Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 10156–10160, December 1989
Neurobiology

Link between D_1 and D_2 dopamine receptors is reduced in schizophrenia and Huntington diseased brain

(Alzheimer disease/Parkinson disease/quinpirole/guanine nucleotide-binding protein)

PHILIP SEEMAN*†, HYMAN B. NIZNIK*†, H.-C. GUAN†, GILLIAN BOOTH†, AND CARLA ULPIAN†

Departments of *Psychiatry and †Pharmacology, Medical Sciences Building, University of Toronto, Toronto, ON Canada M5S 1A8

Communicated by Alfred P. Wolf, September 25, 1989 (received for review June 15, 1989)

ABSTRACT **Dopamine receptor types D_1 and D_2 can oppose or enhance each other's actions for electrical, biochemical, and psychomotor effects. We report a D_1–D_2 interaction in homogenized tissue as revealed by ligand binding. D_2 agonists lowered the binding of [^{3}H]raclopride to D_2 receptors in striatal and anterior pituitary tissues. Pretreating the tissue with the D_1-selective antagonist SCH 23390 prevented the agonist-induced decrease in [^{3}H]raclopride binding to D_2 sites in the striatum but not in the anterior pituitary, which has no D_1 receptors. Conversely, a dopamine-induced reduction in the binding of [^{3}H]SCH 23390 to D_1 receptors could be prevented by the D_2-selective antagonist eticlopride. Receptor photolabeling experiments confirmed both these D_1–D_2 interactions. The blocking effect by SCH 23390 was similar to that produced by a nonhydrolyzable guanine nucleotide analogue, and SCH 23390 reduced the number of agonist-labeled D_2 receptors in the high-affinity state. Thus, the D_1–D_2 link may be mediated by guanine nucleotide-binding protein components. The link may underlie D_1–D_2 interactions influencing behavior, since the link was missing in over half the postmortem striata from patients with schizophrenia and Huntington disease (both diseases that show some hyperdopamine signs) but was present in human control, Alzheimer, and Parkinson striata.**

Many psychomotor signs and symptoms are influenced by drugs that act selectively on brain dopamine receptors. These include rigidity in Parkinson disease, dyskinesias in Huntington disease, hallucinations in schizophrenia, and oral dyskinesia in the elderly. Since dopamine can excite or inhibit neurons (1), and stimulate or inhibit adenylate cyclase (2), it has been proposed (3) that there are two types of dopamine receptors, D_1 and D_2 (see ref. 4 for review). These two receptors enhance each other's action for many psychomotor mechanisms, including body motion (5) and neuron firing (6). The two receptors can also oppose each other, as in controlling mouth motion (7) or cAMP efflux from tissue slices (8). Despite numerous such examples (9), there is little information on possible molecular mechanisms explaining these D_1–D_2 interactions. We here report a link between D_1 and D_2 receptors in homogenized tissue, revealed by the method of ligand binding, and that this link mechanism is similar to, or part of, the guanine nucleotide-binding protein (G-protein) system (10) that mediates some of the actions of dopamine receptors (2, 4). The link may underlie D_1–D_2 interactions influencing behavior, since the link was missing in more than half the postmortem striata from patients with schizophrenia or Huntington disease but was present in postmortem striata from individuals without neurodegenerative disease or with Alzheimer or Parkinson disease.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Binding of [^{3}H]Raclopride to D_2 Receptors. The centrifugation method (11) was used for [^{3}H]raclopride binding. Frozen canine brain striata were from Pel-Freez Biologicals. The tissues were washed twice in buffer (50 mM Tris·HCl, pH 7.4/1 mM EDTA/120 mM NaCl/5 mM KCl/1.5 mM $CaCl_2$/4 mM $MgCl_2$). Porcine anterior pituitary tissues (Bocknek Organic Materials, Rexdale, ON) were prepared as described (12). The final concentrations (in 1 ml) were 1 mg of original tissue per ml (with 0.1% ascorbic acid for canine tissues only) and from 20 pM to 15 nM [^{3}H]raclopride (64–87 Ci/mmol; DuPont; 1 Ci = 37 GBq) with or without 10 μM (*S*)-sulpiride (Ravizza, Milan) to define nonspecific binding. The filtration method was used in some experiments; the tube contents were filtered and rinsed with 7 ml of buffer through a 7034 filter (Skatron, Sterling, VA), using a Skatron cell harvester. The saturation data were analyzed by both the LIGAND program (references in ref. 4) and simple regression analysis. The LIGAND-assisted analysis indicated that a two-site fit was not significantly better than a one-site fit.

Photolabeling of Receptors. D_1 receptors were photolabeled by the method of Niznik *et al.* (13) and D_2 receptors by that of Jarvie *et al.* (14). Canine striatal membranes were Teflon/glass-homogenized in 25 mM Tris·HCl/250 mM sucrose buffer (pH 7.4, 4°C) with protease inhibitors (EDTA, 20 mM; benzamidine, 15 μg/ml; leupeptin, 5 μg/ml; soybean trypsin inhibitor, 5 μg/ml; phenylmethanesulfonyl fluoride, 1 mM) and centrifuged for 10 min at 400 × *g*. The supernatant was subsequently recentrifuged at 48,000 × *g* for 20 min. Membrane pellets were resuspended in Tris·HCl buffer containing 120 mM NaCl and protease inhibitors (without sucrose), to yield a D_1 or D_2 receptor concentration of ≈500 pM, and preincubated with either 200 nM eticlopride or 200 nM SCH 23390‡ for 60 min at 22°C. Following preincubation, homogenates (1 ml) were incubated with either (±)-7-[^{125}I]iodo-8-hydroxy-3-methyl-1-(4-azidophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine ([^{125}I]IMAB, 250 pM) or *N*-(*p*-azido-*m*-[^{125}I]iodophenethyl)spiperone ([^{125}I]N_3-NAPS, 50 pM) and various concentrations of dopamine in a final volume of 5 or

Abbreviations: G protein, guanine nucleotide-binding protein; [^{125}I]IMAB, (±)-7-[^{125}I]iodo-8-hydroxy-3-methyl-1-(4-azidophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine; [^{125}I]N_3-NAPS, *N*-(*p*-azido-*m*-[^{125}I]iodophenethyl)spiperone.

‡Compounds identified by manufacturer code names are as follows: LY 156525, (+)-(4a*S*)-*trans*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline L-tartrate; LY 171555, (−)-(4a*R*)-*trans*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline hydrochloride (quinpirole); LY 275947, (+)-*trans*-3,4,4a,5,6,10b-hexahydro-4-allyl-2*H*-naphth[1,2-*b*]-1,4-oxazin-9-ol; LY 647339, (+)-*trans*-3,4,4a,5,6,10b-hexahydro-4-propyl-2*H*-naphth[1,2-*b*]-1,4-oxazin-9-ol; SCH 23388, (*S*)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol; SCH 23390; (*R*)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol; SKF 38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine.

10 ml, respectively, photolabeled, and processed for SDS/PAGE and autoradiography as described (13, 14).

RESULTS

To search for D_1–D_2 interactions *in vitro*, [^{3}H]raclopride (15) is ideal, since it is highly selective for D_2 receptors (4) but is loosely bound to D_2 ($K_d \approx 1900$ pM) and thus readily sensitive to the presence of dopamine (16).

The addition of dopamine lowered the binding (16) of [^{3}H]raclopride to D_2 receptors, in agreement with previous observations (12, 17, 18). The novel aspect, however, was that this apparent decrease in D_2 density was inhibited by preincubation with a D_1-selective drug (SCH 23390) that by itself had no effect on the properties of [^{3}H]raclopride binding (Fig. 1*A*). In other words, the noncompetitive inhibition of [^{3}H]raclopride binding by dopamine was converted into a competitive type of interaction between dopamine and [^{3}H]raclopride in the SCH 23390-treated tissue. The [^{3}H]raclopride dissociation constant was unaffected by pretreatment with SCH 23390 (Fig. 1*A Inset*). In the presence of dopamine, however, SCH 23390-treated tissue had a higher [^{3}H]raclopride dissociation constant than the control tissue.

This indirect effect of the D_1 drug SCH 23390 on the D_2 receptor required the presence of a D_1 receptor. For example, anterior pituitary, which contains D_2 receptors but no D_1 receptors (19, 20), revealed the dopamine-induced decrease in [^{3}H]raclopride binding but did not show the inhibition of this effect by SCH 23390 (Fig. 1*D*) as seen in striatum (Fig. 1*A*). However, the dopamine-induced depression of [^{3}H]-raclopride binding could be inhibited in both the striatum and anterior pituitary homogenates by preincubation with 5′-guanylyl imidodiphosphate (Fig. 1 *B* and *D*), indicating that the G-protein system was operative, an effect seen previously (12).

To determine the drug selectivity of the dopamine-induced depression of [^{3}H]raclopride to the D_2 sites, the rank order of agonist potency in depressing [^{3}H]raclopride binding was examined and found to be dopamine > norepinephrine > epinephrine > serotonin (Fig. 2). Although no D_2 depression was caused by the D_1-selective agonist SKF 38393 or the D_1-selective antagonist SCH 23390, drugs that were com-

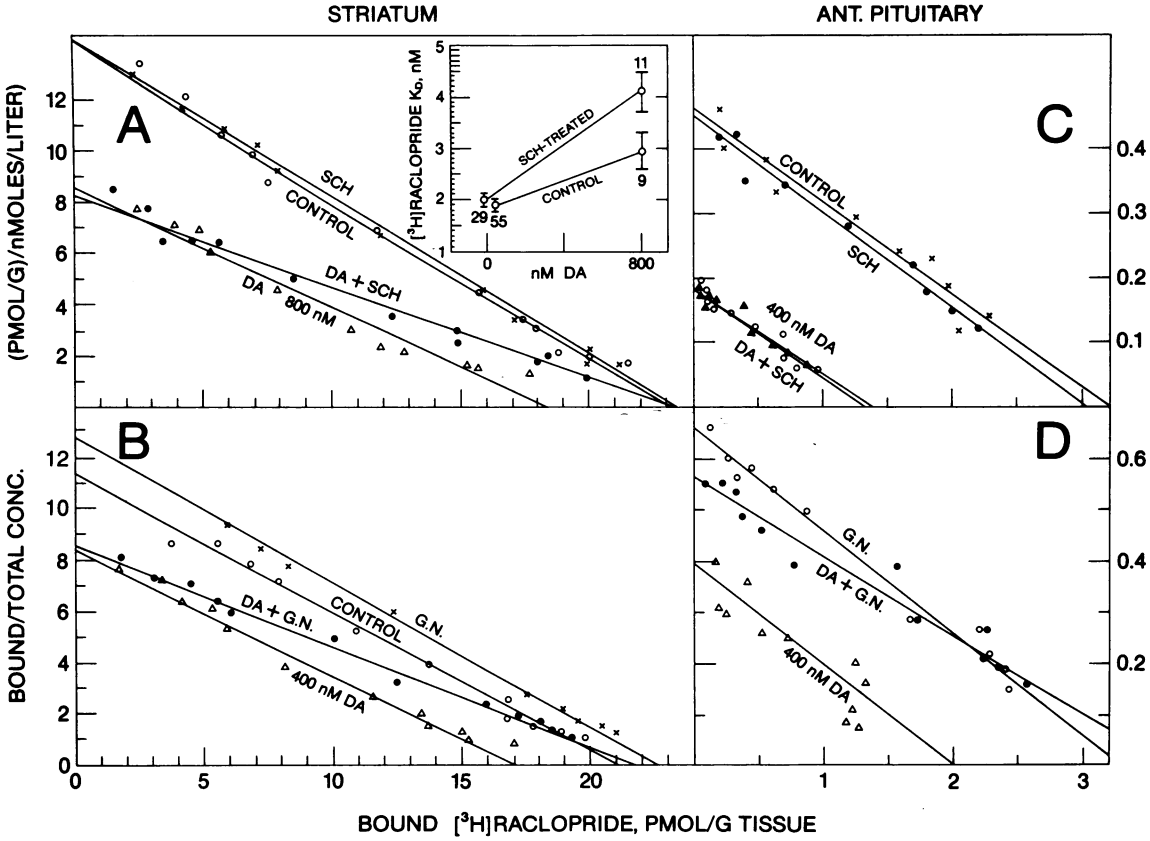

FIG. 1. (*A*) Dopamine (DA, 800 nM), added during the incubation period, noncompetitively depressed the binding of [^{3}H]raclopride to canine striatum. However, when the homogenate had been preincubated with 200 nM SCH 23390 for 1 hr and subsequently incubated with 800 nM dopamine in the presence of 50 nM SCH 23390, the decrease in [^{3}H]raclopride binding was prevented. The preincubation buffer was the same as the incubation buffer, with the exception of SCH 23390, which was 200 nM in the preincubation buffer and 50 nM in the final buffer. Since SCH 23390 has a K_d of 0.17 nM for D_1 (4), concentrations of 50 nM or higher were considered to be an excess for D_1 blockade. The control tissue and the SCH 23390-pretreated tissues came from the same original homogenate, and the two batches of homogenate were treated side-by-side in an identical fashion, except that one beaker contained 200 nM SCH 23390 during the preincubation hour. The final concentration of tissue during preincubation was 4-fold higher than that in the final incubation. Since SCH 23390 by itself had no effect on the binding of [^{3}H]raclopride, the data indicate that SCH 23390, acting on the D_1 receptor, influenced the D_2 receptor in the presence of dopamine. This is one representative experiment, each point done in duplicate; lines were obtained by regression analysis; the program LIGAND yielded identical results. *Inset:* The K_d of [^{3}H]raclopride was 1880 ± 60 pM (n = 55 experiments) in control tissue, while that in homogenates pretreated with SCH 23390 had a similar K_d of 2000 ± 130 pM (n = 29 experiments). In the presence of 800 nM dopamine, however, the two K_d values differed significantly, indicating competition between dopamine and [^{3}H]raclopride in the SCH 23390-pretreated tissue. (*B*) Similar to *A*, except that the tissue was pretreated for 1 hr at room temperature with 400 μM guanylyl imidodiphosphate (G.N.) instead of SCH 23390. (*C*) Dopamine noncompetitively suppressed the binding of [^{3}H]raclopride to D_2 receptors in the anterior pituitary. However, unlike the striatum, pretreatment with 200 nM SCH 23390 could not prevent the suppression of [^{3}H]raclopride binding to D_2. (*D*) Although dopamine suppressed the binding of [^{3}H]raclopride to D_2 sites, this was prevented by prior incubation with 400 μM guanylyl imidodiphosphate (same as *B*, except anterior pituitary).

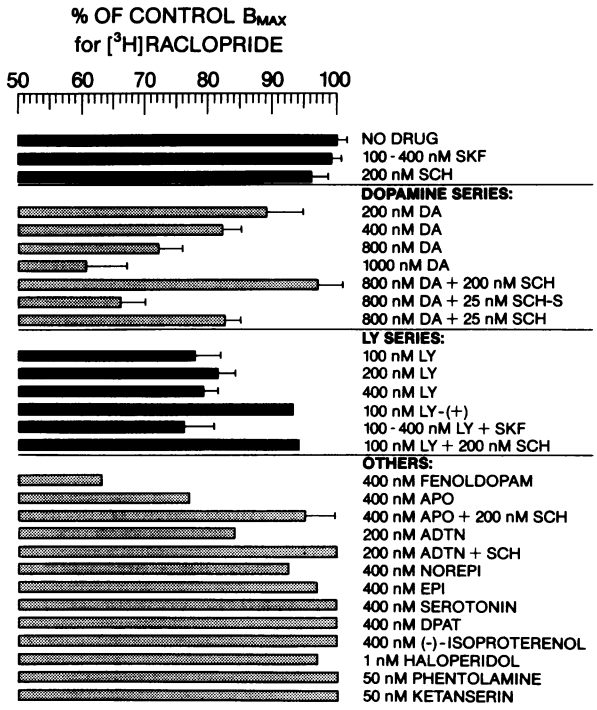

FIG. 2. The inhibition of [^{3}H]raclopride binding to D_2 receptors in canine striatum was selective for D_2 agonists, such as LY 171555 (LY), but not for its inactive (+) enantiomer LY 156525 [LY-(+)]. D_1-selective drugs, such as SKF 38393 (SKF) and SCH 23390 (SCH), as well as serotonin receptor or adrenoceptor blockers (ketanserin or phentolamine, respectively), did not depress [^{3}H]raclopride binding. Preincubation with SCH 23390, however, selectively blocked the dopamine-induced suppression of [^{3}H]raclopride binding, while its inactive (*S*) enantiomer, SCH 23388 (SCH-S), did not. The SCH concentrations indicate those during the 1-hr pretreatment; the final concentrations during the final incubation were 75% lower. DA, dopamine; APO, apomorphine; ADTN, (±)-2-amino-6,7-dihydroxytetralin; NOREPI, norepinephrine; EPI, epinephrine; DPAT, 8-hydroxy-2-(dipropyl)aminotetralin. All data were obtained by the centrifugation method. Bars with SEM markers are for three or more experiments; those bars without SEM markers indicate the average of duplicates. The absolute density of [^{3}H]raclopride D_2 receptors was 22.3 ± 0.3 pmol/g of original tissue in the control samples (n = 55). Although the density of [^{3}H]raclopride sites in the homogenate preincubated with 200 nM SCH 23390 was 96 ± 4% (n = 29) of control, this value served as the 100% value for samples preincubated with SCH 23390.

bined D_1/D_2 agonists (apomorphine and (±)-2-amino-6,7-dihydroxytetralin; see ref. 4) inhibited the binding of [^{3}H]raclopride to D_2 sites. Fenoldopam, which has about the same K_d value for the high-affinity state of D_1 (D_1^{High}) as for D_2^{High} (1.3 and 2.8 nM, respectively; ref. 4), decreased the amount of [^{3}H]raclopride bound by 38% (Fig. 2). The D_2-selective agonist LY 171555 depressed [^{3}H]raclopride binding by only about 22% at all concentrations tested (100–400 nM). Since the strongest effects were obtained with D_1/D_2 agonists, it may be that the residual endogenous dopamine served as a D_1 agonist in the experiments with LY 171555 (or fenoldopam). Serotoninergic agonists and antagonists were ineffective.

While the suppression of [^{3}H]raclopride D_2 sites required D_1/D_2 agonists, the inhibition of this suppression was D_1-selective, as illustrated by the stereoselective action of the active *R* enantiomer SCH 23390 and not its inactive *S* enantiomer, SCH 23388 (Fig. 2). Furthermore, preincubation with 50 nM ketanserin or 50 nM phentolamine for 1 hr had no effect on [^{3}H]raclopride binding or on the dopamine-induced suppression of [^{3}H]raclopride binding.

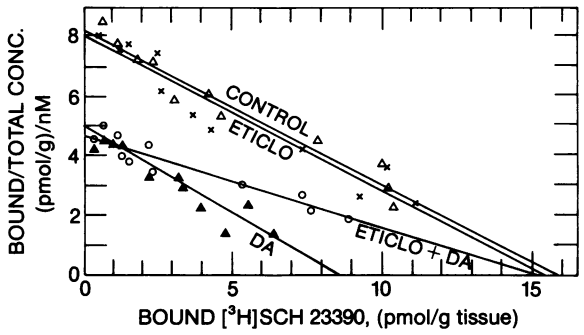

FIG. 3. Example of the D_1–D_2 link in human brain (C320 putamen), as detected using [^{3}H]SCH 23390. Data were obtained by the filtration method, using final concentrations of 0.02 to 6 nM [^{3}H]SCH 23390. Specific binding was defined by 1 μM (+)-butaclamol. Although 400 nM dopamine (DA) added during the incubation period inhibited the binding (B_{max}) of [^{3}H]SCH 23390 to D_1 receptors, this effect did not occur if the tissue had been preincubated with 200 nM eticlopride (ETICLO) and then coincubated (in the presence of 400 nM dopamine) with a final concentration of 50 nM eticlopride. The control tissue (without eticlopride) was treated in an identical fashion, except that eticlopride was not present.

Since the data suggested that D_2 agonists were occupying the D_2^{High} sites and thereby occluding these sites for the binding of [^{3}H]raclopride, we tested this idea more directly. We used three types of experiments to examine whether SCH 23390 could alter the proportion or density of D_2^{High} sites in canine striatum.

First, by means of dopamine competition with 3 nM [^{3}H]raclopride, the presence of SCH 23390 (200 nM during preincubation; 50 nM during incubation) decreased the proportion of D_2^{High} sites from 60 ± 5% to 44 ± 5% (n = 4 experiments, each in duplicate; $P < 0.05$, LIGAND program).

Second, (+)-*trans*-3,4,4a,5,6,10b-hexahydro-4-[^{3}H]propyl-2*H*-naphth[1,2,-*b*]-1,4-oxazin-9-ol {[^{3}H]PHNO (LY 647339), 33 Ci/mmol}, an agonist that is 67-fold more avid for D_2^{High} than for D_1^{High} (4), was custom-synthesized (P.S., A. N. Jones, and P. S. Anderson, unpublished work). We found that SCH 23390 (200 nM during preincubation; 50 nM during incubation) lowered the density of [^{3}H]PHNO sites by 50% (of the amount lowered by 400 μM guanylyl imidodiphosphate; conditions of Fig. 1).

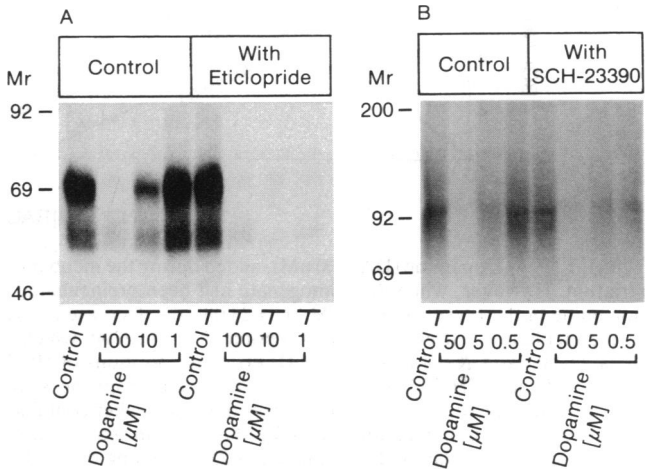

FIG. 4. The ability of dopamine to inhibit the photoincorporation of [^{125}I]IMAB (*A*) or [^{125}I]N_3-NAPS (*B*) into the ligand subunits of canine striatal D_1 and D_2 dopamine receptors was enhanced by pretreating receptor preparations with either eticlopride or SCH 23390, respectively. The data are representative of at least three independent experiments with qualitatively similar results. Markers indicate $M_r \times 10^{-3}$.

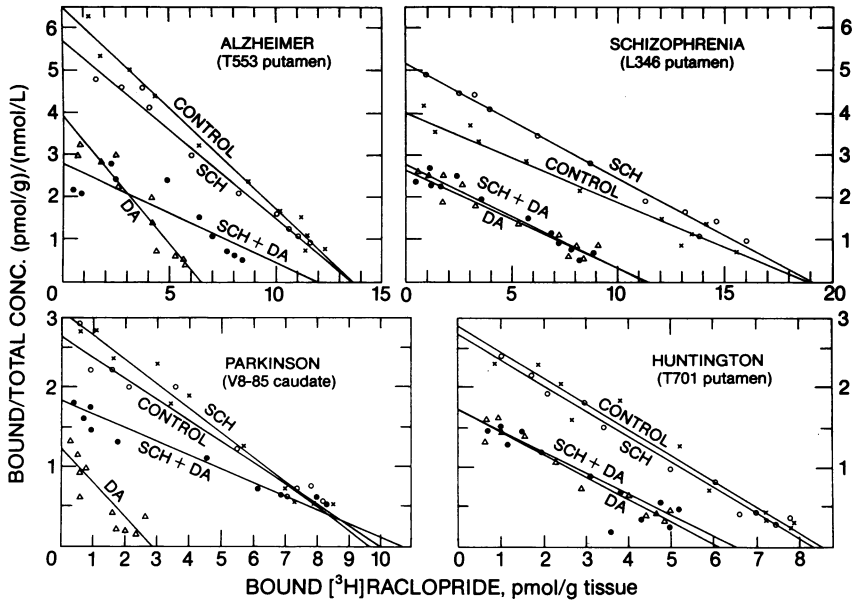

FIG. 5. Examples of data indicating a D_1–D_2 link in Alzheimer (case T553 putamen) and Parkinson (case V8-85 caudate) diseased striata but an absence of this link in schizophrenia (case L346 putamen) and Huntington disease (case T701 putamen). Data were obtained by the centrifugation method as in Fig. 1*A* except that the tissues were washed only once and 400 nM dopamine (DA) was used. Ascorbic acid was never included when human tissues were used. SCH, SCH 23390.

Third, [^{3}H]quinpirole (LY 171555 hydrochloride; 61 Ci/mmol) was custom-synthesized by using *N*-allyl-despropylquinpirole (LY 275947) as a precursor (P.S., C.U., and J. M. Schaus, unpublished work). This dopamine agonist is 250 times more selective for D_2^{High} than for D_1^{High} (4). SCH 23390 caused the binding of [^{3}H]quinpirole (0.8–6 nM) to decrease 33 ± 5% (n = 3, in quintuplicate), indicating a decrease in the density of D_2^{High}, even though SCH 23390 had no effect on the total density of D_2 sites as detected by [^{3}H]raclopride (Fig. 1).

Moreover, we tested for a reciprocal effect of D_2 receptors on D_1 receptors. Here, too, dopamine (400 nM) suppressed the density of [^{3}H]SCH 23390-labeled D_1 sites, only to have these sites restored by preincubation of the tissue with the D_2-selective eticlopride (200 nM during preincubation; 50 nM during incubation) (Fig. 3).

Although the D_1–D_2 effect (Fig. 1) was obtained in rat, canine, and human striatum, there was variability between experiments in the magnitude of the effect. We found that the most unambiguous data were obtained with human tissues and ^{3}H-labeled ligands with high dissociation constants. Hence, [^{3}H]raclopride (K_d = 1900 pM) was better than [^{3}H]spiperone (K_d = 50 pM). Moreover, human tissue has a high K_d (881 pM) for [^{3}H]SCH 23390 (21), compared to canine striatum (K_d = 170 pM; ref. 4), thus permitting the binding of [^{3}H]SCH 23390 to D_1 receptors to be more sensitive to the indirect effects of D_2 receptors. It is important to note that it was essential to preincubate the tissue with SCH 23390.

In summary, the noncompetitive action of dopamine at each receptor was converted into a competitive action by the presence of an antagonist at the other receptor. We confirmed this conversion by receptor photolabeling experiments. Eticlopride enhanced the ability of dopamine to block the photolabeling of D_1 receptors ($M_r \approx$ 74,000) by [^{125}I]IMAB (Fig. 4*A*). SCH 23390 enhanced the potency of dopamine to block the photolabeling of D_2 receptors ($M_r \approx$ 94,000) by [^{125}I]N_3-NAPS (Fig. 4*B*).

Since an important role for the D_1–D_2 link may be in diseases such as schizophrenia and Huntington disease (11) which include hyperdopamine signs and symptoms, we sought evidence for this possibility. We found that the D_1–D_2 link existed in human control striata (11 tissue samples), Alzheimer disease striata (7 samples), and Parkinson disease striata (6 samples). The D_1–D_2 link was missing, however, in 8 of 15 schizophrenia tissue samples (or in 6 of 10 different brains) and in 7 of 13 Huntington samples (or in 6 of 13 different brains) (Figs. 5 and 6). Premortem neuroleptic treatment had no apparent effect on the link, since 2 control and 6 Alzheimer samples contained considerable neuroleptic (as noted in the clinical history and as evidenced by the high K_d for [^{3}H]raclopride).

DISCUSSION

The data suggest the following interpretation (Fig. 7). D_2 agonists occupy the D_2^{High} sites (4), thus occluding these sites for the binding of [^{3}H]raclopride. D_2^{High} can be converted by guanine nucleotide to D_2^{Low}, thus permitting [^{3}H]raclopride to bind to all the D_2 sites present. The similar effects of the nonhydrolyzable guanine nucleotide analogue guanylyl imidodiphosphate and of SCH 23390 (but only when D_1 receptors are present) suggest that the D_1 receptor may influence the D_2 receptor through the G-protein system. For example, the subunits of the stimulatory G protein, G_s (serving D_1), may be in equilibrium with those of the inhibitory G protein, G_i (serving D_2). This D_1–D_2 equilibrium may be unbalanced by SCH 23390.

The *in vitro* mechanisms reported here may account for some of the behavioral and biochemical observations of a D_1–D_2 link. For example, catalepsy produced by SCH 23390 is reversed by D_2-selective agonists (22). This may be based on the ability of SCH 23390 to alter the number of D_2^{High} sites.

Although the brain location and chemical pathology in schizophrenia are no doubt different from those in Huntington disease, some of the hyperdopamine signs and symptoms (psychosis in schizophrenia, chorea in Huntington disease, with both features treated by D_2 blockers) may be based on a similar molecular mechanism of a D_2 action with reduced D_1 influence. Although the data in Fig. 6 suggest that premortem neuroleptic drugs did not eliminate the D_1–D_2 link, a direct test for this link must await a positron-emission tomographic study on nonmedicated individuals.

We thank the following for their excellent cooperation in providing postmortem tissues: Dr. Catherine Bergeron, Dr. T. P. Morley, and Maria Pataki, all at the Canadian Brain Tissue Bank (Banting Institute, University of Toronto, Toronto); Dr. K. Jellinger, Ludwig Boltzmann Institute for Clinical Neurobiology (Lainz Hospital, Vienna); Dr. W. W. Tourtellotte and Iris Rosario, National Neurological Research Bank (Veterans Administration Wadsworth Medical Center, Los Angeles); and Dr. C. M. Wischik, Sue West, Rick Hills, and Anne Futter, all at the Cambridge Brain Bank Laboratory (Cambridge, U.K.). We thank Dr. Susan R. George for helpful suggestions. This work is supported by the National Alliance for Research in Schizophrenia and Depression in association with the Laureate Psychiatric Research Center, Inc. (Tulsa, OK), the Ontario

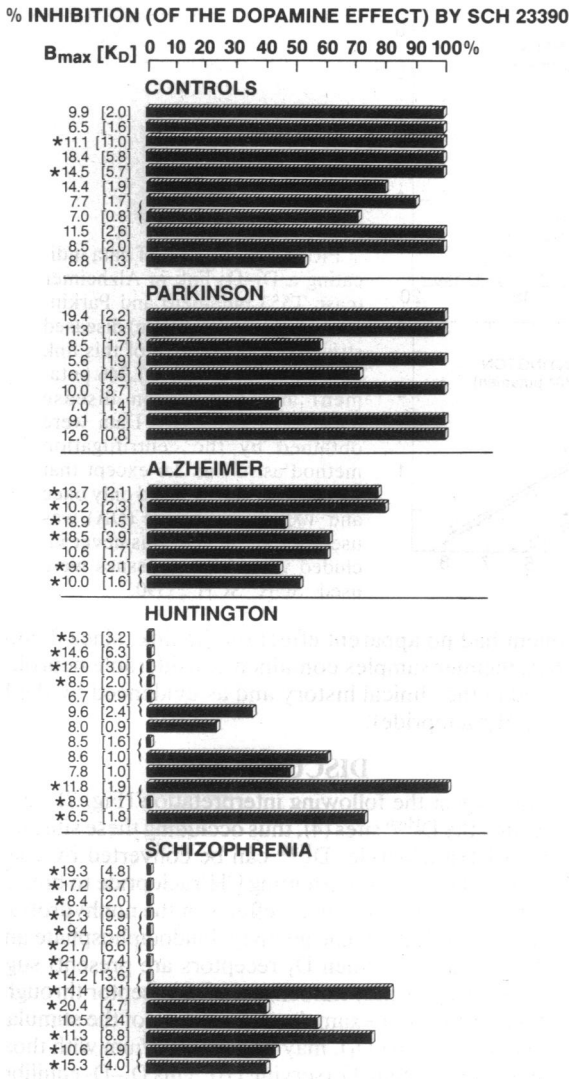

FIG. 6. Human control and diseased striata. Each bar indicates the percent inhibition by SCH 23390 (of the dopamine-induced depression of [^{3}H]raclopride density), defined as $100 - 100(B^S - B^{S+D})/(B^C - B^D)$, where B was the density of [^{3}H]raclopride sites in the control (B^C), in the presence of SCH 23390 (B^S), in the presence of dopamine (B^D), or in the presence of both SCH 23390 and dopamine (B^{S+D}). A star indicates that the individual received neuroleptics in the last year of life, with the tissue generally revealing a high K_d for [^{3}H]raclopride. Tissues were mostly putamens, except where the bracket indicates putamen followed by caudate nucleus from the same brain. B_{max} (in pmol/g) and K_d (nM) values are for [^{3}H]raclopride binding in the control sample (i.e., without added dopamine or SCH 23390). Dopamine was added at 400 nM final concentration in all cases, except in the first set of control tissues (where it was 200 nM, 400 nM, 100 nM, 200 nM, and 800 nM, respectively) and in two schizophrenia tissues (where it was 200 nM).

Mental Health Foundation, and the Medical Research Council of Canada. The National Neurological Research Bank, Wadsworth Veterans Administration Medical Center, Los Angeles, is sponsored by the National Institute of Neurological and Communicative Disorders and Stroke, the National Institute of Mental Health, the National Multiple Sclerosis Society, the Huntington Disease Foundation, and the Veterans Administration. H.B.N. is a Career Scientist of the Ontario Ministry of Health. P.S. is a Laureate Investigator of the Laureate Psychiatric Research Center (Tulsa, OK) in association with the National Alliance for Research on Schizophrenia and Depression.

1. Bloom, F. E., Costa, E. & Salmoiraghi, G. C. (1965) *J. Pharmacol. Exp. Ther.* **150,** 244–252.
2. Onali, P., Olianas, M. C. & Gessa, G. L. (1985) *Mol. Pharmacol.* **28,** 138–145.

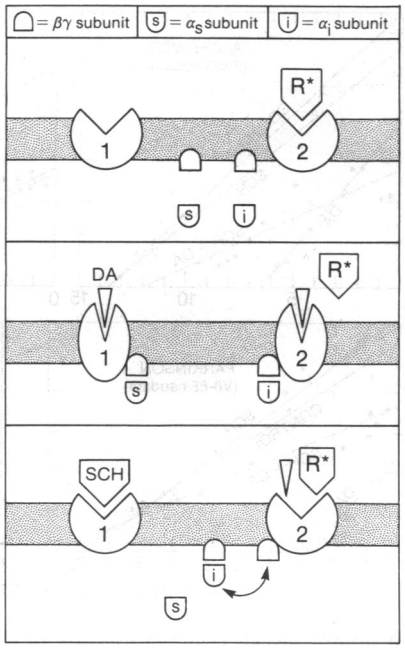

FIG. 7. Possible mechanism whereby D_1 blockade can inhibit the effect of dopamine (on [^{3}H]raclopride binding to D_2 receptors). (*Top*) Binding of [^{3}H]raclopride (R*) to D_2 receptors. (*Middle*) Exogenous dopamine (DA) attaches to the high-affinity state of D_2, inducing a ternary complex of dopamine–receptor–G and thus occluding sites for [^{3}H]raclopride binding. (*Bottom*) In the presence of SCH 23390 (to block D_1 receptors), the subunits of the G proteins may rearrange. This rearrangement converts D_2 receptors to low affinity for dopamine, permitting [^{3}H]raclopride to out-compete dopamine for binding to D_2 receptors. Although D_1 and D_2 receptors are often (but not always) found in the same brain regions, there is as yet no unequivocal autoradiographic evidence that both receptors are located on the same cell membrane. α_s subunit, α subunit of the stimulatory G protein; α_i, subunit, α subunit of the inhibitory G protein; $\beta\gamma$ subunit, subunit common to both G proteins.

3. Spano, P. F., Govoni, S. & Trabucchi, M. (1978) *Adv. Biochem. Psychopharmacol.* **19,** 155–165.
4. Seeman, P. & Niznik, H. B. (1988) *ISI Atlas Sci. Pharmacol.* **2,** 161–170.
5. Gershanik, O., Heikkila, R. E. & Duvoisin, R. C. (1983) *Neurology* **33,** 1489–1492.
6. Walters, J. R., Bergstrom, D. A., Carlson, J. H., Chase, T. N. & Braun, A. R. (1987) *Science* **236,** 719–722.
7. Rosengarten, H., Schweitzer, J. W. & Friedhoff, A. J. (1983) *Life Sci.* **33,** 2479–2482.
8. Stoof, J. C. & Kebabian, J. W. (1981) *Nature (London)* **294,** 366–368.
9. Clark, D. & White, F. J. (1987) *Synapse* **1,** 347–388.
10. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–649.
11. Seeman, P., Bzowej, N. H., Guan, H.-C., Bergeron, C., Reynolds, G. P., Bird, E. D., Riederer, P., Jellinger, K. & Tourtellotte, W. W. (1987) *Neuropsychopharmacology* **1,** 5–15.
12. George, S. R., Watanabe, M. & Seeman, P. (1985) *J. Neurochem.* **44,** 1168–1177.
13. Niznik, H. B., Jarvie, K. R., Bzowej, N. H., Seeman, P., Garlick, R. K., Miller, J. J., Baindur, N. & Neumeyer, J. L. (1988) *Biochemistry* **27,** 7594–7599.
14. Jarvie, K. R., Niznik, H. B. & Seeman, P. (1988) *Mol. Pharmacol.* **34,** 91–97.
15. Kohler, C., Hall, H., Ogren, S.-O. & Gawell, L. (1985) *Biochem. Pharmacol.* **34,** 2251–2259.
16. Seeman, P., Guan, H.-C. & Niznik, H. B. (1989) *Synapse* **3,** 96–97.
17. Sibley, D. R. & Creese, I. (1980) *Eur. J. Pharmacol.* **65,** 131–133.
18. Chatterjee, T. K., Scott, C. E., Vazquez, D. M. & Bhatnagar, R. K. (1988) *Mol. Pharmacol.* **33,** 402–413.
19. Titeler, M., Van Loon, G. R., Seeman, P. & Brown, G. M. (1981) *Eur. J. Pharmacol.* **71,** 143–146.
20. Rovescalli, A. C., Brunello, N., Monopoli, A., Ongini, E. & Racagni, G. (1987) *J. Pharmacol.* **135,** 129–136.
21. Seeman, P., Bzowej, N. H., Guan, H.-C., Bergeron, C., Becker, L. E., Reynolds, G. P., Bird, E. D., Riederer, P., Jellinger, K., Watanabe, S. & Tourtellotte, W. W. (1987) *Synapse* **1,** 399–404.
22. Meller, E., Kuga, S., Friedhoff, A. J. & Goldstein, M. (1985) *Life Sci.* **36,** 1857–1864.