Selective Blockade of Dopamine D-1 Receptor by SCH 23390 Affects Dopamine Agonist Binding to ^3H -Spiperone Labeled D-2 Receptors in Rat Striatum

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Abstract—This study investigated the effects of selective blockade of dopamine D-1 receptors by SCH 23390 and selective stimulation of the receptors by SKF 38393 on the binding characteristics of ^3H -spiperone labeled D-2 receptors in rat striatum. Selective blockade of D-1 receptors by 50 nM SCH 23390 significantly decreased the affinity of dopamine agonist for ^3H -spiperone labeled D-2 receptors, but did not influence dopamine antagonist binding to D-2 receptors. Selective stimulation of D-1 receptors by SKF 38393 (100 nM) did not affect either dopamine agonist or antagonist binding to D-2 receptors. The characteristics of the effect of SCH 23390 on dopamine agonist binding to D-2 receptors was similar to those of GTP, but different from those of sodium ion. This effect could not be due to a direct modification of D-2 receptors by SCH 23390. Pertussis toxin (IAP) treatment significantly decreased the affinity of dopamine agonist for D-2 receptors and reduced the abilities of both SCH 23390 and GTP to decrease the affinity of dopamine agonist for D-2 receptors. These results suggest, therefore, putative interregulatory mechanism between dopamine D-1 and D-2 receptors and the possible involvement of a pertussis toxin sensitive protein in this mechanism.

It has been well-established that in the mammalian central nervous system, dopamine receptors can be classified as D-1 and D-2 subtypes based on their pharmacological properties and association with the enzyme adenylate cyclase (1–3). In rat striatum, the coupling of D-2 receptors with adenylate cyclase is mediated through an inhibitory guanine nucleotide binding protein (G_i). Pertussis toxin treatment, which inactivates the G_i by ADP-ribosylation, blocks D-2 inhibition of adenylate cyclase (4–6). In contrast, the interaction of D-1 receptors with a stimulatory guanine nucleotide binding regulatory protein (G_s) is likely to be involved in dopamine stimulation of adenylate cyclase. D-1 stimulation of cyclic AMP formation is enhanced following the treatment of cholera toxin, which selectively ADP-ribosylates G_s (7–9). Based on radioligand binding experi-

ments, it has been demonstrated that the affinity of agonist for D-2 receptors is remarkably reduced in the presence of guanine nucleotide; however, antagonist binding is insensitive to such treatment (10–14). The regulation of agonist-receptor interaction by guanine nucleotide is thought to reflect the binding of the receptor to a guanine nucleotide binding protein (9).

In rat striatum, the regulation of adenylate cyclase activity is shown as a bimodal regulation by dopamine depending on guanine nucleotide (4, 15, 16). D-1 stimulation and D-2 inhibition of adenylate cyclase require different concentrations of GTP (9). At micromolar concentrations of GTP, dopamine activation of D-2 receptor curtails the dopamine stimulation of the enzyme, which implies that D-1 and D-2 receptors co-exist on the same striatal neurons (16). Behavioral investigations indicate that the systemic treatment of D-1 and D-2 agonists can

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produce additive or synergistic effects. (17–19). These facts suggest a physiological interaction between D-1 and D-2 receptors in the striatum. It has been reported that in rat heart homogenate, occupancy of the β -adrenergic receptor (stimulatory receptor of adenylate cyclase) by an agonist can influence the affinity of muscarinic receptors (inhibitory receptor of adenylate cyclase). This suggests an interdependent linkage between β -adrenergic receptor and muscarinic receptor (20). So far, little is known about if there exists a biochemical interaction between D-1 and D-2 receptors in the central nervous system that could be reflected as a co-regulation of the binding affinities of each dopamine receptor subtype.

In order to gain insight into possible biochemical interactions between D-1 and D-2 receptors in rat striatum, we investigated whether selective blockade of D-1 receptors by SCH 23390 or selective stimulation of the receptors by SKF 38393 could produce any effect on the binding characteristics of D-2 receptors. We found that selective blockade of D-1 receptors by SCH 23390 could reduce the affinity of dopamine agonist for D-2 receptors. No such effect has been observed for SKF 38393. Furthermore, we compared the effect of D-1 receptor blockade by SCH 23390 with that of GTP and sodium ion on the affinity of D-2 receptors for its agonists in order to find out the mechanism of the effect induced by SCH 23390.

Materials and Methods

Materials: ^3H -Spiperone (21.4 Ci/mmol) and ^3H -SCH 23390 (83 Ci/mmol) were purchased from New England Nuclear (MA, U.S.A.) and Amersham (Bucks, U.K.), respectively. The other compounds were from the following sources: o-phenanthroline and dopamine hydrochloride, Nakarai (Kyoto, Japan); S(-)-Sulpiride and chlorpromazine, Fujisawa (Osaka, Japan); (+)Butaclamol hydrochloride and SKF 38393, Research Biochemical, Inc. (MA, U.S.A.); Ketanserin tartrate, Kyowa Hakko Kogyo (Tokyo, Japan); Haloperidol, Dainippon (Tokyo, Japan); Clozapine, a gift from Dr. Yoshida (Dainippon); Lergotril mesylate, Ely Lilly (IN, U.S.A.); Bromocriptine, Sigma (St. Louis,

U.S.A.); Spiperone, gift from Dr. K. Yamazu; LY-171555 and YM-09151, kindly given by Mr. T. Kikuchi (Otsuka); Thymidine, β -NAD and 5'-ATP.2Na, Kohjin (Tokyo, Japan); Dithiothreitol, creatine phosphate, creatine kinase, guanosine-5'-triphosphate (GTP), Boehringer Mannheim (W. Germany); Pertussis toxin (IAP), Kaken (Tokyo, Japan).

Preparation of membranes: The striatal membrane preparations were obtained from male Wistar rats (200–250 g) according to the method described by Creese and Hess (21). Briefly, the striata were homogenized in 100 volumes (wet weight/volume) of ice-cold 50 mM Tris-HCl buffer (pH 7.7). The homogenate was centrifuged at 50,000 $\times$ g for 10 min at 4°C, and the resulting pellet was suspended in an identical volume of Tris-HCl buffer (pH 7.4) containing 5 mM KCl, 2 mM CaCl_2 and 1 mM MgCl_2 , with or without 120 mM NaCl and incubated for 10 min at 37°C. The suspension was then centrifuged again at 50,000 $\times$ g for 10 min at 4°C, and the resulting pellet was washed three more times by intermediate rehomogenization in fresh buffer and recentrifugation. The resulting pellet was suspended in an assay buffer of 50 mM Tris-HCl (pH 7.4) containing 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 1 mM EDTA and 120 mM NaCl. In some cases, as stated in the text, 120 mM NaCl was omitted from the assay buffer.

IAP treatment: IAP treatment of striatal membranes was performed according to Law et al. (22). This method was found to be optimal for the induction of the maximum possible effects of IAP on binding (23). Rat striatum was homogenized (Kinematica Polytron, setting 8, for 20 sec) in 100 volumes (wet weight/volume) of ice-cold 50 mM Tris-HCl buffer containing 5 mM KCl, 1 mM EDTA and 4 mM MgCl_2 (pH 7.4). The homogenate was then centrifuged at 50,000 $\times$ g for 10 min at 4°C, and the resulting pellet was resuspended in an identical volume of the above buffer and incubated for 15 min at 37°C. The homogenate was then centrifuged at 50,000 $\times$ g for 10 min at 4°C, and the resulting pellet was suspended in 1:25 volumes (original wet weight/volume) of 40 mM Tris-HCl buffer containing 16 mM thymidine, 0.4 mM ATP, 16 μM GTP, 4 mM

MgCl₂, 0.8 mM EDTA, 4 mM dithiothreitol, 16 mM creatine phosphate, 4 units of creatine kinase, 0.8 mM *o*-phenanthroline, 10 μ M β -NAD and 10 μ g/ml IAP, pH 8.0. This suspension was then incubated for 45 min at 37°C. At the end of the incubation, the suspension was diluted 1:100 with ice-cold 50 mM Tris-HCl buffer containing 5 mM KCl, 1 mM EDTA and 1 mM MgCl₂ and centrifuged at 50,000 $\times$ g for 10 min at 4°C. The pellet was washed three more times with the same buffer as above before the final suspension in the assay buffer. Control tissue was similarly treated except that IAP was omitted.

Binding assay: Competitive binding experiments between ³H-spiperone and unlabeled dopamine ligands were performed in a final volume of 1.0 ml assay buffer. Membrane preparations (0.15 mg protein per tube) were incubated with ³H-spiperone (0.3 nM) and increasing drug concentrations for 15 min at 37°C. Ketanserin (50 nM) was added to the assay system to mask serotonin-2 (5HT-2) receptor. SCH 23390 (50 nM), GTP (0.03 mM or 0.15 mM), or both SCH 23390 and GTP were present in the binding assay system as indicated in the text. Incubation was terminated by rapid filtration under reduced pressure through Whatman GF/B filters. The filters were then rapidly washed two times with 10 ml of ice-cold Tris-HCl buffer (pH 7.4). Radioactivity trapped on the filters was assessed using a liquid scintillation spectrometer. Specific binding was calculated by subtraction of the value for nonspecific binding in the presence of 0.1 μ M (+)-butaclamol. For the ³H-spiperone equilibrium binding assay, various concentrations of ³H-spiperone from 0.01 nM to 1.0 nM were used.

The ³H-SCH 23390 (0.3 nM) binding assay was performed as previously described by Billard et al. (24).

Data analysis: Data from the competition experiments were analyzed with a computer by a nonlinear least-squares curve fitting program. Data were fitted to single and multiple site models and compared for statistically significant difference. The model chosen was that which best described the data at the 0.05 level of significance. Computer analysis of data from individual ex-

periments always yield the same conclusion: data in the control, SCH 23390, GTP and GTP+SCH 23390 in the absence of sodium chloride fit a two-site model (Table 2), while in any treatment condition as above in the presence of sodium chloride, a one-site model was observed (Tables 1 and 3). Because our curve analysis program can only fit a single curve at a time, and has no provision for the determination of correction factors to simultaneously analyze multiple experiments, in experiments examining the effects of SCH 23390, GTP or co-treatment of both on dopamine and LY-171555 binding to D-2 receptors in the absence of sodium chloride, data from the individual experiments were pooled prior to simultaneous analysis (Table 2). Student's *t*-test was used for statistical comparisons of the IC₅₀s and slopes listed in Tables 1 and 3. Scatchard analysis was used for the saturation data.

Results

Inhibition of ³H-SCH 23390 and ³H-spiperone binding by SCH 23390 and SKF 38393: These experiments were performed to determine what concentrations of SCH 23390 and SKF 38393 could selectively block or stimulate ³H-SCH 23390 labeled D-1 receptor, respectively, without competing with ³H-spiperone binding to D-2 receptors. Figure 1 shows the experimental results. At the concentration of 32 nM, unlabeled SCH 23390 nearly completely inhibited ³H-SCH 23390 (0.3 nM) binding for membrane preparations. At concentrations up to 100 nM, SCH 23390 did not inhibit ³H-spiperone (0.3 nM) binding. SKF 38393 at 100 nM did not inhibit ³H-spiperone (0.3 nM) binding, although it displaced only about 60% of the ³H-SCH 23390 (0.3 nM) binding. In the following experiments, we used 50 nM unlabeled SCH 23390 and 100 nM SKF 38393 to selectively block and stimulate striatal D-1 receptors, respectively.

Effects of SCH 23390, GTP or SKF 38393 on dopamine agonist or antagonist binding to membrane D-2 receptors: The effects of SCH 23390 (50 nM), SKF 38393 (100 nM) and GTP on dopamine agonist and antagonist binding to ³H-spiperone labeled membrane D-2 receptors were first tested in a 50 mM

Fig. 1. Displacement of ^3H -SCH 23390 binding (open symbols) and ^3H -spiperone binding (solid symbols) by SCH 23390 (squares) and SKF 38393 (circles) in rat striatal membrane preparations. Striatal membranes were incubated with ^3H -SCH 23390 (0.3 nM) or ^3H -spiperone (0.3 nM) and increasing concentrations of unlabeled SCH 23390 or SKF 38393 as described in Materials and Methods. Each point represents the mean of three independent experiments carried out in triplicate with a S.E.M. of about 10%.

Fig. 2. Displacement of ^3H -spiperone binding (0.3 nM) to rat striatal membrane preparations by dopamine (A), apomorphine (B), LY-171555 (C), bromocriptine (D) and lergotrile (E) in the presence of SCH 23390 (50 nM, squares), GTP (0.03 mM, triangles), or both (open circles). Solid circles represent the control data. The assay buffer contains 120 mM sodium chloride. Data points are the means of three or four independent experiments carried out in triplicate. The IC_{50} s and slopes are given in Table 1.

Tris-HCl assay buffer containing 50 nM ketanserin, 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 and 1 mM EDTA (pH 7.4). SCH 23390 (50 nM), GTP (0.03 mM

or 0.15 mM) or co-treatment with both significantly decreased the affinities of dopamine and apomorphine, the agonists of both D-1 and D-2 receptors, as well as the

affinity of LY-171555, a selective D-2 agonist, for ^3H -spiperone labeled D-2 receptor, seen as a rightward shift in each of the displacement curves (Fig. 2 A, B and C and Table 1). SCH 23390 (50 nM), GTP (0.03 mM) or co-treatment with both slightly decreased the affinities of bromocriptine and lergotril, but significantly changed the slopes

(Fig. 2 D and E and Table 1). Among all of the agonists studied, co-treatment with SCH 23390 and GTP resulted in values not significantly different to that in the presence of GTP alone (Fig. 2 and Table 1). SKF 38393 (100 nM) did not have any significant effect on the agonist binding to D-2 receptors (Table 1).

Table 1. Effects of SCH 23390 (50 nM), GTP (0.03 nM or 0.15 mM), co-treatment of both SCH 23390 and GTP, and SKF 38393 (100 nM) on dopamine agonists binding to ^3H -spiperone labeled D-2 receptor in rat striatal membranes

	IC ₅₀ (nM)	slope
Dopamine		
Control	1,480±200	0.72±0.04 (4)
SCH 23390	4,240±540 ^a	0.71±0.02 (4)
GTP (0.03 mM)	6,010±650 ^b	0.72±0.03 (4)
GTP (0.03 mM)+SCH 23390	6,700±610 ^b	0.70±0.03 (4)
GTP (0.15 mM)	7,530±290 ^c	0.72±0.03 (3)
GTP (0.15 mM)+SCH 23390	7,590±530 ^c	0.73±0.03 (3)
SKF 38393	1,770±140	0.71±0.04 (3)
Apomorphine		
Control	46± 3	0.76±0.04 (4)
SCH 23390	75± 6 ^a	0.75±0.06 (4)
GTP (0.03 mM)	145± 12 ^b	0.75±0.03 (3)
GTP (0.03 mM)+SCH 23390	160± 7 ^b	0.77±0.04 (3)
GTP (0.15 mM)	198± 13 ^b	0.76±0.03 (3)
GTP (0.15 mM)+SCH 23390	217± 12 ^c	0.75±0.03 (3)
SKF 38393	52± 8	0.73±0.05 (3)
LY-171555		
Control	2,930±240	0.72±0.03 (4)
SCH 23390	3,750±360 ^a	0.75±0.04 (4)
GTP (0.03 mM)	6,340±530 ^b	0.72±0.04 (3)
GTP (0.03 mM)+SCH 23390	6,820±460 ^b	0.75±0.03 (3)
SKF 38393	2,300±110	0.73±0.02 (3)
Bromocriptine		
Control	17.8±1.4	0.71±0.02 (3)
SCH 23390	20.2±0.7	0.84±0.02 ^b (3)
GTP (0.03 mM)	21.8±0.6	0.86±0.07 ^b (3)
GTP (0.03 mM)+SCH 23390	22.7±2.1	0.92±0.05 ^b (3)
SKF 38393	18.4±1.5	0.71±0.03 (3)
Lergotril		
Control	18.2±1.3	0.71±0.03 (3)
SCH 23390	19.8±2.2	0.88±0.04 ^a (3)
GTP (0.03 mM)	23.5±5.8	0.96±0.05 ^b (3)
GTP (0.03 mM)+SCH 23390	23.8±2.4	0.99±0.05 ^b (3)
SKF 38393	19.8±2.4	0.72±0.03 (3)

The binding assays were performed as described in Materials and Methods. Data are expressed as means±S.E.M. of (n) independent experiments carried out in triplicate. ^aP<0.02, ^bP<0.01, ^cP<0.001, versus control.

Fig. 3. Displacement of ³H-spiperone (0.3 nM) binding to rat striatal membranes by YM-09151 (A) and S(-)-sulpiride (B) in the presence of SCH 23390 (50 nM, open squares), GTP (0.03 mM, triangles), a combination of SCH 23390 and GTP (open circles), or SKF 38393 (100 nM, solid squares). Solid circles represent the control data. Each point represents the mean of three independent experiments carried out in triplicate. YM-09151: IC₅₀=0.33 nM, slope=1.14; S(-)-Sulpiride: IC₅₀=77.7 nM, slope=0.93.

Figure 3 shows that the displacements of ³H-spiperone binding by selective D-2 receptor antagonists, YM-09151 and S(-)-sulpiride, were not affected by SCH 23390 (50 nM), GTP (0.03 mM), co-treatment with both SCH 23390 and GTP, or SKF 38393 (100 nM). Similar results were seen for the other dopamine D-2 antagonists such as spiperone, haloperidol, chlorpromazine and clozapine (data not shown). The equilibrium binding of ³H-spiperone was not influenced by SCH 23390 (50 nM), GTP (0.03 mM) or co-treatment with SCH 23390 and GTP (data not shown).

These results obviously indicate that like GTP, SCH 23390 can decrease the affinity of the dopamine agonist for the ³H-spiperone labeled D-2 receptor. It has been thought that guanine nucleotides decrease the affinity of the agonist through a mechanism different from those of sodium ion (14, 25). Thus binding assays were performed in the absence of sodium ion in order to compare the characteristics of the effects induced by SCH 23390, GTP, and sodium ion with each other. In the absence of 120 mM sodium chloride, the displacement curves of ³H-spiperone binding by dopamine and by LY-171555 were best described by a two-site model. The high affinity component accounted for about 45% of the total receptor population (Table 2, data for control). The addition of SCH 23390 (50 nM) or GTP

(0.03 mM) did not alter the biphasic displacement curve for dopamine and that for LY-171555, but resulted in a rightward shift of each of the displacement curves (Fig. 4). The relative proportions of the two sites did not differ from those observed in the control (Table 2). When SCH 23390 was added together with GTP, the result was not different to that when GTP was added alone (Fig. 4 and Table 2).

Effects of SCH 23390 and GTP on dopamine and LY-171555 binding to IAP-treated membranes: We attempted to determine the mechanism for the regulatory effect of SCH 23390 on the affinity of the agonist/antagonist interaction at D-2 receptors by using IAP to inactivate G_i. In IAP-treated membranes the affinities of dopamine and LY-171555 for ³H-spiperone labeled D-2 receptors were significantly decreased compared to the control (Fig. 5 and Table 3), and SCH 23390 only induced a small reduction in the affinity for dopamine and that for LY-171555, respectively; however, the resulting IC₅₀s were not significantly different from those of the untreated preparations (comparing data for IAP treatment+SCH 23390 in Table 3 with the data for SCH 23390 in Table 1). In the presence of GTP (0.15 mM), the affinities of dopamine and LY-171555 were somewhat decreased again to values not significantly different from the untreated preparations (comparing the data for IAP

Fig. 4. Displacement of ^3H -spiperone binding to rat striatal membranes by dopamine (A) and LY-171555 (B) in the presence of SCH 23390 (50 nM, squares), GTP (0.03 mM, triangles) or both (open circles). Solid circles represent the control data. Sodium chloride was omitted from the assay buffer. Each point is the mean of three independent experiments carried out in triplicate. Computer-fitted values are summarized in Table 2.

treatment+GTP in Table 3 with the data for GTP in Table 1). IAP treatment did not cause any change in slope either in the presence or absence of GTP and SCH 23390 (Table 3).

Discussion

The interesting finding of the present study is the demonstration that SCH 23390, a selective dopamine D-1 receptor antagonist, can reduce the affinity of a dopamine agonist for the ^3H -spiperone-labeled D-2

receptor in rat striatal membranes. However, SKF 38393, a selective D-1 agonist, was found to have no such effect. This finding suggests that there might be an interactive, regulatory mechanism between D-1 and D-2 receptors in rat striatum. The modification of the agonist/antagonist interaction at D-2 receptors by the selective D-1 antagonist SCH 23390 is very surprising. This phenomenon has previously been shown to occur often in the presence of guanine nucleotides and some cations such as Na^+ (10–14, 25).

Table 2. Effects of SCH 23390 (50 nM), GTP (0.03 mM) and cotreatment of both drugs on dopamine and LY-171555 binding to rat striatal membranes in the absence of 120 mM sodium chloride

	K _H (nM)	K _L (nM)	%R _H	%R _L
Dopamine				
Control	49± 9	5,623± 404	44.9±7.6	55.1±7.6
SCH 23390	192±11	13,117±1,810	51.8±8.1	48.2±8.1
GTP	168±25	12,656±2,459	59.5±2.6	40.5±4.8
GTP+SCH 23390	157±22	20,173±3,158	51.2±4.5	48.8±4.5
LY-171555				
Control	39± 8	3,294± 397	47.8±3.5	52.2±3.6
SCH 23390	181±19	11,660±4,553	52.4±4.2	47.6±3.9
GTP	165±14	12,140±4,023	51.1±3.1	48.9±3.1
GTP+SCH 23390	174±23	16,240±3,716	52.6±2.7	47.4±2.4

Values in the table represent the computer-fitted parameter estimates for the pooled data±S.E.M. of three independent experiments carried out in triplicate. Under all of the conditions, according to the statistical analyses, the data best-fitted a two-site model.

Table 3. Effects of SCH 23390 (50 nM) and GTP (0.15 mM) on dopamine and LY-171555 binding to IAP-treated rat striatal membranes

	IC50	slope
Dopamine		
control	1,745±183	0.69±0.03
IAP treatment	3,884±520 ^a	0.71±0.02
IAP treatment+SCH 23390	4,342±584 ^a	0.71±0.02
IAP treatment+GTP	6,598±745 ^b	0.70±0.02
LY-171555		
control	2,616±166	0.68±0.03
IAP treatment	3,533±183 ^a	0.71±0.02
IAP treatment+SCH 23390	3,839±478 ^a	0.72±0.02
IAP treatment+GTP	5,928±577 ^b	0.71±0.04

The affinities of dopamine and LY-171555 for ³H-spiperone labeled D-2 receptor were significantly decreased following IAP treatment. SCH 23390 only slightly decreased the affinities of the two agonists, resulting in similar IC50 values as seen in untreated membrane preparations (comparing with Table 1). GTP (0.15 mM) decreased the affinities of these two agonists with a potency similar to that in untreated membrane preparations (comparing with Table 1). Data are means±S.E.M. of three independent experiments carried out in triplicate. ^aP<0.05, vs. control, ^bP<0.05, vs. IAP treatment.

The mechanism for the effect of SCH 23390 on D-2 agonist affinity may be as follows: 1) Direct modification of D-2 receptor and 2) Through another component which originally exists in membrane preparations.

The concentrations of SCH 23390 (50 nM) and SKF 38393 (100 nM) used in this study were those at which SCH 23390 and SKF 38393 can selectively block or stimulate ³H-SCH 23390 (0.3 nM) labeled D-1 receptors, respectively, without competing with ³H-spiperone (0.3 nM) binding to D-2 receptors. The equilibrium binding of ³H-

spiperone was also not affected by SCH 23390 at this concentration. Therefore, the effect observed in SCH 23390 treatment could not be due to their direct modification of the D-2 receptor. On the other hand, spiperone possesses a relatively high affinity for 5HT-2 receptors (26–28), and SCH 23390 also binds to 5HT-2 receptors with 25 times less potency than ketanserin *in vitro* (29). In order to exclude the possible influence of 5HT-2 receptors, according to the previous reports (21, 23), ketanserin (50 nM), a selective 5HT-2 receptor blocker,

Fig. 5. Displacement of ³H-spiperone binding (0.3 nM) to IAP-treated rat striatal membrane preparations by dopamine (A) and LY-171555 (B). Solid circles, with untreated membranes; squares, with IAP-treated preparations; triangles, with IAP-treated preparations in the presence of SCH 23390 (50 nM); open circles, with IAP-treated preparations in the presence of 0.15 mM GTP. Each point represents the mean of three independent experiments carried out in triplicate. Values of IC₅₀s and slopes are summarized in Table 3.

was added to the ³H-spiperone binding assay system. Thus the effect of 50 nM SCH 23390 could not be mediated by the 5HT-2 receptor.

It has been reported that guanine nucleotide and sodium ion decrease the affinity of agonists for the D-2 receptor through different mechanisms. The effect of guanine nucleotide on the agonist/antagonist interaction at the D-2 receptor is the result of the dissociation of guanine nucleotide binding

protein from the receptor; however, sodium ion acts like an allosteric inhibitor of the D-2 receptor (14, 25). We observed that in the absence of 120 mM sodium chloride, the displacement curves of dopamine agonists displayed very shallow profiles, which were best described by a two-site model when analyzed by a nonlinear least-squares curve fitting program (Fig. 4 and Table 2). GTP or SCH 23390 or the addition of both only resulted in a rightward shift in each of the

displacement curves without altering the proportion of the two binding states (Fig. 4 and Table 2). Upon the addition of 120 mM sodium chloride, the agonist high affinity binding state was converted to the low affinity binding state, so that a one-site model was sufficient for the dopamine agonist displacement curves (Fig. 2 and Table 1). We noticed that in either the presence or absence of sodium chloride (120 mM), SCH 23390 (50 nM) was able to significantly decrease the affinities of the studied agonists for the D-2 receptor. However, when striatal membranes were treated by SCH 23390 (50 nM) and GTP (0.03 mM or 0.15 mM) together, the effect induced by SCH 23390 was abolished, and the resulting IC₅₀s were not different to the GTP treatment alone (Fig. 2 and Table 1). In other words, there was no additive or synergistic effect observed between 50 nM SCH 23390 and 0.03 mM GTP. These results suggest that the characteristics of the effect induced by SCH 23390 was very similar to the effect of GTP and different from those of sodium ion.

Putting these findings together, we hypothesized that the mechanism through which SCH 23390 affects the binding of the dopamine agonist for the ³H-spiperone labeled D-2 receptor may be similar to that through which GTP acts. In light of our existing knowledge that GTP decreases the affinity of the dopamine agonist for the D-2 receptor because of an association of G-protein with the D-2 receptor, we therefore used IAP (a pertussis toxin) to inactivate G_i which is involved in the D-2 inhibition of the enzyme adenylylase. Generally, the IAP treatment could not completely inactivate G_i, as shown by the fact that the D-2 mediated inhibition of adenylylase was partially decreased and the ability of guanine nucleotide to reduce the affinity of dopamine agonist for D-2 receptor was reduced, but not abolished following IAP treatment (5, 6, 30). Our results are consistent with the partial inactivation of G_i by *in vitro* IAP treatment. In IAP-treated membranes, dopamine and LY-171555 displaced ³H-spiperone binding with significantly lower affinities than control membranes. GTP (0.15 mM) or SCH 23390 (50 nM) produced a smaller rightward shift

in the displacement curves in IAP-treated membranes than in control membranes (Fig. 5 and Table 3). This result suggests that an IAP-sensitive, regulatory component may play a role in the modification of the agonist/antagonist interaction at the D-2 receptor by SCH 23390. The nature of this component may have the characteristics of a guanine nucleotide binding protein. This is the first report that D-1 receptor antagonist can modify the agonist/antagonist interaction at D-2 receptors. A few reports have suggested an interactive, regulatory mechanism between receptors that affects either the activity of adenylylase (31, 32) or the affinity of the agonist-receptor interaction (20, 32), but in all cases, these regulatory effects are mediated by receptor agonists. On the basis of receptor-activation leading to the dissociation of G-proteins whose subunits may interact with other receptor G-protein complexes, it is easy to understand the speculation that G protein is involved in these cases.

It seems difficult to understand that a D-1 receptor antagonist can modify the agonist/antagonist interaction at D-2 receptors according to the classical, pharmacological concept of an antagonist. However, some data suggest that the antagonist not only passively occupies the binding site of the receptor but also does anything else (31, 33, 34). Moreover, the formation of a high affinity ligand-receptor-G-protein ternary complex induced by binding of the antagonist spiperone to the D-2 receptor has been reported in a recent literature (23). Unlike the characteristics of agonist/receptor binding, the formation of a ternary complex induced by the antagonist is not destabilized by guanine nucleotide. The G_i subclass of G-proteins can be presumed to be the prime candidate for inducing the formation of this high affinity ternary complex because G_i is involved in D-2 receptor-mediated inhibition of striatal adenylylase (23). On the other hand, it has been reported that pertussis toxin produced a small, but significant increase in D-1 stimulated cyclic AMP accumulation in striatal slices. This indicates that stimulatory receptors not only interact with G_s but possibly also G_i (5). Furthermore, there is evidence that the D-2 receptor is

capable of interacting with both Gi and Go (o=other proteins whose function is not clear) (35, 36), and that the action of IAP is not only specific on Gi but also Go (36). In light of these results, we speculate that if the SCH 23390/D-1 receptor interaction can induce a formation of ligand-receptor-G-protein ternary complex (like spiperone/D-2 receptor interaction) and the ternary complex can share the same G-protein (Gi ?, Go ?) with a ternary complex induced by the agonist/D-2 receptor interaction, this ternary complex may play a role like GTP to destabilize the ternary complex induced when the D-2 receptor is occupied by an agonist. Since it is currently unknown if SCH 23390 can induce a ternary complex, the consequences of SCH 23390/D-1 receptor interaction is yet to be elucidated. It is also interesting to investigate the effect of membrane treatment with cholera toxin, which selectively ADP-ribosylates Gs, on the effect of SCH 23390 on D-2 binding properties.

In this study, we did not find any effect of SKF 38393, a selective D-1 stimulator, on either agonist or antagonist binding to D-2 receptors. This may be because the concentration of SKF 38393 (100 nM) used in this study is too low to induce any visible effect. At this concentration, SKF 38393 only competed for about 60% of the specific ³H-SCH 23390 (0.3 nM) binding sites. Increasing the concentration of SKF 38393 may interrupt ³H-spiperone binding, making the data impossible to interpret. The treatments of GTP and SCH 23390 induced a relatively smaller reduction in affinity for bromocriptine and lergotril than for dopamine, apomorphine and LY-171555, but significantly changed the slopes of the latter two drugs. This difference may be because bromocriptine and lergotril are both a D-1 antagonist and a D-2 agonist (17, 37–40). A previous study indicated that binding of bromocriptine to the D-2 receptor is not sensitive to guanine nucleotide treatment (23).

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