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The Interaction between D-2 Dopamine Receptors and GTP-Binding Proteins

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SUMMARY

D-2 dopamine receptors were solubilized from porcine striatal membranes with 0.3% sodium cholate/1 M NaCl and separated from the bulk of the guanine nucleotide-binding regulatory proteins (G-proteins) by Ultrogel AcA34 gel filtration chromatography. The partially purified D-2 receptors were reconstituted in phospholipid vesicles with G_i or G_o purified from porcine brain. The dissociation constant (K_d) of the D-2 receptors in the reconstituted vesicles for [^{3}H]spiperone binding was 82–89 pM, which was not affected by the presence or absence of G-proteins. The displacement curve for [^{3}H]spiperone/dopamine was analyzed, assuming that there are two populations of binding sites. The K_d values for the binding sites with high affinity for agonists (HAS) and that for the binding sites with low affinity for agonists (LAS) were approximately 1 μM and 100 μM, respectively. The proportion of HAS was 8% when the receptor preparation was reconstituted into phospholipid vesicles without G-proteins, but it increased to 58–64% with increasing G-protein concentrations. The potency of G_o was a little higher than that of G_i. The proportion of HAS in the presence of G-proteins decreased to about 11% on addition of GTP. When G-proteins were treated with islet-activating protein, GTP-sensitive HAS were not observed. These results indicate that at least 50% of the partially purified D-2 receptors interact with both G_i and G_o.

Dopamine receptors are classified into the D-1 and D-2 subtypes, the former being coupled to the activation of adenylate cyclase and the latter not (1). D-2 dopamine receptors (D-2 receptors) have been shown to be related to inhibition of adenylate cyclase in the anterior pituitary (2, 3), striata (4–7), and retina (8), inhibition of inositol phospholipid metabolism (9, 10), inhibition of phosphorylation of synaptic vesicle proteins (11), and inhibition of prolactin secretion (10, 12). Recently, several lines of evidence have indicated that the function of D-2 receptors is mediated by activation of guanine nucleotide-binding regulatory proteins (G-proteins). The inhibition of adenylate cyclase through D-2 receptors requires the presence of GTP (7) and is abolished on treatment with IAP (pertussis toxin) (13). Cote *et al.* (14) showed that IAP prevented the inhibition by D-2 receptors of pro-opiomelanocortin synthesis in rat intermediate lobe tissue. The interaction of D-2 receptors with G-proteins has also been suggested by the results of experiments showing that guanine nucleotides decrease the affinity of D-2 receptors for agonists in membranes (15–17) or solubilized (18) preparations, and that the effect of guanine nucleotides on agonist binding of D-2 receptors in the retina is eliminated on treatment with IAP (19).

These results are consistent with the idea that one of the functions of D-2 receptors is the activation of G-proteins, and the activated G-proteins mediate most, if not all, of the diverse responses. The relevant G-protein has been supposed to be the inhibitory G-protein (G_i), because the activation of D-2 receptors has been shown to result in the inhibition of adenylate cyclase. However, direct evidence for the interaction between D-2 receptors and G_i has not been obtained. In addition, the possibility still remains that D-2 receptors also interact with G-proteins other than G_i. The most abundant G-protein in the brain is G_o, and G_o as well as G_i has been shown to interact with muscarinic acetylcholine (20) and γ-aminobutyric acid receptors (21).

In the present study at least 50% of the partially purified D-2 receptors in reconstituted phospholipid vesicles were found to interact with both G_i and G_o.

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Experimental Procedures

Materials. [^{3}H]Spiperone (76–89 Ci/mmol) and [^{35}S]GTPγS (1100 Ci/mmol) were purchased from Amersham; bovine serum albumin,

ABBREVIATIONS: IAP, islet-activating protein (pertussis toxin); G_i, the G-protein associated with inhibition of adenylate cyclase; G_o, a G-protein of unknown function discovered in brain with an α-subunit of 39,000 daltons that is ADP-ribosylated by islet-activating proteins; HAS, binding sites with high affinity for agonists; LAS, binding sites with low affinity for agonists; DTT, dithiothreitol; GTPγS, guanosine 5′-(3-*O*-thio)triphosphate; 5HT, 5-hydroxytryptamine (serotonin); Hepes, 4-(2-hydroxyethyl)-1-piperazinesthanesulfonic acid; EDTA, ethylenediaminetetraacetate; dimethyl POPOP, 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene; Dotite DPO, 2,5-diphenyloxazole.

GTP, and the bovine brain extract (Folch Fraction I) from Sigma; deoxycholic acid from Aldrich; DTT, ascorbate, and pargyline from Wako Pure Chemical Industries; dopamine from Nakarai Chemicals; Ultrogel AcA34 from Pharmacia LKB Biotechnology; and Sephadex G-50 (fine) from Pharmacia. Ketanserin was donated by Kyouwa-Hakko Co. and sulpiride by Fujisawa Pharmaceutical Co. and Sumitomo Pharmaceutical Co.

Partial purification of D-2 receptors. All procedures were carried out at 4° unless otherwise stated. Synaptic membranes (640 ml; protein concentration, 19 mg/ml) were prepared from 600 g of fresh or frozen porcine striata by the procedure described previously (22) and stored at −80° before use. A portion of the preparation (170 ml) was thawed and mixed with 330 ml of medium A [1 M NaCl, 20 mM Hepes-NaOH buffer (pH 8.0), 4 mM $MgCl_2$, 1 mM Na_3-EDTA, 1 mM DTT, 100 nM ketanserin] supplemented with 0.1 mM sulpiride. To the suspension was added slowly with stirring 500 ml of medium A containing 0.6% sodium cholate. After stirring for an additional 1 hr, the suspension was centrifuged at 95,000 × *g* for 1 hr. The supernatant was collected and concentrated in dialysis tubes with solid polyethylene glycol 20,000 as a water adsorbent. The concentrated solution (approximately 50 ml) was applied to a column (4.5 × 74 cm) of Ultrogel AcA34 that had been equilibrated with medium A supplemented with 0.3% sodium cholate and 0.1 mM sulpiride. Elution was carried out with the same buffer, and 15-ml fractions were collected at a flow rate of 50 ml/hr. The fractions exhibiting [^{3}H]spiperone binding but not appreciable [^{35}S]GTPγS-binding activity were combined and concentrated to approximately 50 ml with polyethylene glycol 20,000 as above. The molar concentration of D-2 receptors was calculated from the [^{3}H]spiperone binding.

Purification of G-proteins. A mixture of G_i and G_o was obtained from porcine brain as described by Sternweis and Robishaw (23), and then G_i and G_o were separated by DEAE-Toyopearl chromatography as described previously (20). The molar concentrations of the purified G_i and G_o were calculated from the [^{35}S]GTPγS binding.

ADP ribosylation of G-proteins. G_i and G_o were ADP-ribosylated with IAP essentially by the method of Bokoch *et al.* (24, 25).

Reconstitution of D-2 receptors with G-proteins. The reconstitution of D-2 receptors with G-proteins was carried out essentially as described previously (20). Bovine brain extract (Folch Fraction I) and porcine total lipids (1.5 mg each) were suspended by sonication in 1.7 ml of medium B [100 mM NaCl, 20 mM Hepes-KOH buffer (pH 8.0), 1 mM Na_3-EDTA, and 1 mM DTT] containing 0.3% sodium cholate, and then partially purified D-2 receptors and G-proteins (1.7 ml each) were added to the suspension. After vortexing, the mixture was applied to a column of Sephadex G-50 (fine) (1.2 × 18 cm) preequilibrated with medium B, and the void volume fractions were collected as the reconstituted vesicles.

Binding assay. Membranes or reconstituted preparations were mixed with [^{3}H]spiperone with or without dopaminergic ligands in a buffer solution [25 mM potassium phosphate buffer (pH 7.0), 4 mM Hepes-KOH buffer (pH 8.0), 100 mM NaCl, 4 mM $MgCl_2$, 0.6 mg/ml of bovine serum albumin, 1 mM Na_3-EDTA, 100 nM ketanserin, 1 mM DTT] in a total volume of 2 ml. Each mixture was incubated at 30° for 60 min, and then the bound form of [^{3}H]spiperone was separated from free [^{3}H]spiperone by rapid filtration (about 5 sec) through a Whatman GF/B filter in a Cell-Harvester (Skatron Co., Lier, Norway). Following two washes with about 2 ml each of 20 mM potassium phosphate buffer (pH 7.0), the filters were dried and counted with a scintillation spectrometer (Aloka type LSC-671) in a scintillation cocktail consisting of 330 ml of Triton X-100, 100 mg of dimethyl POPOP, 4 g of Dotite DPO, and 670 ml of toluene per liter. Ketanserin (5HT-2 antagonist) was included in the reaction mixture because spiperone may bind with the 5HT-2 sites besides the D-2 sites (26–28). The solubilized D-2 receptors were first freed of sulpiride by passage through a Sephadex G-50 (fine) column and then incubated at 30° for 1 hr with [^{3}H]spiperone (300 pM) in medium C [20 mM Hepes-NaOH (pH 8.0), 1 M NaCl, 1 mM Na_3-EDTA, 4 mM $MgCl_2$, 1 mM DTT, 100 nM ketanserin] in a total volume of 500 μl. Then the bound form of [^{3}H]spiperone was separated from free [^{3}H]spiperone on a Sephadex G-50 (fine) column, followed by counting as above. Nonspecific binding was defined as the binding in the presence of 1 mM sulpiride and was subtracted from the total binding to obtain the specific binding. All experiments were carried out in duplicate. The binding of [^{35}S]GTPγS was assayed as described previously (20).

Results

D-2 receptors were solubilized from porcine striatal membranes with a solution containing 0.3% sodium cholate, 1 M NaCl, and 0.1 mM sulpiride. The [^{3}H]spiperone-binding activity of the solubilized preparations decreased to less than 20% after 10 hr of storage, even at 4°, unless sulpiride was added to the solubilization medium. In the presence of sulpiride, the binding activity was stable for at least 25 hr at 4°. The added sulpiride could be removed with a Sephadex G-50 column just before the binding assay. The recovery of the [^{3}H]spiperone binding activity in the solubilized preparation was approximately 30% of that in the membrane preparations.

In good agreement with previous results (29, 30), the displacement curve of dopamine but not that of sulpiride for the [^{3}H]spiperone binding of membrane receptors shifted to the right (in the direction of a higher concentration of dopamine) on the addition of GTP. However, the effect of GTP on the dopamine binding of solubilized receptors was dependent on the presence of sodium cholate in the incubation medium. The effect of GTP was not observed when the solubilized preparation was incubated with [^{3}H]spiperone and dopamine in the presence of sodium cholate, but it could be detected when sodium cholate was absent (Fig. 1). This result indicates that sodium cholate interferes with the interaction of D-2 receptors with G-proteins. D-2 receptors were separated from G-proteins by gel permeation chromatography in a medium containing sodium cholate. Fig. 2a shows the elution patterns for the [^{3}H]spiperone- and

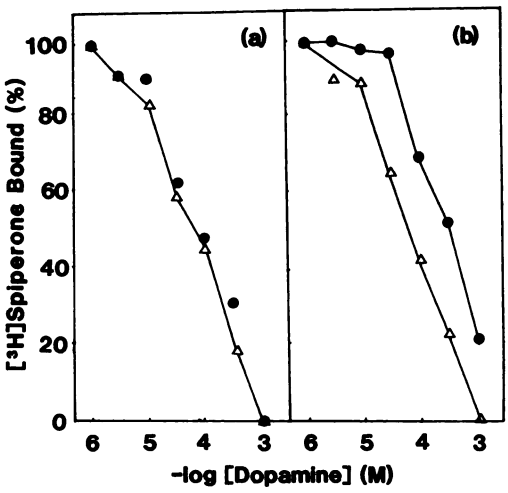
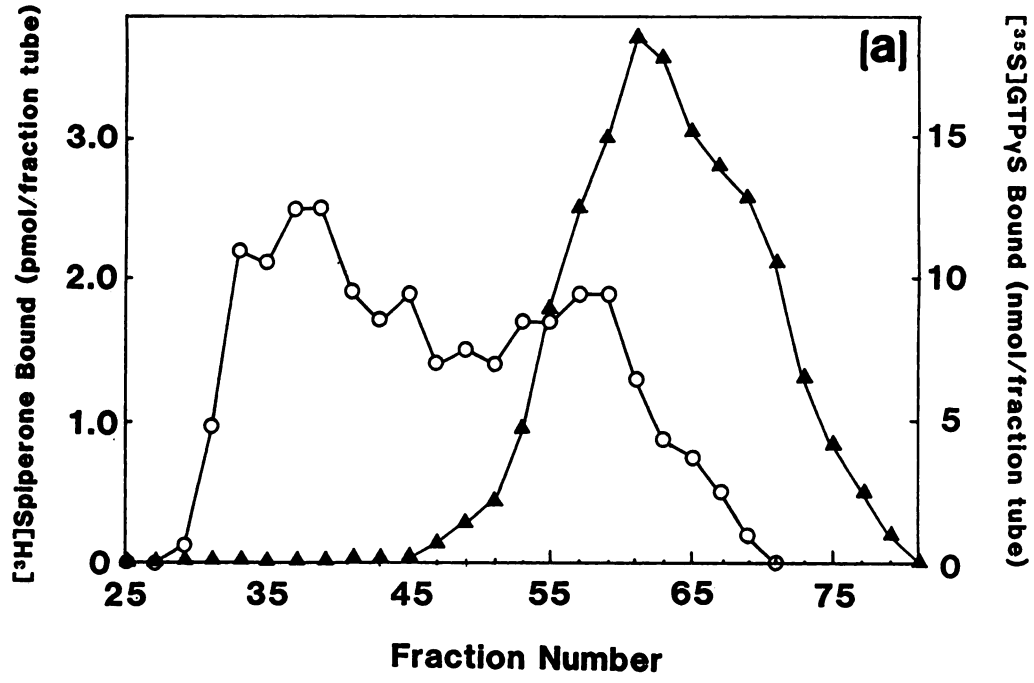


Fig. 1. The effect of GTP on [^{3}H]spiperone/dopamine competition for solubilized receptors. a. The solubilized preparation containing 0.3% sodium cholate (400 μl) was diluted to 1 ml with medium A containing [^{3}H]spiperone (final concentration, 1 nM) and different concentrations of dopamine. The mixtures were incubated at 30° for 1 hr in the presence (●) or absence (△) of 0.1 mM GTP. b. The solubilized preparation was passed through a Sephadex G-50 (fine) column preequilibrated with medium A, and the void volume fraction (800 μl) was diluted with medium A containing [^{3}H]spiperone and dopamine in the presence (●) or absence (△) of 0.1 mM GTP. Assaying of the bound form of [^{3}H]spiperone was carried out as described in a.



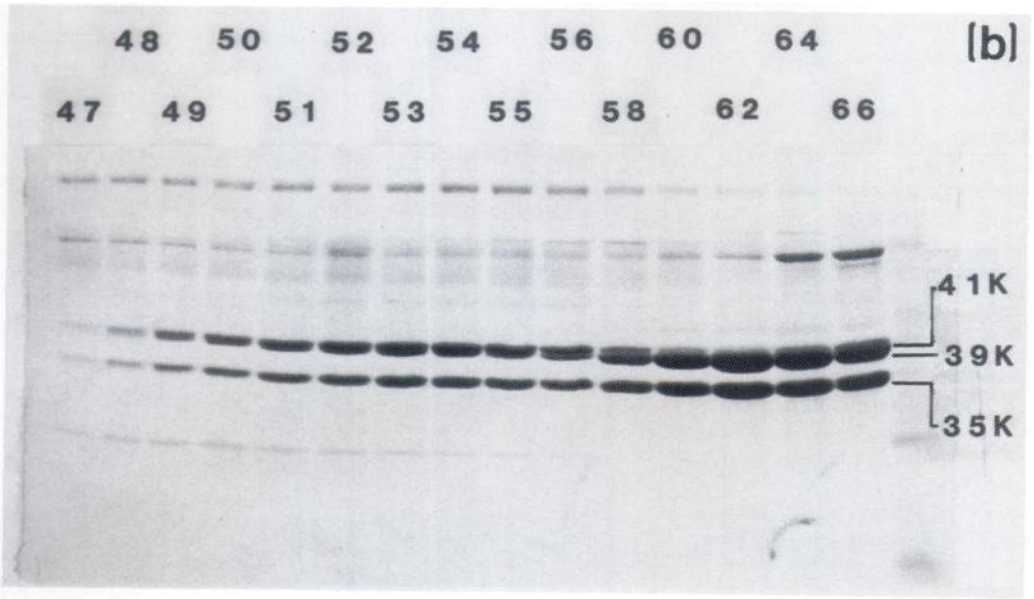


Fig. 2. Partial purification of D-2 receptors (a) and the purity of isolated G-proteins (b). a. Separation of D-2 receptors from GTPγS-binding activity by Ultrogel AcA34 chromatography. Ultrogel AcA34 chromatography and the assaying of the [^{3}H]spiperone (○) and [^{35}S]GTPγS (▲) binding were carried out as described under Experimental Procedures. Fractions 29–45 (225 ml) were collected, concentrated to about 50 ml, and used as the partially purified D-2 receptors. More than 95% of the guanine nucleotide-binding activity was removed from the D-2 receptor preparation by the chromatographic procedure. b. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of G-proteins in the eluate from DEAE-Toyopearl chromatography (the final step for purification of G-proteins). The purification of G-proteins from porcine cerebral membranes and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (acrylamide concentration, 12%) of the purified G-proteins was carried out as described previously (20). Fractions 50–53 and 62–66 were used as the G_i and G_o preparations, respectively.

[^{35}S]GTPγS-binding activities. The broad distribution of [^{3}H]spiperone-binding activity indicates the heterogeneity in the apparent size of solubilized D-2 receptors, which probably reflects aggregation of the receptors among themselves and/or with other components. The [^{35}S]GTPγS-binding activity was eluted as a single peak, but with a shoulder. The recoveries of the [^{3}H]spiperone- and [^{35}S]GTPγS-binding activities were 25 and 60%, respectively. The fractions exhibiting the [^{3}H]spiperone-binding activity but without appreciable [^{35}S]GTPγS-binding activity were collected, concentrated, and used as partially purified D-2 receptors for the reconstitution with purified G-proteins, G_i or G_o (Fig. 2b). The [^{35}S]GTPγS-binding activity of the condensed D-2 receptor preparation was slightly higher than the nonspecific binding, but the agonist binding of the receptor preparation was not affected by GTP unless G-proteins were added.

D-2 receptors and G-proteins (G_i or G_o) were reconstituted in phospholipid vesicles essentially by the same method as that used previously for the reconstitution of muscarinic acetylcholine receptors with G-proteins (20). Fig. 3 shows the results of Scatchard analysis of the [^{3}H]spiperone binding of the membrane and reconstituted preparations. The dissociation constants (K_d) were estimated to be 65 pM for the membrane preparations, and 89, 82, and 84 pM for the receptor preparations reconstituted in phospholipid vesicles without G-proteins, and with G_i and G_o (molar ratio of D-2 receptors to G-proteins = 1:1000), respectively. The addition of 0.1 mM GTP did not affect the [^{3}H]spiperone binding. The displacement curves of sulpiride for the [^{3}H]spiperone binding with the reconstituted D-2 receptor preparations were essentially the same whether G-proteins (G_i and G_o) were present or not; the curves were not affected by GTP and fitted the one-site model (Fig. 4).

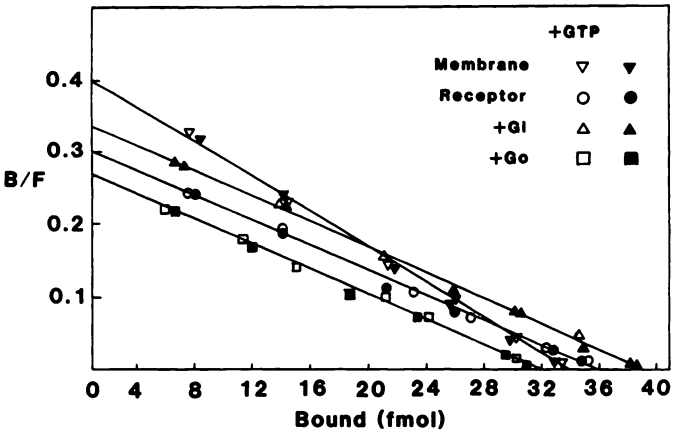


Fig. 3. Scatchard analysis of [^{3}H]spiperone binding with membrane and reconstituted preparations in the absence or presence of G_i or G_o. The assaying of [^{3}H]spiperone binding was performed as described under Experimental Procedures. The binding was carried out in the presence (▽, ○, △, □) or absence (▼, ●, ▲, ■) of 0.1 mM GTP in a total volume of 2 ml. The molar ratio of D-2 receptors to G_i (△, ▲) or G_o (□, ■) in the reconstituted preparations was 1:1000. The [^{3}H]spiperone concentration ranged from 20 pM to 1 nM. Nonspecific binding was defined as the binding in the presence of 1 mM sulpiride, and was subtracted from the total binding. The data fitted the one-site model. The maximum bindings were 36 fmol, 39 fmol, and 32 fmol for the receptor preparations reconstituted without G-proteins; and with G_i or G_o, respectively, which should be 50 fmol if the recovery of receptors through the reconstitution procedure was 100%.

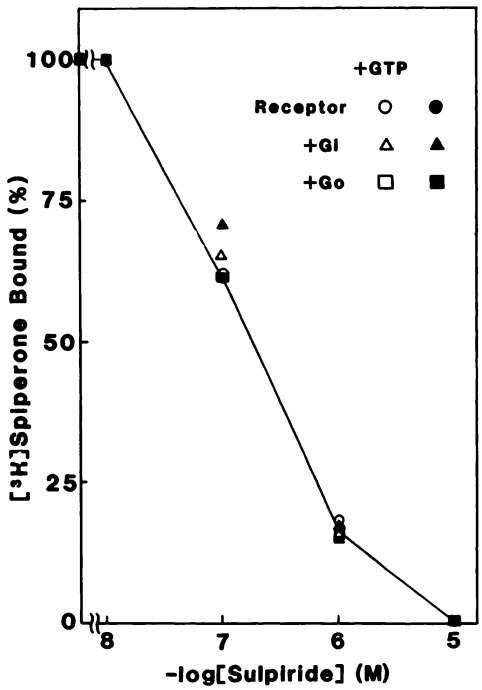


Fig. 4. Displacement curves of sulpiride for [^{3}H]spiperone binding with receptor preparations reconstituted in phospholipid vesicles in the absence or presence of G_i or G_o. The assaying of [^{3}H]spiperone binding was performed as described under Experimental Procedures. The binding was carried out in the presence (○, △, □) or absence (●, ▲, ■) of 0.1 mM GTP. The molar ratio of D-2 receptors to G_i (△, ▲) or G_o (□, ■) in the reconstituted preparations was 1:1000. The dissociation constants were 82 nM, 103 nM, and 92 nM for the receptor preparations reconstituted without G-proteins, and with G_i or G_o, respectively. Nonspecific binding was measured in the presence of 1 mM sulpiride and subtracted from the total binding.

Fig. 5 shows the displacement curves of dopamine for the [^{3}H]spiperone binding of the membrane and reconstituted preparations. The membrane preparations showed heterogeneous affinities for the agonist and the displacement curves fitted the two-site model, whether GTP was present or not; and the addition of GTP decreased the proportion of HAS from 60% to 14% (Fig. 5a, Table 1). When reconstituted in phospholipid vesicles without the addition of G-proteins, the partially purified D-2 receptors also showed heterogeneous affinities for the agonists, but the displacement curves were not affected by GTP (Fig. 5b). When the D-2 receptors were reconstituted with either G_i or G_o, however, the displacement curves were clearly affected by GTP (Fig. 5, c and d). In the absence of GTP, the displacement curves shifted more to the left as the concentration of G-proteins was increased. In contrast, the displacement curves in the presence of GTP were hardly affected by the presence or absence of G-proteins. All of these displacement curves were analyzed on the basis of a two-site model. The results of the analysis are summarized in Table 1. The K_d values of HAS and LAS of the reconstituted preparations were 0.74–1.29 μM and 55.6–145.6 μM, respectively. There was no correlation between the concentration of G-proteins and the K_d value of either HAS or LAS. The K_d values of the reconstituted preparations were approximately 10 times as high as those of the original membrane preparations. The proportion of HAS was 8% in the absence of G-proteins, but it increased to 58–64% in the presence of excess G-proteins, i.e., thousands-fold more than D-2 receptors. The proportion of HAS was higher than G_o was used than when the same concentration of G_i was used, suggesting that the D-2 receptors have higher affinity for G_o than for G_i. The proportion of HAS in the presence of G-proteins decreased to 8–15% on the addition of GTP. These results for the D-2 receptors reconstituted with G-proteins were comparable with those for the membrane preparations. In addition, the effect of GTP could not be observed when the G-proteins (both G_i and G_o) were ADP-ribosylated with IAP followed by reconstitution (Fig. 6). This observation was consistent with the finding that the ADP-ribosylation by IAP of the α-subunits of G_i and G_o interfered with their interaction with muscarinic receptors (31). However, in the experiment in Fig. 6, the shift to the left of the [^{3}H]spiperone/dopamine competition curves was less than that in Fig. 5, even when D-2 receptors were reconstituted with G-proteins that had not been treated with IAP. This may have been due to the fact that, in the experiment of Fig. 6, G-proteins were reconstituted in phospholipid vesicles after incubation with IAP in the presence of L-α-dimyristoyl phosphatidylcholine, which was necessary in order to obtain the full ADP-ribosylation of G-proteins. Cholate has been added in the solvent of G-proteins. Bokoch *et al.* (24) suggested that detergents which affect the labeling of G-proteins by IAP might be sequestered by the L-α-dimyristoyl phosphatidylcholine. Control G-proteins were also incubated with this phospholipid and then subjected to be reconstituted with the receptor preparations. It was possible that some fraction of G-proteins had been incorporated into L-α-dimyristoyl phosphatidylcholine resulting in an insufficient interaction with D-2 receptors upon reconstitution into phospholipid vesicles.

The results of these experiments thus suggested that about 50% of the partially purified D-2 receptors interact with both

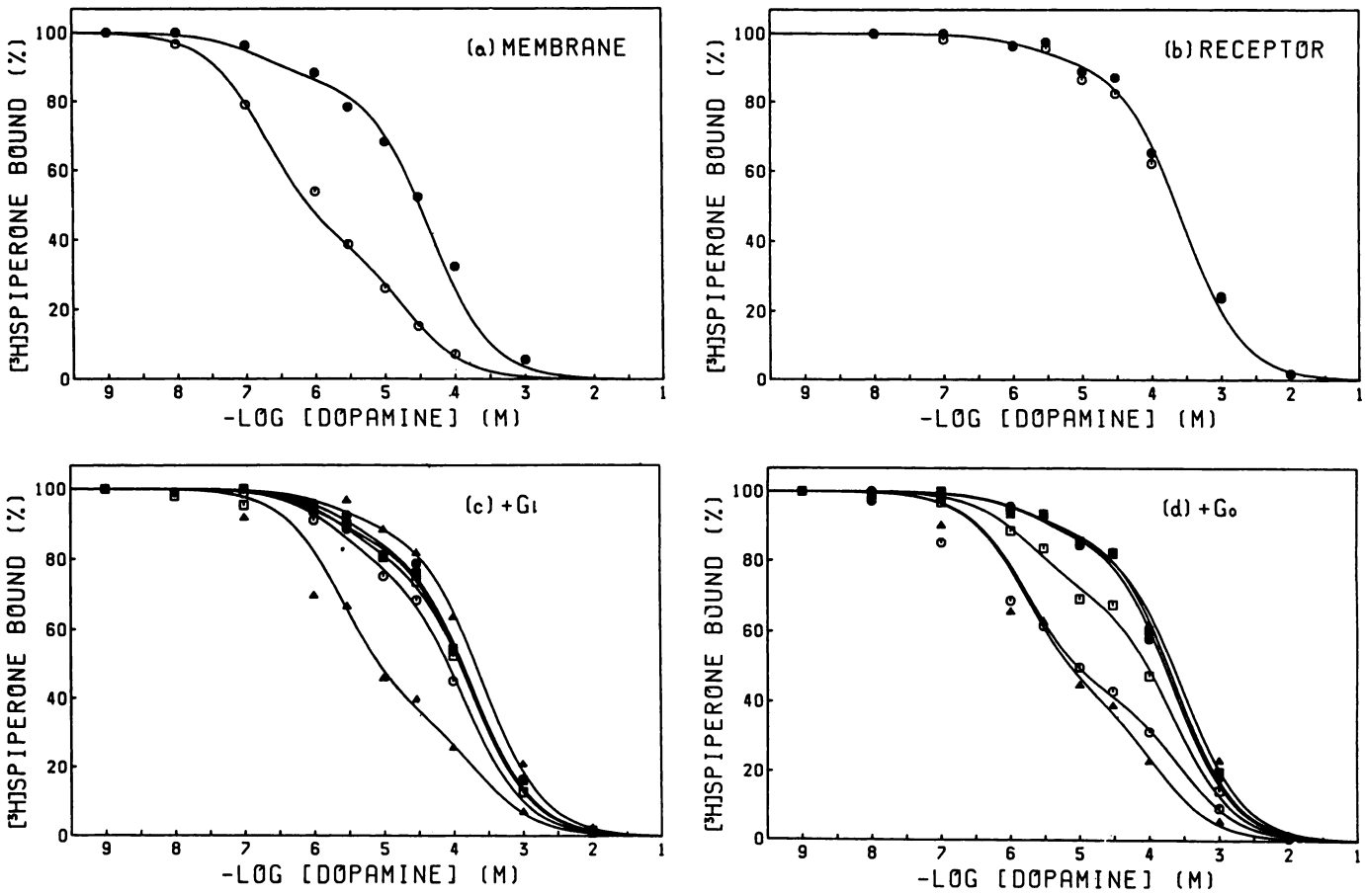


Fig. 5. Displacement curves of dopamine for [^{3}H]spiperone binding with membrane (a) and receptor preparations reconstituted in the absence (b) or presence of different concentrations of G_i (c) and G_o (d). Reconstitution of the partially purified receptors and G-proteins in phospholipid vesicles was performed as described under Experimental Procedures. The [^{3}H]spiperone binding was carried out in the absence (*open symbols*) or presence (*solid symbols*) of 0.1 mM GTP. The concentration of D-2 receptors was 5 pM. The molar ratio of D-2 receptors to G-proteins in the preparations reconstituted with G_i (c) and G_o (d) were 1:100 (□, ■), 1:300 (○, ●), and 1:1000 (△, ▲), respectively. The data are the means for three separate experiments, for each of which the assays were carried out in duplicate. The results of the analysis are summarized in Table 1.

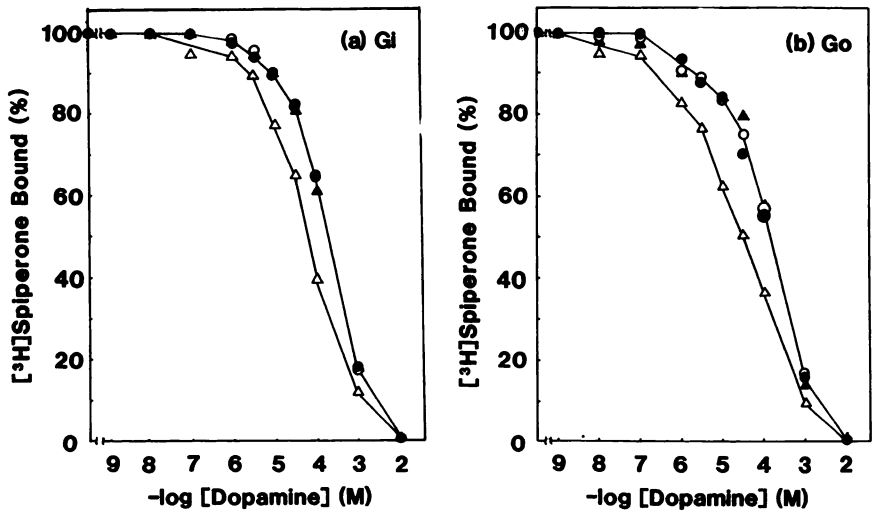


Fig. 6. Effects of IAP treatment of G-proteins (G_i and G_o) on dopamine binding with the reconstituted D-2 receptors. The G-proteins were diluted to a final concentration of 1 μM with 75 mM Tris-HCl (pH 8.0) containing 10 mM thymidine, 2.5 mM $MgCl_2$, 1 mM Na_3-EDTA, 1 mM DTT, 1 mM ATP, 10 μg/ml L-α-dimyristoyl phosphatidylcholine, and 100 nM NAD (final volume, 2 ml), and then incubated for 60 min at 30° in the presence (○, ●) or absence (△, ▲) of 25 μg of IAP. Then the IAP-treated and control G-proteins were separately reconstituted in phospholipid vesicles with a 1000 times lower concentration of partially purified D-2 receptors, and [^{3}H]spiperone/dopamine competition binding was carried out in the presence (●, ▲) or absence (○, △) of 0.1 mM GTP as described in the legend to Fig. 5.

G_i and G_o in an IAP-sensitive manner in the reconstituted preparations.

Discussion

In this study, D-2 dopamine receptors were partially purified from porcine striatal membranes and reconstituted with purified G-proteins (G_i and G_o) into phospholipid vesicles. The reconstituted receptors showed guanine nucleotide-sensitive high affinity binding with dopamine as revealed by [^{3}H]spiperone/dopamine displacement curves. The receptor preparation might contain some residual G-proteins since it showed [^{35}S]GTPγS-binding activity which was slightly higher than nonspecific binding, but the observed guanine nucleotide-sensitive

TABLE 1

Analysis of [^{3}H]spiperone/dopamine competition according to the two-site model

The affinities for dopamine of D-2 dopamine receptors determined from the data presented in Fig. 5 were analyzed with a computer program for nonlinear least squares analysis (SALS) (37). The dissociation constants, K_d (*H*) and K_d (*L*), were calculated from the corresponding IC_{50} values according to the method of Cheng and Prusoff (38).

Receptor preparation	(−) GTP			0.1 mM GTP		
	K_d (*H*)	K_d (*L*)	*p*[a]	K_d (*H*)	K_d (*L*)	*p*[a]
	μM		%	μM		%
Membrane preparation	0.08	7.9	60	0.08	16.9	14
Reconstituted vesicles: Molar ratio of *G* vs. *R*[b]						
G_i						
0	0.94	129.8	8	0.94	129.8	8
100	1.24	91.9	19	0.74	81.3	14
300	1.06	67.5	22	1.29	89.6	15
1000	1.24	97.9	64	1.15	121.3	10
G_o						
100	0.87	103.4	29	0.83	112.6	11
300	0.78	124.1	57	1.10	104.8	12
1000	0.74	55.6	58	1.15	145.6	14
Average	0.98	95.7		1.03	112.1	11.4
±SD	0.21	27.2		0.20	22.5	2.9

[a] Proportion of HAS.

[b] *R*, D-2 receptors; *G*, G-proteins. The concentration of receptors was held constant at 5 pM.

high affinity binding was considered not to be due to the residual G-proteins for the following reasons: (1) the displacement curves were not affected by GTP when partially purified D-2 dopamine receptors were reconstituted without addition of purified G-proteins as shown in Fig. 5b; (2) when D-2 receptors were reconstituted with purified G-proteins, however, the displacement curves shifted more to the left as the concentration of G-proteins was increased, as shown in Fig. 5, c and d. Similar results were obtained for partially purified muscarinic receptors by Florio and Sternweis (32). They used DEAE-Sephacel chromatography to separate the receptors from 95% of the guanine nucleotide-binding activity and reported that the effect of guanine nucleotides was restored after the addition of purified G-proteins, whereas guanine nucleotide alone had no effect on the binding of agonists to these resolved receptors.

Analysis of the [^{3}H]spiperone/dopamine displacement curves of the D-2 receptor preparations reconstituted with G_i or G_o indicated that there are three different sites or states of D-2 receptors: guanine nucleotide-insensitive HAS (about 10%), guanine nucleotide-sensitive HAS-LAS convertible sites (about 50%) and guanine nucleotide-insensitive LAS (about 40%). Similar proportions of these sites were found in the membrane preparations, although the apparent K_d values were 10 times lower than those for the reconstituted preparations. These results indicate that at least 50% of the D-2 receptors can interact with both G_i and G_o to form guanine nucleotide-sensitive HAS-LAS convertible sites. At present the differences between the guanine nucleotide-sensitive and -insensitive sites are unknown. They may reflect different subtypes of D-2 receptors, different conformational states, or binding of the solubilized receptors with some other components. It should be noted that a similar distribution of the three different populations of binding sites was observed for muscarinic receptors purified to apparent homogeneity and reconstituted with G-proteins (20).

The present results show that D-2 receptors are capable of interacting with both G_i and G_o, although this does not necessarily mean that D-2 receptors interact with both G_i and G_o *in situ*. The possibility cannot be excluded that D-2 receptors are segregated from one of the G-proteins spatially or functionally *in situ*. It is tempting to speculate, however, that D-2 receptors interact with and activate both G_i and G_o *in situ*. Consistent with the present results, Senogles *et al.* (33) recently purified D-2 receptors from bovine anterior pituitary in bound forms with G_i and G_o.

Recently, several reports indicated that G_i activated by muscarinic receptors allowed the opening of potassium channels in the heart (34). The function of G_o has not been determined yet but is suggested to be related to the control of calcium channels (35). Recently, Sasaki and Sato (36) showed that potassium channels in abdominal ganglion of *Aplysia* were allowed to open by dopamine, histamine, and acetylcholine in an IAP-sensitive manner. It would be interesting to know if the activation of G_i and G_o by D-2 receptors in mammalian nervous tissues is also related to the regulation of ion channels.

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