min, 4 °C and the yield of D_2-receptors was 18.6%.

3 Addition of some sulphobetain detergents increased the extent of solubilization, 125 mM NaCl and 0.25 M sucrose decreased it, while SH-group protecting agents (2 mM dithiothreitol and 6 mM β-mercaptoethanol), as well as MEGA-9 and MEGA-12 were almost ineffective.

4 $-\log IC_{50}$ values for solublized dopamine D_2-receptors are in linear correlation with the corresponding values for membrane-bound receptors ($r = 0.962$, slope factor 0.96) and K_d value of solubilized receptors was 3.61 ± 0.94 nM, while that of membrane-bound receptors was 1.25 ± 0.10 nM.

5 Specific binding of [^{3}H]-spiperone to the solubilized receptors resolved by linear sucrose density gradient centrifugation shows two maxima, one in the first several fractions from the bottom and the other with an apparent S value of 7.3.

Introduction

Solubilization of plasma membrane receptors is one of the necessary steps for their isolation and characterization. Dopamine receptors were isolated from several sources and solubilization was achieved with different detergents (Laduron & Ilien, 1982; Hall *et al.*, 1983; Kuno *et al.*, 1983). Using plant glycoside digitonin, Gorissen & Laduron (1979) reported the solubilization of binding sites from dog striatum which retained the characteristics of membrane-bound dopamine receptors. Gorissen *et al.* (1980) showed that digitonin-solubilized dopamine receptors from rat striatum are masked by a high number of non-stereospecific spirodecanone sites, the number of which was much higher in the frontal cortex than in the striatum. The same authors noticed more spirodecanone sites in the rat than in the corresponding preparations from dog and supposed that this difference was the result of species variability. Madras *et al.* (1982) compared binding characteristics of solubilized preparations from human, calf and canine brains and demonstrated that the binding characteristics of the calf D_2-receptors were considerably altered upon digitonin solubilization. They concluded that calf caudate is a poor choice for dopamine-receptor solubilization using digitonin. Gorissen & Laduron (1978) and Tam & Seeman (1978) suggested that rat and calf striatum are not good sources of soluble dopamine receptors, since primarily non-dopaminergic binding sites were solubilized (Gorissen *et al.*, 1980) and the correlation between IC_{50} values of solubilized and native receptors was rather low (Madras *et al.*, 1981; 1982). However, calf caudate nucleus may serve as a suitable tissue for biochemical investigations, since it provides a rich source of dopamine binding sites which have been studied by numerous authors (Seeman *et al.*, 1976; Burt *et al.*, 1976; Creese *et al.*, 1977; 1979; Hartley & Seeman, 1978; Šoškić *et al.*, 1983). Therefore we have found it of interest to examine the possibilities for the improvement of the solubilization

of dopamine D_2-receptors using this brain structure. Investigations of different solubilizing agents and alterations of experimental conditions showed that CHAPS, previously used for soubilization of opiate receptors (Simonds *et al.*, 1980), brain dopamine D_2-receptors (Lew *et al.*, 1981) and recently for solubilization of dopamine D_2-receptors from the bovine caudate nucleus (Kuno *et al.*, 1983) represents the best solubilizer among several detergents from different chemical categories.

Methods

Brain membrane preparation

Calf brains were obtained from a local slaughterhouse within 1 h after death. Nuclei caudata were dissected immediately from the fresh tissue. Synaptosomal membranes were obtained as described by Nishikori *et al.* (1980) for preparation of the M_1 fraction. The final pellet was resuspended in 50 mM Tris: HCl, 5 mM Na_4EDTA, pH 7.4 to give a protein concentration of 10 mg ml^{-1}. The membrane suspension was divided into 4.0 ml aliquots which were frozen in liquid nitrogen and kept at −20 °C.

Solubilization of the membranes

Thawed synaptosomal membrane suspensions were diluted with the Tris HCl-Na_4EDTA solution to give a protein concentration of 7.5 mg ml^{-1} and mixed with half of their volume of different concentrations of the detergents dissolved in the same buffer supplied with 0.06% ascorbic acid and 0.03% sodium azide. When optimal solubilization conditions were defined and CHAPS was chosen as the best solubilizer, suspensions of the membranes were diluted to 5.2 mg protein ml^{-1}. Solubilization was performed for 25 min at 4 °C with gentle stirring, followed by centrifugation (180,000*g*, 30 min, Ti-50 rotor, Beckman L_{3-50} ultracentrifuge). Proteins were determined in the clear supernates by the procedure of Lowry *et al.* (1951).

Binding assay

[^{3}H]-spiperone binding to the native synaptosomal membranes was carried out according to Clement-Cormier & George (1978) and binding to solubilized membrane proteins by a slight modification of the method of Chan *et al.* (1981). Incubation mixtures contained 50 µl of 9 nM [^{3}H]-spiperone in solubilizing buffer, 50 µl of buffer or the drug examined in the same buffer and 400 µl of solubilized membranes (0.2–0.6 mg protein ml^{-1}). After 16 h of incubation at 4 °C, 100 µl of human gamma-globulin (0.45%) followed by 300 µl of 30% polyethylene glycol 6000 were added; 15 min later, aliquots of these mixtures were vacuum filtered through Whatman GF/B filters, twice rinsed with 7 ml polyethylene glycol solution, dried and transferred into toluene-based scintillation

Table 1 Yield of solubilized [^{3}H]-spiperone binding sites from synaptosomal membranes of the bovine caudate nucleus treated with different detergents

Detergent	*Concentration of detergent (%)*	*Solubilization of* [^{3}H]-*spiperone receptors (%)*
CHAPS	0.750	1.8
	0.500	4.0
	0.375	12.7
	0.250	9.7
	0.125	8.5
Digitonin	1.000	6.1
	0.750	5.0
	0.500	3.2
NAPS	1.000	2.2
MEGA-12	0.125	4.6
	0.062	2.0
MEGA-9	1.000	1.3
	0.500	1.3
	0.250	2.2
Brij 35	0.250	2.8

After solubilization with indicated concentrations of the detergents dissolved in 50 mM tris HCl, 5 mM Na_4EDTA, 0.06% ascorbic acid, 0.03% Na-azide, membrane suspensions (7.5 mg ml^{-1} protein) were centrifuged (30 min, 180,000 *g*, Tr-50 rotor, Beckman L_{3-50} ultracentrifuge). In the supernates [^{3}H]-spiperone binding was checked at 0.9 nM of the radioligand. Yield in fmol was calculated as follows: (specific binding to soluble fraction/specific binding to membrane fraction) × 100. Specific binding (fmol) was examined at the same concentration of the radioligand under identical conditions both in membranes and in soluble preparations and was calculated as the difference between total binding and binding in the presence of 1 µM (+)-butaclamol. The data from representative experiments were used. Each value represents mean of three assays performed in triplicate. The variation of triplicate samples for a single experiment was about 10%. No solubilization was achieved with 0.250 and 0.125% digitonin, 1.000, 0.500, 0.250 and 0.125% Na-deoxycholate, N-(+)-gluco-N-methylcholamide, LAPS, Triton X-100 and Lubrol PX, 0.250, 0.125 and 0.062% PAPS, 0.500, 0.250 and 0.125% NAPS, 0.250% MEGA-12, 0.125% MEGA-9, 1.000, 0.500 and 0.125% Brij 35. Higher concentrations of PAPS and MEGA-12 were not used because of their insolubility.

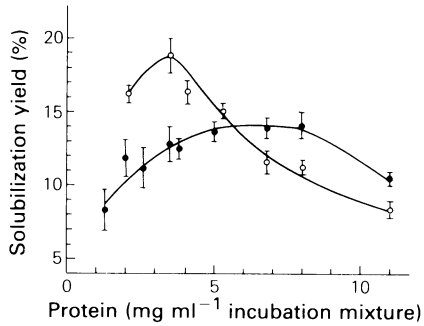


Figure 1 Yield of solubilization of dopamine D_2-receptors as a function of membrane protein concentration. Various concentrations of the membrane proteins were incubated with 0.25% (○) and 0.375% (●) CHAPS in 50 mM Tris HCl, 5 mM Na_4 EDTA, 0.02% ascorbic acid, 0.01% sodium azide, pH 7.4 for 25 min at 4 °C with gentle stirring. After centrifugation (180,000 *g*, 30 min) specific [^{3}H]-spiperone binding was determined in 400 µl aliquots of the supernates at 0.9 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. Yield was calculated in fmol.

liquid. 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 and specific binding was the difference between total and non-specific binding.

Sucrose density-gradient centrifugation

An aliquot (0.3 ml) of the solubilized membrane preparation (0.15 mg protein) was layered on linear sucrose density gradient (15–30% sucrose in 50 mM

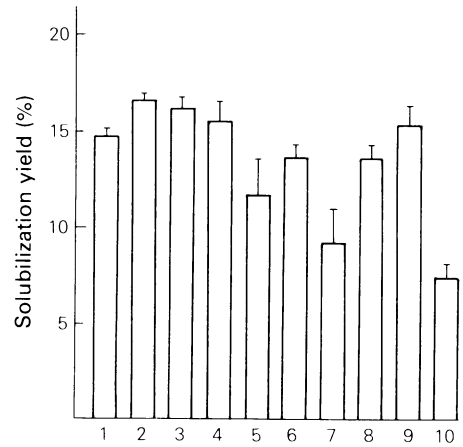


Figure 2 The effect of addition of different compounds on the yield of solubilization of dopamine D_2-receptors achieved by CHAPS: 0.25% CHAPS in 50 mM Tris HCl, 5 mM Na_4EDTA, 0.02% ascorbic acid, 0.01% sodium azide, pH 7.4 with the addition of 0.05% solution of various detergents, 125 mM NaCl, 0.25 M sucrose, 2 mM dithiothreitol or 6 mM β-mercaptoethanol was used. Solubilization was performed for 25 min at 4 °C. Specific [^{3}H]-spiperone binding was determined as described in legend to Figure 1. 1 = CHAPS (3-[3-cholamidorpropyl) dimethyl-ammonio]-1-propanesulphonate); 2 = CHAPS + PAPS (3-[(3-palmitoylamidopropyl) dimethylammonio] -1-propanesulphonate); 3 = CHAPS + LAPS (3-[(3-laurylamidopropyl) dimethylammonio] -1-propanesulphonate); 4 = CHAPS + NAPS (3-[3-nonanoylamidopropyl) dimethylammonio] -1-propanesulphonate); 5 = CHAPS + MEGA-12 (N-(+)-gluco-methyllaurylamide); 6 = CHAPS + MEGA-9 (N-(+)-gluco-N-methylnonanoylamide); 7 = CHAPS + 125 mM NaCl; 8 = CHAPS + 2 mM dithiothreitol; 9 = CHAPS + 6 mM β-mercaptoethanol; 10 = CHAPS + 0.25 M sucrose. The results are means of three experiments done in triplicate.

Table 2 Recovery of [^{3}H]-spiperone binding sites and protein after CHAPS solubilization

	Protein (mg ml^{-1})	*Vol* (ml)	*Total protein* (mg)	*Receptor density* (B_{max}) (pmol mg^{-1} prot.)	K_d (nM)	*Total receptors* (pmol)
Membrane	5.22	6.0	31.32	1.33 ± 0.17	1.25 ± 0.10	42.60
Soluble	0.50	9.0	4.56	1.74 ± 0.26	3.61 ± 0.94	7.92
Recovery	9.60%		14.28%	130%		18.6%

Solubilized membranes (0.250% CHAPS, 50 mM Tris HCl, 5 mM EDTA, 0.02% ascorbic acid, 0.01% sodium azide, pH 7.4, 25 min, 4 °C) were separated from insoluble residues by centrifugation (180,000 *g*, 30 min, Ti 50 rotor, Beckman L_{3-50} ultracentrifuge). Specific [^{3}H]-spiperone binding was examined in both clear supernates and original membrane preparations at 0.9 nM of the radioligand in the absence and in the presence of 1 µM (+)-butaclamol. Values are means of three experiments done in triplicate.

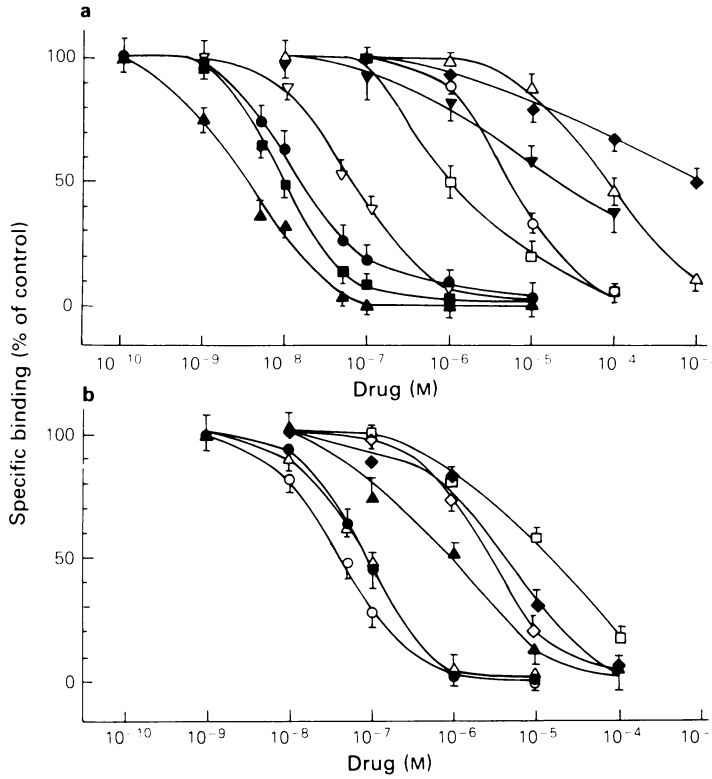


Figure 3 Inhibition of [^{3}H]-spiperone binding to soluble preparations of calf caudate nuclei by dopamine agonists and antagonists. Aliquots (400 μl) of soluble membrane preparations were incubated with various concentrations of drugs and 0.9 nM [^{3}H]-spiperone for 16 h at 4 °C. After addition of 0.45% human gamma-globulin, samples were precipitated with 30% polyethylene glycol 6000 and vacuum filtered through Whatman GF/B filters. Specific binding of [^{3}H]-spiperone was defined as that inhibited by 1 μM (+)-butaclamol. At a final concentration of 0.9 nM [^{3}H]-spiperone the total c.p.m. bound (after subtracting the amount bound to filter) was 290 c.p.m. of which 79% was specific. The amount bound non-specifically to the filter was approximately 110 c.p.m. Points are mean of at least three experiments done in triplicate vertical lines show s.e.mean. (a) Spiperone (▲); (+)-butaclamol (●); (−)-butaclamol (○); dopamine (△); *cis* (Z) fluphentixol (■); *trans* (E) flupenthixol (□); clozapine (▼); noradrenaline (◆) ergosine methanesulphonate (▽). (b) Fluphenazine (○); sulpiride (□) haloperidol (●); chlorpromazine (△); metoclopramide (▲); apomorphine (◇); mianserin (◆).

Tris HCl, 5 mM Na_4EDTA, 0.02% ascorbic acid, 0.01% sodium azide, 0.025% CHAPS, pH 7.4) and centrifuged at 206,000*g*, 17 h, 4°C, SW 50.1 rotor in a Beckman L_{3-50} ultracentrifuge. Tubes were punctured at the bottom and 10 drop fractions were collected. After protein content determination (Spector, 1978), fractions from two tubes were pooled and 300 μl aliquots were taken for determination of specific [^{3}H]-spiperone binding at 4 nM of the radioligand. Marker proteins (Collection MS-II, Serva, Heidelberg, West Germany) were run under the same conditions and their position in the gradients was determined on the basis of protein content in individual fractions after centrifugation.

Analysis of data

Saturation curves were analysed by the Eadie-Hofstee technique as recommended by Zivin & Waud (1982).

Chemicals

[^{3}H]-spiperone (sp. act. 21 Ci mmol^{-1}) was obtained from the Radiochemical Centre, Amersham.

The following drugs were generously provided as gifts: (+)- and (−)-butaclamol (Ayerst, Canada); haloperidol and spiroperidol (Janssen Pharmaceutica, Beerse, Belgium); *cis*(Z)- and *trans* (E)- flupen-

thixol (H. Lundbeck & Co., Copenhagen, Denmark); chlorpromazine and fluphenzine (E.R. Squibb & Sons, Princeton, U.S.A.); metoclopramide and sulpiride (Delagrange, Paris, France); mianserin (Chemical Industry "Zorka", Šabac, Yugoslavia); clozapine and apomorphine (Sandoz Ltd., Basel, Switzerland) and ergosine methanesulphonate (Chemical and Pharmaceutical Industry "Lek", Ljubljana, Yugoslavia).

Detergents

CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonate), PAPS (3-[3-palmitoylamidopropyl) dimethyl-ammonio]-1-propanesulphonate), LAPS (3-[(3-laurylamidopropyl) dimethylammonio]-1-propanesulphonate) and NAPS (3-[(3-nonanoylamido-propyl) dimethylammonio]-1-propanesulphonate) were synthesized in our laboratory according to the method for the synthesis of CHAPS (Hjelmeland, 1980). Detergents of N-(+)-gluco-N-methylamide type (N-(+)-gluco-N-methylnonanoylamide [MEGA-9], N-(+)-gluco-methyl-laurylamide [MEGA-12] and N-(+)-gluco-N-methylcholamide were prepared as described by Hildreth (1982).

All other chemicals were products of Sigma, analytical grade.

Results

Solubilizing agents used for solubilization of synaptosomal membranes of the bovine caudate nuclei and their efficiencies are listed in Table 1.

As seen (Table 1) solubilizing agents from several chemical categories were used: (a) cardiac glycoside digitonin; (b) cholic acid derivatives (CHAPS, Na-deoxycholate and N-(+)-gluco-N-methylcholamide); (c) polyoxyethylene compounds (Lubrol PX, Triton X-100 and BRIJ 35); (d) N-(+)-gluco-N-methylalkaneamide (MEGA-9 and MEGA-12) and (e) sulphobetain detergents (NAPS, LAPS and PAPS). From the data presented in Table 1 it is obvious that CHAPS was the most efficient solubilizer of D_2-receptors among all detergents checked.

Yield of solubilization strongly depends on the ratio of the amount of membrane protein and the concentration of detergent, as illustrated in Figure 1.

Optimal solubilization was achieved with 0.25% CHAPS at a protein concentration of 3.5 mg ml^{-1} of incubation mixture. The receptor and protein recoveries are indicated in Table 2.

Examination of optimal conditions for membrane solubilization showed that the best yield was obtained by incubation of membrane suspensions at 4 °C with gentle stirring for 25 min.

In Figure 2, the effect of combined detergents, NaCl, sucrose and protectors of SH-groups on the yield of solubilization is depicted. The addition of sulphobetain detergents to CHAPS led to an increase in the amount of solubilized dopamine D_2-receptors. Sodium chloride and sucrose diminished the yield of solubilization, while protectors of sulphydryl groups, as well as N-(+)-gluco-N-methylalkaneamide detergents had almost no effect.

$-\log IC_{50}$ values for different dopamine agonists and antagonists used in competition experiments with solubilized and membrane preparations are listed in Table 3 and corresponding displacement curves are depicted in Figure 3.

Figure 4 represents correlation of $-\log IC_{50}$ values for solubilized and membrane-bound dopamine D_2-receptors. The correlation between values obtained in our experiments for the membrane-bound and the solubilized receptor was very good (a correlation coefficient r of 0.962).

The saturation curve of solubilized D_2-sites is given in Figure 5. Analysis of this curve performed by Eadie-Hofstee technique (Zivin & Waud, 1982) gave values of 3.61 ± 0.94 nM and

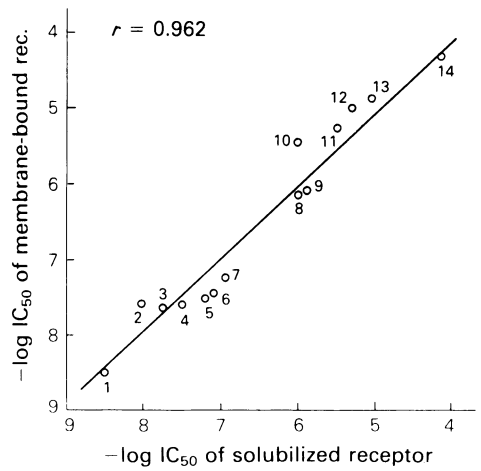


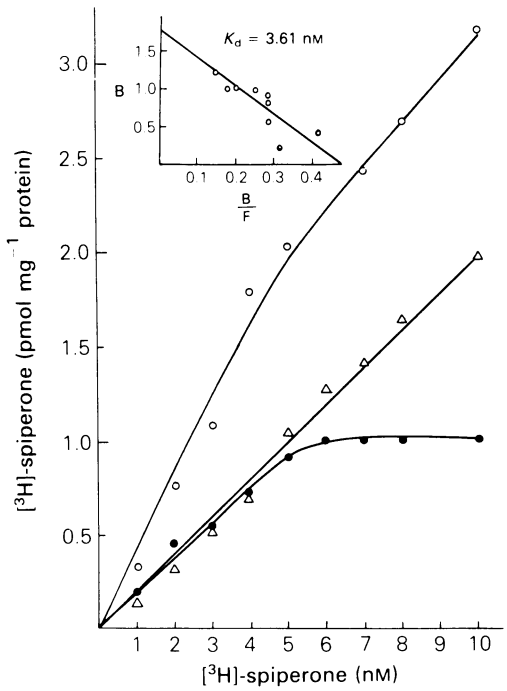
Figure 4 Correlation between $-\log IC_{50}$ values of CHAPS solubilized and membrane-bound receptors. IC_{50} values were calculated from competition curves shown in Figure 3. 1 = Spiperone; 2 = *cis*(Z) flupenthixol; 3 = (+)-butaclamol; 4 = fluphenazine; 5 = ergosine methanesulphonate; 6 = haloperidol; 7 = chlorpromazine; 8 = *trans*(E) flupenthixol; 9 = apomorphine; 10 = metoclopramide; 11 = mianserin; 12 = (−)-butaclamol; 13 = sulpiride; 14 = dopamine. IC_{50} values were determined as the concentrations of unlabelled drugs which protected 50% of dopamine binding sites against the binding of [^{3}H]-spiperone (correlation coefficient 0.962, s.e.mean about 10%).

Table 3 $-\log IC_{50}$ values for various dopamine agonists and antagonists on the membrane-bound and solubilized receptors

Competitor	$-log\ IC_{50}$ (membrane-bound receptors)	$-log\ IC_{50}$ (solubilized receptors)
Spiperone	8.50±0.11	8.51±0.10
cis (Z) Flupenthixol	7.70±0.10	8.04±0.18
(+)-Butaclamol	7.66±0.19	7.77±0.14
Fluphenazine	7.58±0.12	7.48±0.28
Ergosine methanesulphonate	7.45±0.14	7.22±0.18
Haloperidol	7.40±0.22	7.10±0.22
Chlorpromazine	7.20±0.04	6.96±0.21
trans (E) Flupenthixol	6.10±0.12	6.00±0.30
Metoclopramide	5.50±0.11	6.00±0.22
Apomorphine	6.08±0.05	5.91±0.15
Mianserin	5.32±0.37	5.48±0.31
(−)-Butaclamol	5.07±0.34	5.30±0.10
Sulpiride	5.13±0.07	4.96±0.28
Dopamine	4.29±0.25	4.10±0.24
Clozapine	4.20±0.18	4.10±0.43

IC_{50} values were calculated from competition curves obtained at 0.15 nM and 0.9 nM of [^{3}H]-spiperone for membrane-bound and solubilized receptors, respectively, and various concentrations of different drugs. Competition curves for membrane-bound receptors were analysed by two-site model to exclude 5-hydroxytryptamine receptors.

1.74 ± 0.26 pmol mg^{-1} protein for K_d and B_{max}, respectively. Corresponding values for membrane-bound receptors were: $K_d = 1.25 \pm 0.10$ nM, $B_{max} = 1.33 \pm 0.17$ pmol mg^{-1} protein.



[^{3}H]-spiperone association and dissociation kinetics performed according to Weiland & Molinoff (1981) are presented in Figure 6. $(R)_t$ was determined by Eadie-Hofstee analysis of the saturation data using the same solubilized membrane preparation. Association kinetics was analysed by pseudo-first order kinetics and k_1 calculated from k_{obs} was $(3.80 \pm 0.46) \times 10^{-3}$ min^{-1} nM^{-1}, $t_{\frac{1}{2}} = 21.0 \pm 2.2$ min. Dissociation kinetics was of first order, $k_{-1} = (1.66 \pm 0.17) \times 10^{-2}$ min^{-1}, $t_{\frac{1}{2}} = 34.5 \pm 4.0$ min. K_d calculated on the basis of these data was $k_{-1}/k_1 = 4.4 \pm 1.0$ nM and this value agrees well with that calculated from the saturation curve.

Examination of thermal stability of solubilized receptors (Figure 7) showed that they were much more heat labile than the membrane-bound receptors. Thermal inactivation of solubilized D_2 sites follows

Figure 5 Saturation curve of solubilized dopamine rceptors. Aliquots (400 µl) of soluble receptors preparation prepared with 0.25% CHAPS were assayed in the presence of varying concentrations of [^{3}H]-spiperone and in the presence or absence of 1 µM (+)-butaclamol. After 16 h at 4 °C 0.45% human gamma-globulin was added and samples were precipitated with 30% polyethylene glycol followed by vacuum filtration through Whatman GF/B filters. Total binding (○); specific binding (●); non-specific binding (△). Inset shows Eadie-Hofstee analysis of the same data. Each point represents mean of three experiments done in triplicate.

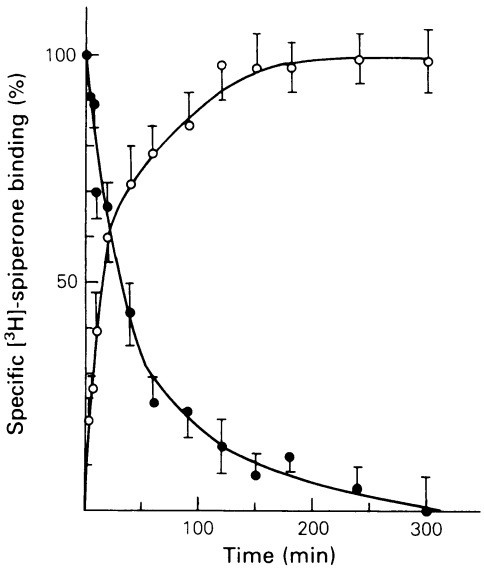


Figure 6 Association (○) and dissociation (●) curves for specific [^{3}H]-spiperone binding to solubilized receptors. Association kinetics: after solubilization under optimal conditions (0.25% CHAPS, 50 mM Tris HCl, 5 mM Na_4EDTA, 0.02% ascorbic acid, 0.01% sodium azide, pH 7.4, 25 min, 4 °C) solubilized proteins were separated by centrifugation (180,000 *g*, 30 min), 18 ml of solubilized receptor preparation was incubated with [^{3}H]-spiperone (final concentration 0.9 nM) in the presence or absence of 1 μM (+)-butaclamol at 4 °C. At indicated time intervals, aliquots of 400 μl were taken and specific binding was determined as the difference between total binding and binding in the presence of 1 μM (+)-butaclamol. Points are means of three experiments done in triplicate; s.e.mean shown by vertical lines.

Dissociation kinetics: 18 ml of solubilized receptors preparation were incubated with 0.9 nM [^{3}H]-spiperone for 20 h at 4 °C after which at zero point, 1000 fold excess of unlabelled spiperone was added. Aliquots of incubation mixtures were taken at different time intervals and specific binding was determined as described above. Each point is mean of three experiments done in triplicate; s.e. mean shown by vertical lines.

zero order kinetics and $t_{\frac{1}{2}}$ values were 15.0 ± 2.8 min, 121.7 ± 9.1 min and approximately 10 h, at 37 °C, 20 °C and 4 °C, respectively. Corresponding values for membrane-bound receptors were about 97 min at 37 °C and more than 6 h at 20 °C.

In order to determine sedimentation properties of solubilized D_2-sites membrane extracts were subjected to linear sucrose density gradient centrifugation (Figure 8). Specific binding of [^{3}H]-spiperone was determined in individual fractions at 4 nM of the radioligand. A higher concentration of [^{3}H]-spiperone was used in these experiments in order to saturate most of the available binding sites, because

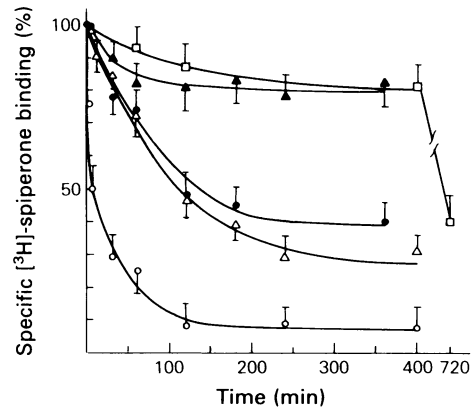


Figure 7 Comparison of the thermal stability of solubilized and membrane-bound dopamine D_2-receptors. Solubilized membrane preparations were incubated with 0.9 nM of [^{3}H]-spiperone at 4 °C (□), 20 °C (△) and 37 °C (○). Membrane suspensions were incubated at the same time with the same concentration of the radioligand at 20 °C (▲) and 37 °C (●). Aliquots of incubation mixtures were taken at varying time intervals and specific [^{3}H]-spiperone binding was determined as the difference between total binding and binding in the presence of 1 μM (+)-butaclamol. Each point is mean of three experiments performed in triplicate.

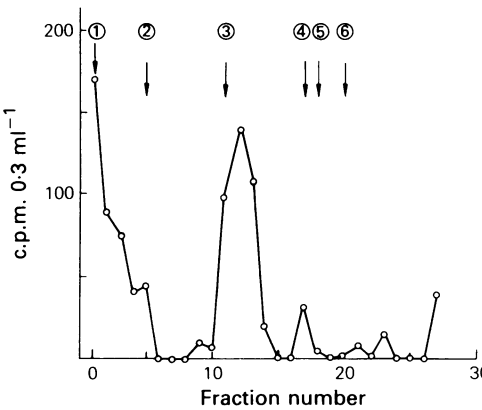


Figure 8 Sedimentation profile of CHAPS solubilized [^{3}H]-spiperone binding sites: 0.3 ml of solubilized membrane preparation (0.15 mg protein) was layered on linear sucrose density gradient (15–30% sucrose in 50 mM Tris HCl, 5 mM Na_4EDTA, 0.02% ascorbic acid, 0.01% sodium azide, 0.25% CHAPS). After centrifugation (206,000 *g*, 17 h, 4 °C, SW 50.1 rotor, Beckman L_{3-50} ultracentrifuge) tubes were punctured at the bottom and 10 drop fractions collected. Fractions from two tubes were pooled and 300 μl aliquots were incubated with [^{3}H]-spiperone at 4 nM of the radioligand in the presence or in the absence of 1 μM (+)butaclamol and specific binding was determined. Marker proteins were run simultaneously on separate gradients. Their position in the gradients was determined on the basis of protein content: 1 = ferritin; 2 = catalase; 3 = aldolase; 4 = bovine serum albumin; 5 = ovalbumin; 6 = chymotrypsinogen A.

of the small amount of protein (0.15 mg) applied to the gradient.

As seen from Figure 8, specific binding of [^{3}H]-spiperone showed two maxima, one in the first several fractions from the bottom which very probably represents agglomerates of D_2-sites and the other which sedimented between aldolase and bovine serum albumin, with apparent sedimentation coeffecient of 7.3 S.

Discussion

CHAPS appears to be the best solubilizing agent of dopamine D_2-receptors, among all detergents used throughout our studies. Under defined conditions described in this paper, the yield of solubilization of D_2-receptors of the bovine caudate nuclei was 18.6% or about three fold higher than the yield achieved with digitonin (Madras *et al.*, 1982). However, Hall *et al.* (1983) using cholate-NaCl, solubilized 36% of D_2-receptors from the same brain structure. In our experiments non-specific binding represents about 21% of total binding and the extract obtained was rich in D_2-receptors, as judged by the number of binding sites per mg protein. Addition of several sulphobetain detergents to CHAPS (PAPS, LAPS or NAPS) increased the extent of solubilization, and these results deserve more detailed studies. One of the explanations for such an increase could be the similarity in structure of these detergents to some phospholipid constituents of the membranes. Working with bovine striatum, Kuno *et al.* (1983) solubilized 26% of the original binding capacity with 10 mM CHAPS and 0.72 M NaCl, while we observed a decrease of the yield of solubilization when the membranes were treated with 0.250% CHAPS and 125 mM NaCl. This discrepancy of the data could be attributed to the difference in the conditions of solubilization. Addition of 0.25% sucrose to CHAPS also decreased the yield of solubilization, while MEGA-9, MEGA-12 and protectors of sulphydryl groups were almost completely ineffective.

D_2-sites solubilized by CHAPS fulfil most of the criteria suggested by Laduron & Ilien (1982) for solubilized receptors such as: (a) high affinity to the best ligands with binding constants within the nM range; (b) stereospecificity, as a significant difference in affinity for (+)- and (−)-butaclamol (more than 1000 times), as well as for *cis* (Z)- and *trans* (E)-flupenthixol (about 100 times) was observed; (c) saturability achieved in [^{3}H]-spiperone binding experiments; (d) reversibility; (e) chemical specificity checked by different agonists and antagonists and (f) correlation of $-\log IC_{50}$ values for solubilized and membrane-bound receptors. Besides these criteria, solubilized receptors did not sediment during centrifugation at 180,000g for 60 min, they were not retained at small size pore filters (Millipore, 0.45 μ) and they showed decreased thermostability in comparison with membrane-bound receptors.

$-\log IC_{50}$ values calculated from the competition curves obtained with different dopamine agonists and antagonists on solubilized receptors are in linear correlation with the corresponding values for membrane-bound receptors ($r = 0.962$, slope factor 0.96). This can be taken as the evidence that [^{3}H]-spiperone receptors solubilized by CHAPS retain their native properties.

The K_d value for solubilized receptors was 3.61 ± 0.94 nM, i.e. almost three times higher than the corresponding value for membrane-bound receptors which was 1.25 ± 0.10 nM. Similar effect was observed by several authors working on receptor solubilization (Gorissen *et al.*, 1979; Madras *et al.*, 1982; Davis *et al.*, 1982; Witkin & Harden, 1982; Kuno *et al.*, 1983) and it very probably results from the change in chemical environment of the receptor molecule.

The Hill coefficient for the saturation curve ($n = 1.22 \pm 0.02$) suggests the existence of only one class of solubilized receptors for [^{3}H]-spiperone. Shapes of [^{3}H]-spiperone association and dissociation curves demonstrate also the existence of one class of the receptors. Thermal stability of solubilized receptors was much lower than that of membrane-bound receptors and thermal inactivation proceeded rapidly at 37 °C ($t_{\frac{1}{2}} = 15.0 \pm 2.8$ min).

Behaviour of solubilized receptors on sucrose density gradients suggests either a heterogeneous nature of these structures or formation of aggregates. Similar results were obtained by several authors who analysed some other plasma membrane receptors (Sobel *et al.*, 1977; Guellaen *et al.*, 1979; Sherman-Gold & Dudai, 1980).

Our results show that CHAPS represents a very suitable agent for solubilization of dopamine-receptors from the bovine caudate nucleus, yield of D_2-receptors is relatively high and these receptors remain in their native form. Besides, CHAPS has a high critical micellar concentration and is neutral (Hjelmeland, 1980), so that further analysis of the solubilized receptors either by means of ion-exchange chromatography, or isoelectric focusing could be performed. This detergent can also be easily removed by simple dialysis. Earlier data showed that CHAPS can be successfully used for solubilization of opiate (Simonds *et al.*, 1980) and dopamine receptors (Lew *et al.*, 1980; Kuno *et al.*, 1983).

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