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Reconstitution of affinity-purified dopamine D_2 receptor binding activities by specific lipids

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The role of lipids in maintaining ligand binding properties of affinity-purified bovine striatal dopamine D_2 receptor was investigated in detail. The receptor, purified on a haloperidol-linked Sepharose CL6B affinity column, exhibited low [^{3}H]spiroperidol binding unless reconstituted with soybean phospholipids. In order to understand the role of individual phospholipids in maintaining the receptor binding activity, the purified preparation was reconstituted separately with individual phospholipids and assayed for [^{3}H]spiroperidol binding. Except for phosphatidylcholine and phosphatidylethanolamine, that respectively restored 30 and 20% binding as compared to that obtained with soybean lipids, reconstitution with other lipids had very little effect. When various combinations of phospholipids were used for reconstitution, a phosphatidylcholine and phosphatidylserine mixture seemed to almost fully restore the receptor binding. A mixture of phosphatidylcholine and phosphatidylethanolamine was as effective as phosphatidylcholine alone in reconstituting ligand binding; however, when phosphatidylserine was also included in the mixture, there was a pronounced increase in binding (about 2-fold compared to the soybean lipids and about 6-fold compared to the phosphatidylcholine-phosphatidylethanolamine mixture). Substitution of other phospholipids or cholesterol for phosphatidylserine in phosphatidylcholine and phosphatidylethanolamine mixture had little effect. Maximal reconstitution of [^{3}H]spiroperidol binding was obtained with phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine mixture (2:2:1, w/w) at a concentration of 0.5 mg/ml. The reconstituted receptor exhibited high affinity binding for [^{3}H]spiroperidol which was comparable to that obtained with membrane or solubilized preparations. Various dopaminergic antagonists and agonists showed appropriate order of potency for the reconstituted receptor. The presently described reconstitution data suggest a role of specific phospholipids in preserving the binding properties of dopamine D_2 receptor and should prove useful in studies on functional reconstitution of the receptor.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid.

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Introduction

The role of lipids in modulating hormone and neurotransmitter receptor mechanisms is now well recognized; however, the question of specific lipid requirement for the maintenance of receptor func-

tions remains unresolved [1,2]. Lipid involvement in receptor-ligand interactions and the subsequent transduction mechanisms has largely been suggested by treatment of memrbanes with various phospholipases. The interpretation of results obtained by such methods is made difficult by the presence of cleavage products in the preparation. Thus, in order to study the requirement of specific lipids in receptor functions, it is desirable to obtain a purified preparation of the receptors and reconstitute them in lipid vesicles of defined composition. Data on specific lipid requirement of isolated receptors are limited, but for those receptors that have been reconstituted in lipid vesicles or planar bilayers, the importance of particular lipids was noted in some instance but not in others [3]. For example, no specificity for the phospholipid polar head or its alkyl chain could be defined for β-adrenergic receptor [4] or nicotinic cholinergic receptor [5], although in the latter case, cholesterol and negatively charged lipids were found to improve the functional reconstitution in terms of ion-gating properties [5–7]. Using a delipidated preparation of β-adrenergic receptor that showed no antagonist binding, it has been reported [8] that reconstitution of the receptor with phosphatidylethanolamine restored maximal binding compared to other phospholipids, and that cholesterol ester in mixture with acidic phospholipids dramatically increased the antagonist binding. Similarly, isolation of opiate receptors by affinity chromatography has been shown to result in a loss of ligand binding which could be restored by reconstituting the receptor with an acidic lipid fraction from the column [9]. The same group of workers has recently reported [10] a change in opiate receptor characteristics with alteration of membrane lipid composition by a new enzymatic reconstitution method.

Brain dopamine receptors have been classified into two subtypes, D_1 and D_2, based on their affinity towards dopaminergic ligands and relationship to adenylate cyclase [11]. The bovine caudate D_2 receptor has been solubilized with cholate and reconstituted in crude soybean phosphatidylcholine [12]. An enhancing effect of phosphatidylcholine and phosphatidylserine on D_2-antagonist binding to crude cholate extracts of rat brain synaptic membranes has also been observed [13]. Since in the above studies, the effects of lipid were studied in crude extracts, it is difficult to assess the requirement of specific lipids by the receptor.

We have recently purified bovine-striatal dopamine D_2 receptor by affinity chromatography on haloperidol-Sepharose and have found that inclusion of lipids was necessary in the elution buffer to obtain active receptor [14]. In another recent report on the purification of anterior pituitary dopamine D_2 receptor [15], an absolute requirement of reconstitution with soybean phospholipids had been noted for the demonstration of receptor characteristics; however, no lipid specificity for the receptor was evaluated. In our goal toward functional reconstitution of dopamine D_2 receptor with its effector system, we first set out to define the role of various phospholipids in reconstituting the ligand-binding properties of the purified receptor. In the present communication, we describe the reconstitution of purified dopamine D_2 receptor in phospholipid vesicles of defined composition and demonstrate that a combination of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine offers an optimal system for the study of receptor characteristics.

Materials and Methods

Materials. [^{3}H]Spiroperidol (25 Ci/mmol) and [^{3}H]haloperidol (18 Ci/mmol) were purchased from New England Nuclear. The following unlabelled drugs were generous gifts: haloperidol (McNeil, Canada); spiroperidol and domperidone (Janssen, Belgium); N0434 (Nelsen, U.S.A.). *N*-Propylnorapomorphine, (+)- and (−)-butaclamol were obtained from Research Biochemicals Inc., Wayland, MA, U.S.A. Apomorphine, phenylmethylsulphonyl fluoride (PMSF), and all the phospholipids were purchased from Sigma Chemical Co., St. Louis, U.S.A. Sepharose CL6B and cholic acid were from Pharmacia (Uppsala, Sweden) and Calbiochem, (La Jolla, CA, U.S.A.) respectively. Bio-Bead SM-2 was from Bio-Rad and was washed with methanol and water prior to use. All other chemicals were of the highest available grade.

Solubilization and purification of D_2 receptor. Bovine striatal membranes and soluble prepara-

tions were obtained as described previously [16]. Briefly, solubilization was carried out by stirring equal volumes of the membranes and a detergent solution (0.5% cholic acid/1.8 M NaCl/1 mM dithiothreitol/0.2 mM PMSF) in an ice-bath for 1 h and then centrifuging the mixture at 100 000 × *g* for 1 h. The clear supernatant was diluted 4-fold with 50 mM Tris-HCl (pH 7.4) containing 1 mM EDTA, and used for purification.

The receptor was purified using an affinity column of haloperidol-Sepharose, essentially as described earlier [14]. The receptor was eluted from the column either by 500 nM spiroperidol in a buffer containing 50 mM Tris/1 mM EDTA/0.225 M NaCl/0.06% cholic acid at pH 7.4 or by 0.5 M Tris containing 1 mM EDTA/0.225 NaCl/0.06% cholic acid at pH 8.5. When spiroperidol was used for elution the ligand was removed from the eluate on Sephadex G-50 after concentrating it in Amicon centriflo cones. The Tris elution protocol was followed in most of the reconstitution studies described herein. In either case, no appreciable ligand binding (assayed with [^{3}H]spiroperidol) could be detected unless crude soybean phospholipids or bovine brain total lipids (0.1–0.5 mg/ml) were present in the elution buffer. The receptor thus eluted showed a specific activity of 150–170 pmol/mg protein, representing about 2000-fold purification from the membranes.

Reconstitution with lipids. The reconstitution of the purified receptor was performed according to the method of Cerione et al. [17] with modifications that included omission of ultracentrifugation step. The various phospholipids used in reconstitution were dispersed as follows. The phospholipid solution (in chloroform) was evaporated to dryness under nitrogen and resuspended in 50 mM Tris (pH 7.4) containing 1 mM EDTA by vigorous vortexing to give a final concentration of 5 mg/ml unless otherwise specified. Phosphatidylserine or other dry lipids were dissolved in the organic solvent (or added to other phospholipid solutions, if used in mixture), evaporated and suspended as above. Phosphatidic acid was directly suspended in the Tris-EDTA buffer. All lipid suspensions were sonicated in a sonicator bath (Bransonic) for 10–15 min until no further decrease in turbidity was noted.

Unless otherwise mentioned, the phospholipid dispersions were added to the receptor preparation at a final concentration of 0.5 mg/ml lipid and 0.4 μg/ml protein, respectively. After incubation for 30 min at 0–4° C, washed Bio-Bead SM-2 (0.04 g/ml) was added and the mixture was stirred gently for about 30 min. The Bio-Beads were then removed by centrifugation at 1000 × *g*.

Receptor binding assay. The binding of [^{3}H]spiroperidol to the reconstituted preparations was assayed as previously described for purified preparation [14]. The total binding was determined in 1.0 ml assay volume containing 1 nM [^{3}H]spiroperidol in 50 mM Tris/1 mM EDTA (pH 7.4), and the nonspecific binding was measured in parallel assays containing 1 μM (+)-butaclamol. A titrated amount of HCl was added to the buffer so that the final pH, after addition of receptor preparation, was 7.4. Incubation was carried out at 4° C for 16 h and the bound ligand was separated on GF/C filters after precipitation with poly(ethylene glycol) (10% final concentration). The binding of [^{3}H]spiroperidol to various lipid dispersions alone was also determined the same way and, in all cases, the specific binding was always found to be less than 10% of the corresponding lipid reconstituted preparation.

Protein determination. Protein was determined by the Amido schwartz method [18] using bovine serum albumin as a standard.

Results

Purification and reconstitution of the receptor with individual lipids

The receptor eluted from the affinity column showed low specific binding when assayed with the specific antagonist [^{3}H]spiroperidol. However, reconstituted with crude soybean phospholipids, it showed a specific binding of about 160 pmol/mg protein that represented a 2000-fold purification of the receptor. In order to understand the role of individual phospholipids in supporting the reconstitution, the receptor preparation was reconstituted separately with different purified lipids and assayed for [^{3}H]spiroperidol binding (Fig. 1). Except for phosphatidylcholine (PC) and phosphatidylethanolamine (PE), that restored about 30 and 20% binding respectively as compared to the

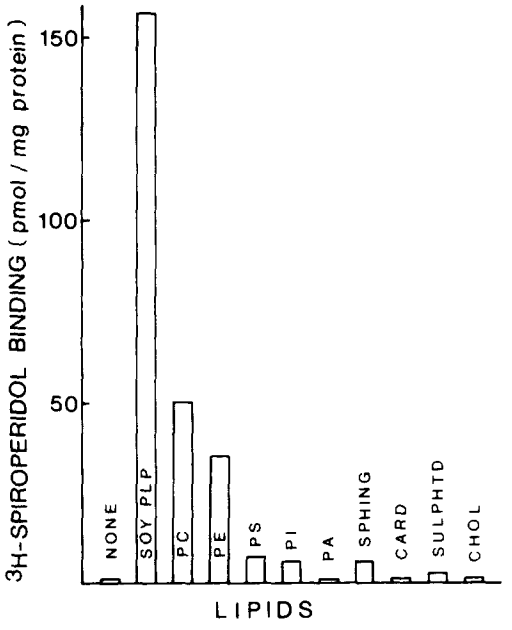

Fig. 1. [^{3}H]Spiroperidol binding by purified D_2 receptor reconstituted with different lipids. NONE, no lipids added; SOY PLP, soybean phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; SPHING, sphingomyelin; CARD, cardiolipin, SULPHTD, sulphatide; CHOL, cholesterol. The receptor preparation was reconstituted with the different lipids (0.5 mg/ml, final concentration) as described under Materials and Methods. The results are means of tripicate determinations carried out on the same receptor preparation and are representative of three separate experiments with different batches of receptor preparations, (S.E. $\leq$ 10%).

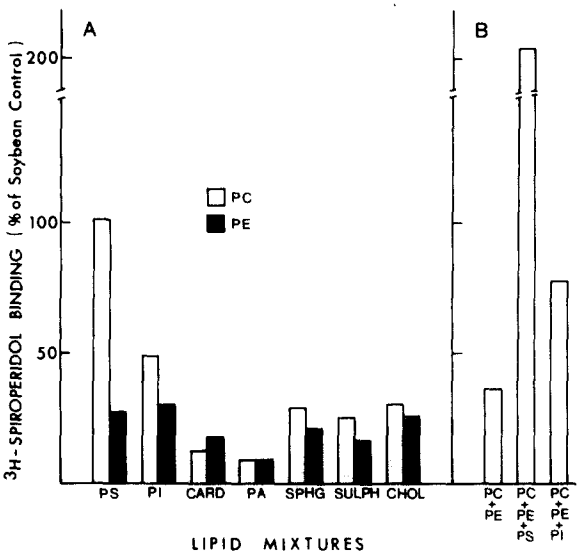

Fig. 2. Effect of a combination of different lipids on reconstitution of [^{3}H]spiroperidol binding by purified dopamine D_2 receptor. A. Reconstitution of the receptor by different lipids in mixture with either PC or PE. The different lipid additions with PC or PE were done at a proportion of 2:1, w/w (PC or PE: lipid added) and the final concentration of lipids in reconstitution medium was 0.5 mg/ml. The results are expressed relative to that obtained with soybean phospholipids (100%). B. Effect of reconstitution with a PC, PE mixture (1:1, w/w) and addition of PS or PI to PC and PE mixture. The PC/PE/PS and PC/PE/PI mixtures were in 2:2:1 (w/w) ratio. The final concentration of lipid was kept constant at 0.5 mg/ml. Results are relative to that obtained with soybean phospholipids (100%). Results are means of triplicate determinations carried out on the same receptor preparation and are representative of three separate experiments. See Fig. 1 for abbreviations.

soybean lipids, none of the other lipids had any significant effect. The binding increased as a function of lipid (PC or PE) concentration from 0.05 mg/ml to 0.2 mg/ml and remained more or less constant up to a concentration of 2 mg/ml (data not shown). Assuming that more than one type of lipid may be necessary to support the optimum reconstitution of the receptor, various combinations of other lipids with PC or PE were tested (Fig. 2a). A PC and phosphatidylserine (PS) mixture almost fully restored the binding activity when compared to the soybean control. Phosphatidylinositol (PI) with PC also restored 50% of binding but inclusion of other lipids with PC or PE had only little effect. In fact, some acidic phospholipids, like phosphatidic acid and cardiolipin inhibited the antagonist binding when mixed with PC or PE. PC and PE together were as good as PC alone in the reconstitution of [^{3}H]spiroperidol binding (Fig. 2b), but interestingly, when phosphatidylserine was included in the PC/PE mixture, there was a dramatic increase in the binding (about 2-fold compared to the soybean control and about 6-fold compared to PC/PE mixture). A mixture of PI with PC and PE, although much less effective than PC/PE/PS mixture, did cause an increase in the antagonist binding. None of the other lipids – phosphatidic acid, cardiolipin, sulphatide, sphingomyelin or cholesteryl hemisuccinate – caused any appreciable increase in binding when used in combination with PC/PE mixture (data not shown).

In order to test whether phospholipids are indispensable for the reconstitution of receptor, a dispersion of cholesteryl hemisuccinate and monolein glycerol (1 : 1, w/w) was used; however, no appreciable increase in the binding above that obtained with cholesterol ester itself could be noted (data not shown).

To investigate the proportional requirement of different phospholipids in the PC/PE/PS, PC/PE/PI and PC/PS mixtures to reconstitute the receptor, different ratios of individual lipids in the mixtures were used for reconstitution at a total phospholipid concentration of 0.5 mg/ml. A broad range of optimal ratio from 5 : 5 : 1 to 2 : 2 : 1 (w/w) was observed for PC/PE/PS mixture; for PC/PE/PI mixture, the ratio of individual lipids from 10 : 10 : 1 to 1 : 1 : 1 was found optimal. Equal proportions of the three lipids in PC/PE/PS mixture decreased the receptor binding. For PC/PS mixture, an optimum ratio of 10 : 1 to 2 : 1 was observed. Various phospholipids were added to PC or PE (Fig. 2a) at two different ratios (5 : 1 and 2 : 1); no significant difference was noted and the data therefore are reported for 2 : 1 ratio.

To determine the optimal phospholipid-to-protein ratio, a constant amount of receptor was titrated with increasing amount of a fixed proportion of PC/PE/PS, PC/PE/PI or PC/PS mixture (Fig. 3). It was found that PC/PE/PS, at a concentration of 0.5 mg/ml (which was used in experiments of Fig. 2) produced maximal binding. PC/PS and PC/PE/PI mixtures were less efficient at all concentrations used. Higher concentrations of PC/PE/PS and PC/PS mixtures appeared inhibitory.

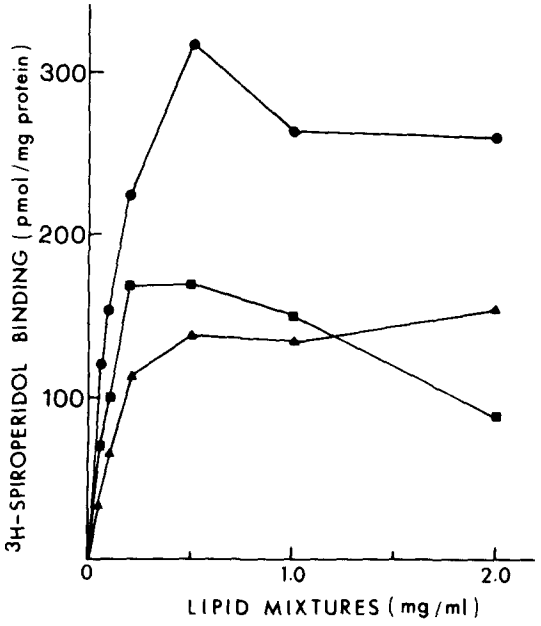

Fig. 3. Concentration of different phospholipid mixtures required for reconstitution of maximal binding of purified dopamine D_2 receptor. ●, PC/PE/PS (2:2:1, w/w); ▲, PC/PE/PI (2:2:1, w/w); ■ PC/PS (2:1, w/w). Different amounts of the phospholipid mixtures at the indicated proportions were added to the receptor preparation and the volume was brought to the same level with 50 mM Tris/1 mM EDTA buffer (pH 7.4). The values are means of triplicate determinations that are representative of two or three experiments.

Competition of the reconstituted receptor with membrane-bound receptors

An important test of the integrity of the isolated receptors is their ability to compete for ligands with the membrane-bound receptors [19]. As shown in Fig. 4, the purified receptor reconstituted with PC/PE/PS mixture inhibited [^{3}H]spiroperidol binding to bovine striatal mem-

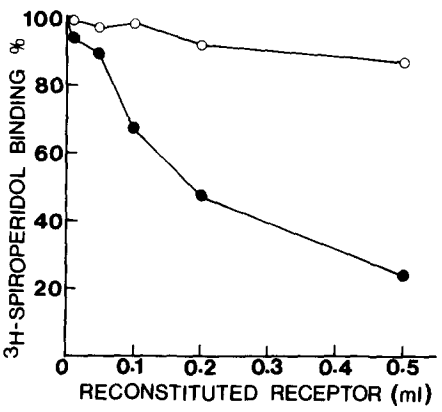

Fig. 4. Inhibition of [^{3}H]spiroperidol binding to bovine striatal membrane by the purified receptor reconstituted with PC/PE/PS mixture. ●, reconstituted receptor; ○, lipid mixture. The receptor was reconstituted as described in Materials and Methods, and various amounts of the reconstituted receptor or lipid mixture were added directly to the binding assay system for membrane receptor. Membrane receptor assays were performed essentially as described in Materials and Methods except that incubation was carried out at 25 °C for 60 min and the contents filtered on GF/B filters. Results are means of triplicate determinations and are representative of two or three experiments.

branes in a concentration-dependent manner. Lipid mixture alone showed only a small inhibition at the highest amount used. However, no inhibition could be observed with the purified receptor preparation alone (data not shown).

Binding specificity of the reconstituted receptor

The receptor reconstituted with PC/PE/PS revealed binding parameters that were similar to those observed for the solubilized or purified preparations [14]. The binding to antagonist [^{3}H]spiroperidol was saturable and the Scatchard analysis of the data (Fig. 5) yielded an apparent dissociation constant (K_d) of 0.65 nM. Displacement of [^{3}H]spiroperidol with various dopaminergic antagonists and agonists followed a similar rank order of potency as that observed with the solubilized or purified receptor. The IC_{50} values of different drugs are compared in purified and reconstituted preparations (Table I) and it is shown that, although the rank order of potency is similar in both the preparations, there are differences in the absolute values. In general, the IC_{50} values for antagonists and agonists were slightly higher for the reconstituted preparation.

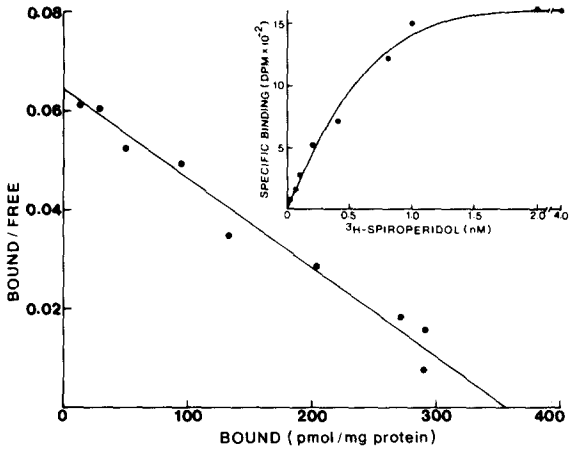

Fig. 5. Scatchard plot and saturation isotherm (inset) of [^{3}H]spiroperidol binding to reconstituted dopamine D_2 receptor. The receptor was reconstituted with a mixture of PC/PE/PS (2:2:1, w/w) at a final concentration of 0.5 mg/ml as described in Materials and Methods. Assays were done as described in Materials and Methods at [^{3}H]spiroperidol concentrations varying from 0.025 nM to 4 nM. Results are means of triplicate determinations and are representative of three separate determinations with different batches of receptor preparations.

TABLE I

BINDING SPECIFICITY OF VARIOUS DOPAMINERGIC LIGANDS TO RECONSTITUTED DOPAMINE D_2 RECEPTOR

The receptor was reconstituted with a mixture of PC, PE and PS as described in Materials and Methods. The IC_{50} values (concentration of ligands that inhibit 50% of [^{3}H]spiroperidol binding) were obtained by using 0.5 nM [^{3}H]spiroperidol and 7 to 9 concentrations of the competing ligands (from 10^{-10} to 10^{-4} M) and were calculated by log probit analysis. The values are means of two or three experiments carried out in triplicate. Results for purified preparations are from Ref. 14.

Ligand	Affinity purified IC_{50} (nM)	Reconstituted IC_{50} (nM)
Antagonists		
spiroperidol	0.7	2.1
(+)-butaclamol	9.66	18.7
domperidone	15.0	44.0
haloperidol	24.0	47.0
(−)-butaclamol	> 2000.0	> 2000.0
Agonists		
N-propylnorapomorphine	33.0	85.0
N0434	167.0	410.0
apomorphine	500.0	520.0
Others		
propranolol	> 10000	> 10000
phentolamine	> 10000	> 10000
ketanserine	1100	1285

Discussion

The present study shows that ligand binding to purified striatal dopamine D_2 receptor can be restored modestly by reconstituting with crude soybean lipids and to a great extent by a mixture of PC/PE/PS. None of the purified lipids was able to restore the binding to any appreciable extent when used individually in reconstituting the receptor, although PC and PE did show some promise. The dramatic increase in the receptor binding by inclusion of PS with PC or with PC/PE mixture may suggest a role of the acidic lipid in the expression of ligand binding. No stringent specificity of phospholipids can be defined from the results, since PI, substituting for PS, showed a similar effect, though to a much lesser extent. Although Moroi and Hsu [13] too reported an increase in [^{3}H]spiroperidol binding to synaptic

membrane cholate extract by PC, their results regarding the enhancing effect by PS alone do not quite agree with the present studies. Since in our experiments with purified receptor preparation, PS strongly stimulated binding only in combination with PC or PC/PE mixture, it may be possible that the cholate extract already contains some amount of neutral lipids. Acidic phospholipids, especially phosphatidic acid and PS have been reported to have marked influence on the properties of some other receptor systems, when used in combination with PC or PE [7,20], but it is surprising to note that in the present experiments, besides PS and PI, neither acidic phospholipids nor cholesterol was able to stimulate the ligand binding to the receptor. The significance of this observation is not clear at present.

Although little is known about the molecular processes by which lipids and proteins interact during reconstitution to form vesicles, there could be several possibilities for the differential effects of lipids. Different lipids may not associate to the same extent with the receptor, or if they do, the conformation formed by such an association may not be suitable for ligand binding. It may also be possible that some of the incorporated receptors are trapped within the vesicles or oriented towards its interior, thereby masking the ligand binding sites. Thus, it cannot be said with any certainty if the stimulatory action of PS in PC/PE/PS mixture is due to a direct specific requirement of this phospholipid for the ligand binding to the receptor or due to its effect on receptor protein conformation by providing a negatively charged environment.

The receptor reconstituted with PC/PE/PS mixture demonstrated high affinity for [^{3}H]spiroperidol (K_d = 0.65 nM) which is almost similar to that observed for solubilized or purified preparations [14–16]. The receptor thus reconstituted also competed with membrane-bound receptors for the antagonist and exhibited the characteristic rank order of potency for different dopaminergic antagonists and agonists. However, the IC_{50} values for different drugs were generally somewhat higher with the reconstituted preparation than those observed with the purified preparation [14]. Although the reasons are not clear, a change in the affinity of ligands has generally been reported by several research groups for different receptor systems reconstituted in lipid vesicles [8,9,21]. The changes in affinity may possibly have been caused by a higher weight ratio of lipid to protein (1000 : 1) used in the present experiments to achieve most efficient reconstitution of the receptor. The lack of any effect of cholesterol upon reconstitution may suggest that membrane fluidity is not an important parameter in dopamine D_2 receptor binding characteristics.

Since the receptor did not show any appreciable binding in the absence of lipids, it is difficult to assess the true efficiency of reconstitution. However, in terms of relative efficiency, the presence of zwitterionic and acidic lipids appears to offer the most suitable reconstitution medium for the expression of ligand binding properties. A requirement of complex mixture of phosphatidylserine, phosphatidylethanolamine and cholesterol has indeed been shown for efficient reconstitution of nicotinic acetylcholine receptor [22] and β-adrenergic receptor [23]. In this context, it is relevant to take note of a recent report [24] that acidic phospholipid species, viz., PS and phosphatidic acid inhibit fluoride and glucagon-activated adenylate cyclase in rat liver membrane, presumably by acting at guanine nucleotide regulatory proteins. Although no direct bearing of such findings on the present experiments can be related at present, it would be worthwhile to investigate if these lipids have similar action on striatal adenylate cyclase where dopamine D_2 receptor is negatively coupled to the cyclase.

In summary, the present studies define the lipid requirements for successful reconstitution of purified dopamine D_2 receptor binding properties, which should be helpful in our efforts to functionally reconstitute the purified D_2 receptor with guanine nucleotide regulatory protein(s) and adenylate cyclase.

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References

1 Loh, H.H. and Law, P.Y. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 201–234
2 Fain, J.N. (1986) In The Receptors (Conn. P.M., ed.), Vol. 3, pp. 213–241, Academic Press, New York
3 Levitzki, A. (1985) Biochim. Biophys. Acta 822, 127–153
4 Leftkowitz, R.J., Cerione, R.A., Codina, J., Birnbaumer, L. and Caron, M.G. (1985) J. Membrane Biol. 87, 1–12
5 Ochoa, E.L.M., Dalziel, A.W. and McNamee, M.G. (1983) Biochim. Biophys. Acta 727, 151–162
6 Criado, M., Eibl, H. and Berrantes, F.T. (1984) J. Biol. Chem. 259, 9188–9198
7 Fong, T.M. and McNamee, M.G. (1986) Biochemistry 25, 830–840
8 Kirilovsky, J. and Schramm, M. (1983) J. Biol. Chem. 258, 6841–6849
9 Cho, T.M., Ge, B.L., Yamato, C., Smith, A.P. and Loh, H.H. (1983) Proc. Natl. Acad. Sci. USA 80, 5176–5180
10 Farahbakhsh, Z.T., Deamer, D.W., Lee, N.M. and Loh, H.H. (1986) J. Neurochem. 46, 953–962
11 Mishra, R.K. (1986) in Neuromethods: Neurochemistry (Boulten, A., Baker, G. and Hrdina, P., eds.), Vol. 4, pp. 23–54, Humana Press, Clifton, NJ
12 Wheatley, M. and Strange, P.G. (1984) FEBS Lett. 166, 389–392
13 Moroi, K. and Hsu, L.L. (1985) Int. J. Biochem. 17, 637–644
14 Ramwani, J. and Mishra, R.K. (1986) J. Biol. Chem. 261, 8894–8898
15 Senogles, S.E., Amlaiky, N., Johnson, A.L. and Caron, M.G. (1986) Biochemistry 25, 749–753
16 Kazmi, S.M.I., Ramwani, J., Srivastava, L.K., Rajakumar, G., Ross, G.M., Cullen, M. and Mishra, R.K. (1986) J. Neurochem. 47, 1493–1502
17 Cerione, R.A., Strulovici, B., Benovic, J.L., Strader, C.D., Caron, M.G. and Leftkowitz, R.J. (1983) Proc. Natl. Acad. Sci. USA 80, 4899–4903
18 Schaffner, W. and Weissman, C. (1973) Anal. Biochem. 56, 502–514
19 Cuatrecasas, P. (1973) Biochemistry 12, 3547–3557
20 Bach, R., Gentry, R. and Nemerson, Y. (1986) Biochemistry 25, 4007–4020
21 Asano, T., Pedersen, S.E., Scott, C.W. and Ross, E.M. (1984) Biochemistry 23, 5460–5467
22 Dalziel, A.W., Rolins, E.S. and McNamee, M.G. (1980) FEBS Lett. 122, 193–196
23 Smigel, M.D., Ross, E.M. and Gilman, A.G. (1984) in Cell Membranes, Methods and Reviews (Elson, E.L., Frazier, W.A. and Glaser, L., eds.), pp. 247–294, Plenum Press, New York
24 Houslay, M.D., Needham, L., Dodd, N.J.F. and Grey, A.M. (1986) Biochem. J. 235, 237–243