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Characterisation of G_s activation by dopamine D1 receptors using an antibody capture assay: antagonist properties of clozapine

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Abstract

Herein, we examined the direct coupling of human dopamine D1 receptors to G_s proteins using an antibody capture assay together with a detection technique employing scintillation proximity assay beads. Using a specific antibody, dopamine (DA) and the selective dopamine D1 receptor agonists, 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF81297) and 3-allyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF82958), behaved as high-efficacy agonists (~100%) in stimulating guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding to G_s in L-cells, whereas 2,3,4,5,-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SKF38393) displayed partial agonist properties (70%). The action of dopamine was specifically mediated by human dopamine D1 receptors inasmuch as the selective human dopamine D1 receptor antagonist, (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (SCH23390), blocked dopamine-induced [^{35}S]GTPγS binding to G_s with a pK_B (9.29) close to its pK_i (9.33). The antipsychotic agents, clozapine and haloperidol, displayed no intrinsic activity when tested alone and inhibited dopamine-stimulated G_s activation with pK_B's of 6.7 and 7.3, respectively, values close to their pK_i values at these sites. In conclusion, the use of an anti-G_s protein immunoprecipitation assay coupled to scintillation proximity assays allows direct evaluation of the functional activity of dopamine D1 receptors ligands at the G protein level. Employing this novel technique, the typical and atypical antipsychotics, clozapine and haloperidol, respectively, both exhibited antagonist properties at dopamine D1 receptors.

Keywords: Dopamine D1 receptor; G_s protein; Scintillation proximity assay; Clozapine; Haloperidol

1. Introduction

Dopamine receptors (D1–D5) can be grouped into two receptor families. Dopamine D2-like receptors include the dopamine D2, D3 and D4 subtypes, whereas dopamine D1-like receptors include the dopamine D1 and D5 subtypes (Neve and Neve, 1997). Dopamine D1 receptors couple to the G_s protein family ($G_{\alpha s}$ and $G_{\alpha olf}$ isoforms) in recombinant cell systems (Dearry et al., 1990; Zhou et al., 1990) as well as in tissue (Zhuang et al., 2000; Corvol et al., 2001; Jin et al., 2001). The efficacy of ligands at dopamine D1 receptors has generally been measured by determination of adenylyl cyclase activity (O'Boyle et al., 1989; Lewis et al., 1998; Cai et al., 1999). However, depending on the type of adenylyl cyclase under study (whether activated or not by $G_{\beta\gamma}$ subunits, in addition to $G_{\alpha s}$, see Watts, 2002), interpretation of ligand efficacy and potency at distal, dopamine D1 receptor-coupled targets such as adenylyl cyclase may be more complicated than direct measures of G protein activation. Thus, it would appear desirable to directly explore the influence of drugs at G_s-proteins coupled to human dopamine D1 receptors.

In this light, experimental assays of [^{35}S]GTPγS-labelled-G-proteins are of potential interest since they have been extensively used to study the coupling of other G protein coupled receptors to their respective G proteins. Though classical filtration assays are principally limited to detection of members of the $G_{i/o}$ protein family (Milligan, 2003), an antibody-capture assay of [^{35}S]GTPγS-labelled G proteins combined with a detection technique employing anti-immunoglobulin G-coated scintillation proximity assay beads efficiently permitted determination of the activation of G_q and G_s proteins (DeLapp et al., 1999; Bymaster et al., 2001; Cussac et al., 2002a; Porter et al., 2002). Thus, one goal of the present study was to develop and pharmacologically validate an antibody capture/[^{35}S]GTPγS binding approach

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for exploration of the functional coupling of human dopamine D1 receptors to endogenous G_s proteins. To this end, we undertook studies of human dopamine D1 receptors expressed in L-cells employing the prototypical dopamine D1 receptor agonists, 2,3,4,5,-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SKF38393), 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF81297) and 3-allyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF82958), and the dopamine D1 receptor antagonist, (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (SCH23390), drugs which have been widely used to characterise the functional role of dopamine D1 receptors in both in vitro as well as in vivo paradigms (O'Boyle et al., 1989; Gessa et al., 1991; Lewis et al., 1998).

While the clinical efficacy of antipsychotics against positive symptom of schizophrenia involves blockade of mesolimbic populations of dopamine D2 receptors (Seeman, 1980; Kapur and Seeman, 2001), there is continuing interest in the significance of dopamine D1 receptors. SCH23390 failed to show antipsychotic activity in clinical trials (Gessa et al., 1991; Farde and Sedvall, 1995), and contradictory results have been reported concerning levels of dopamine D1 receptors in schizophrenic patients (Okubo et al., 1997; Domyo et al., 2001), but there is increasing evidence that dopamine D1 receptors in the prefrontal cortex are implicated in the perturbed cognitive function displayed by psychotic patients (Lynch, 1992; Lidow et al., 1998; Goldman-Rakic, 1999; Okubo et al., 1997). Thus, it is of importance to evaluate the role of dopamine D1 receptors in the therapeutic properties of antipsychotic agents. Of particular pertinence in this regard is the atypical antipsychotic, clozapine, the clinical response to which is a function of dopamine D1 receptor genotype, suggesting a role of these receptors in its action (Potkin et al., 2003). As concerns its interactions with dopamine D1 receptors, contradictory results have been reported employing a variety of techniques: that is, it has shown agonist (Ahlenius, 1999; Oerther and Ahlenius, 2000) or antagonist (Andersen and Braestrup, 1986; Kozell and Neve, 1997; Cai et al., 1999) properties. Thus, we set out to evaluate the actions of clozapine at human dopamine D1 receptors coupled to G_s using this novel antibody capture assay, Its actions were compared to those of the typical antipsychotic, haloperidol, which likewise interacts with dopamine D1 receptors, though far less potently than its antagonist actions at dopamine D2 receptors (Kapur and Seeman, 2001).

2. Materials and methods

2.1. Competition binding assays

Binding affinity was determined by competition binding experiments from L-cells-human dopamine D1 receptor membranes in presence of [^{3}H]SCH23390 (0.5 nM, PerkinElmer Life Science, Paris, France) in a buffer containing TrisHCl (50 mM) pH 7.5, NaCl (120 mM), KCl (5 mM), $CaCl_2$ (2 mM) and $MgCl_2$ (5 mM). Incubations in 96-well plates lasted 1 h at 22 °C and non-specific binding was defined by butaclamol (3 μM). Experiments were terminated by rapid filtration through Unifilter-96 GF/B filters (pretreated with polyethyleneimine 0.3%) using a Filtermate harvester (PerkinElmer Life Science, Boston, MA). Radioactivity retained on the filters was determined by liquid scintillation counting using a Top-Count microplate scintillation counter (PerkinElmer Life Science). Isotherms were analysed by non-linear regression to yield IC_{50} values. Inhibition constants (K_i values) were derived from IC_{50} values according to the Cheng–Prusoff equation.

2.2. Characterisation of antibody used in scintillation proximity assays (SPA)

About 25 ng of purified recombinant rat $G_{\alpha o}$, $G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha q}$ and $G_{\alpha s}$ (short splice variant), (VWR International S.A.S, Fontenay-sous-Bois, France) and 10 μg of L-cell membranes were loaded onto 10% polyacrylamide gel and transferred to nitrocellulose. Immunoblotting of G_α subunits was performed using the polyclonal anti-$G_{\alpha s/\alpha olf}$ (C18) antibody from Santa Cruz Biotechnology (Santa Cruz, CA) at 0.4 μg/ml, followed by enhanced chemiluminescence detection with horseradish peroxydopaminese as secondary antibody (1/6000) (Amersham Biosciences, Saclay, France).

2.3. Measurement of agonist efficacy and antagonist potency at human dopamine D1 receptors coupled to G_s

Human dopamine D1 receptor-linked specific G_s-protein activation by agonists was determined by measuring the stimulation of [^{35}S]GTPγS (1332 Ci/mmol; PerkinElmer Life Sciences, Paris, France) binding coupled to an antibody capture assay, essentially as previously described for $G_{q/11}$ and G_{i3} proteins (Cussac et al., 2002a). L-cell-human dopamine D1 receptor membranes (~2 μg per well) were pre-incubated 30 min with agonists and antagonists in a buffer containing 20 mM HEPES, pH 7.4, 1 μM GDP, 50 mM $MgCl_2$ and 100 mM NaCl, and the reaction was started with [^{35}S]GTPγS (~0.3 nM in a final volume of 200 μl in 96-well optiplates (PerkinElmer Life Sciences) for 60 min at room temperature. At the end of the incubation period, 20 μl of Nonidet P-40 (Merck Eurolab, Fonteney sous Bois, France, 0.27% final concentration) was added to each well and the plates incubated with gentle agitation for 30 min. Polyclonal antibodies anti-$G_{\alpha s/\alpha olf}$ (1.74 μg/ml final dilution) were added to each well in a volume of 10 μl before 30 min of additional incubation period. Scintillation proximity assay beads coated with secondary anti-rabbit antibodies

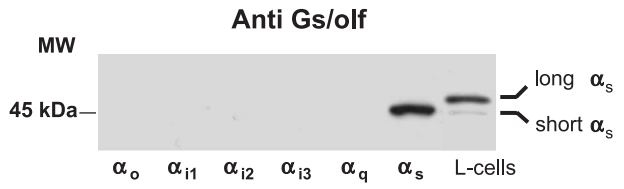

Fig. 1. Immunodetection of Gα subunits. Purified $G_{\alpha o}$, $G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha q}$, $G_{\alpha s}$ (short splice variant, $G_{s\alpha S}$) and L-cell membrane were separated on a gel and submitted to immunodetection using polyclonal antibody anti-$G_{\alpha s/\alpha olf}$ as described in Materials and methods. The long splice variant of Gαs subunits is principally expressed in L-cell.

from Amersham Biosciences UK (Little Chalfont, Buckinghamshire, UK) were added in a volume of 50 μl at a dilution indicated by the manufacturer and the plates incubated for 3 h with gentle agitation. The plates were then centrifuged (10 min, 1300×*g*) and radioactivity was detected on a Top-count microplate scintillation counter (PerkinElmer Life Sciences). Agonist efficacy is expressed relative to dopamine, which was tested at a maximal concentration in each experiment. Thus, basal binding (which includes both non-specific radioactivity detection and endogenous guanine nucleotide turnover on G_s) is defined as 0%, whilst dopamine-stimulated [^{35}S]GTPγS binding to G_s is defined as 100%. All data are expressed as means±S.E.M. of at least three independent determinations.

2.4. Analysis of data

Isotherms were analysed by non-linear regression, using the program "PRISM" (Graphpad Software, San Diego, CA) to yield EC_{50} and IC_{50} values. K_B values of antagonists for inhibition of dopamine (1μM)-stimulated G_s protein activation were calculated according to the Cheng-Prusoff equation: K_B=IC_{50}/(1+(Agonist/EC_{50})), where IC_{50}=Inhibitory Concentration$_{50}$ of antagonist, Agonist=concentration of dopamine, and EC_{50}=Effective Concentration$_{50}$ of dopamine alone.

2.5. Materials

L-cells membranes expressing cloned human dopamine receptor subtype 1 (human dopamine D1, GenBank No. X58987, 7.5 pmol per mg of membrane protein) were purchased from PerkinElmer Life Science (Boston, MA). Haloperidol, clozapine, SCH23390 ((*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol hydrochloride), SKF38393 (2,3,4,5,-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride), SKF81297 (6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) and SFK82958 (3-allyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) were purchased from Sigma/RBI (Natick, MA) and dopamine from Sigma.

3. Results

In order to verify the specificity of the antibody used in the scintillation proximity assay, we tested the polyclonal anti-$G_{\alpha s/\alpha olf}$ antibody against different purified G_α subunits. Fig. 1 shows that anti-$G_{\alpha s/olf}$ antibody recognised the Gαs subunit but not other G_α subunits of G_i and G_q families. Two immunoreactive bands were detected in L-cell membranes, representing the short and long splice variants of $G_{\alpha s}$ subunits, the latter being the major protein expressed in this cell line (Fig. 1).

At human dopamine D1 receptors, dopamine induced a robust signal in stimulating [^{35}S]GTPγS binding at G_s with an EC_{50} of 115 nM (Fig. 2 and Table 1). Dopamine increased [^{35}S]GTPγS binding to G_s proteins by ~1.8 to 2-fold (typically ~2800 dpm for basal up to a maximal level of 5500 dpm) whereas, employing the same membrane

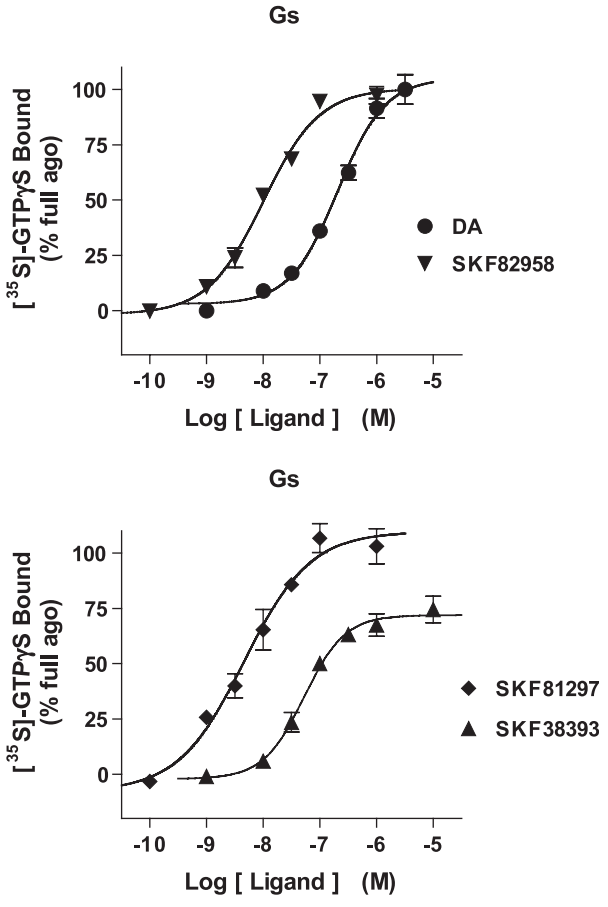

Fig. 2. Concentration-dependent actions of agonists at human dopamine D1 receptor-mediated G_s protein activation. Agonist concentration–responses curves at G_s proteins from L cell-human dopamine D1 receptor membranes was determined with anti-$G_{\alpha s}$ protein antibodies captured via secondary anti-rabbit antibody-coated SPA beads as described in Materials and methods. [^{35}S]GTPγS binding is expressed as a percentage of maximal stimulation with dopamine (100%). Points shown are means of triplicate determinations from representative experiments repeated on at least three occasions. E_{MAX} and pEC_{50} data from these experiments are shown in Table 1.

Table 1
Stimulation by agonists at human dopamine D1 receptors coupled to G_s and comparisons with their respective affinities (pK_i values)

Ligand	G_s			pK_i
	pEC_{50}	EC_{50} (nM)	E_{MAX} (%)	
DOPAMINE	6.94±0.09	115	105.6±1.1	5.46±0.11
SKF38393	7.01±0.10	97.7	70.7±2.0[a]	6.73±0.01
SKF82958	8.00±0.06	10	99.3±3.4	7.49±0.04
SKF81297	8.10±0.11	7.9	100.9±3.0	7.47±0.06

Agonist efficacies at G_s were determined by [^{35}S]GTPγS binding coupled to a scintillation proximity assay. Agonist efficacies are expressed relative to that of dopamine, which was tested at a maximally effective concentration in each experiment (10 μM). EC_{50} values were calculated from mean pEC_{50} values. pK_i values were determined as described in Materials and methods. Values are means±S.E.M. of at least three independent experiments.

[a] E_{MAX} values differed significantly ($p<0.05$, unpaired t-test) versus dopamine.

preparation, a conventional [^{35}S]GTPγS binding approach failed to reveal a marked effect of dopamine (~1.1 fold, data not shown). The dopamine D1 receptor antagonist, SCH23390, blocked dopamine-stimulated [^{35}S]GTPγS binding at G_s with a pK_B identical to its pK_i (9.3, Fig. 3 and Table 2), confirming that engagement of human dopamine D1 receptors mediated G_s activation. The dopamine D1 receptor ligands, SKF82958 and SKF81297, possessed high potency and behaved as full agonists in stimulating G_s proteins, whereas SKF38393 was less potent and exhibited partial agonist properties (70%) as compared to dopamine (Fig. 2 and Table 1).

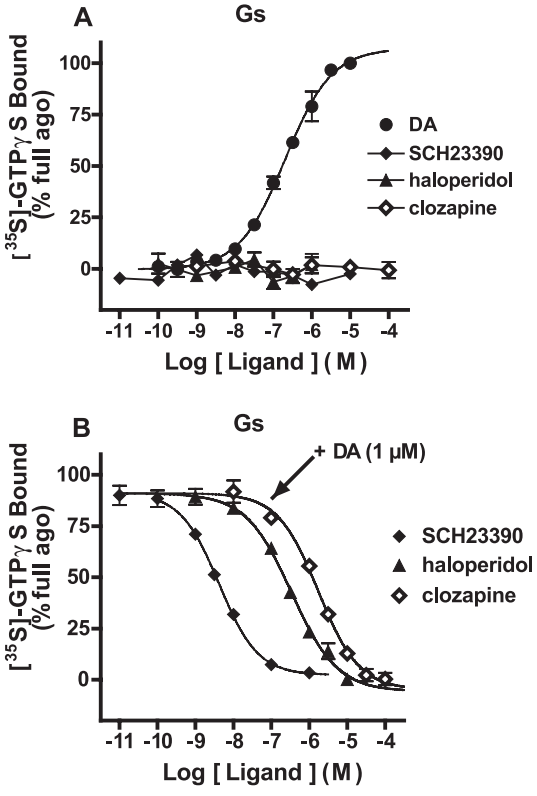

Fig. 3. Concentration-dependent actions of antagonists upon human dopamine D1 receptor-mediated G_s protein activation. Concentration–response curves for SCH23390, haloperidol and clozapine alone (A) and against dopamine (1 μM)-stimulated [^{35}S]GTPγS binding at G_s proteins (B) from L cell-human dopamine D1 receptors membrane were determined using SPA. [^{35}S]GTPγS binding is expressed as a percentage of maximal stimulation with dopamine (100%). Points shown are means of triplicate determinations from representative experiments repeated on at least three occasions. pK_B data from these experiments are shown in Table 2.

Table 2
Inhibition by antagonists at human dopamine D1 receptors coupled to G_s and comparisons with their respective affinities (pK_i values)

Ligand	G_s		pK_i
	pK_B	K_B (nM)	
SCH23390	9.29±0.07	0.51	9.33±0.04
Haloperidol	7.29±0.12	51.3	7.41±0.07
Clozapine	6.71±0.09	195	6.91±0.04

Antagonist potencies (pK_B values) were calculated from IC_{50} values for inhibition of dopamine (1 μM)-stimulated [^{35}S]GTPγS binding at G_s proteins coupled to a scintillation proximity assay. K_B values were calculated from mean pK_B values. pK_i values were determined as described in Materials and methods. Values are means±S.E.M. of at least three independent experiments.

Employing this technique, it was shown in parallel that the atypical antipsychotic, clozapine, as well as the typical neuroleptic, haloperidol, both concentration-dependently and completely blocked dopamine-induced G_s activation with pK_B values similar to their respective pK_i's (Fig. 3 and Table 2). Both drugs were inactive when tested alone (Fig. 3).

4. Discussion

Immunoprecipitation with selective antibodies directed against specific classes of [^{35}S]GTPγS-labelled G proteins, coupled to a recently described scintillation proximity assay, now allows determination of the activation of discrete G proteins (DeLapp et al., 1999; Bymaster et al., 2001; Cussac et al., 2002a; Newman-Tancredi et al., 2002). In particular, this approach efficiently measures G_q and G_s activation in contrast to classical filtration assays where high basal [^{35}S]GTPγS binding to $G_{i/o}$ proteins masks stimulation of G_q and G_s protein families (see Milligan, 2003 for review). Accordingly, using an antibody-capture assay targeting $G_{\alpha s/\alpha olf}$ (Fig. 1), we were able to characterise herein the direct coupling of human dopamine D1 receptors to G_s protein.

By use of this strategy, we observed the same relative efficacy of several selective dopamine D1 receptor ligands at L-cell-transfected human dopamine D1 receptors as previously reported for adenylyl cyclase coupled to endogenous dopamine D1 receptors in rat striatal membranes, and for dopamine D1 receptor expressed in C6-glioma cells (O'Boyle et al., 1989; Lewis et al., 1998). However, in these studies SKF38393 displayed an efficacy of only 30–40% relative to dopamine, probably reflecting a low density of

dopamine D1 receptors in these cell systems. The comparatively high efficacy of SKF38393 reported herein (70%) is likely due to the high level of human dopamine D1 receptors in L cells (about 7.5 pmol per mg of membrane protein). Reflecting signal amplification in cell lines, we and others have shown that greater receptor occupancy is necessary to achieve half-maximal activation of G proteins compared to that of downstream effectors, such as described for α_{2C}-G_i- adenylyl cyclase and 5-HT_{2C}-G_q-phospholipase C signalling pathways in chinese hamster ovary (CHO) cells (Umland et al., 2001; Cussac et al., 2002a,b). Taken together, these results suggest that SKF38393 would probably exhibit full agonist properties at the level of adenylyl cyclase in our L-cell system. Though this remains to be determined, these observations suggest that, by analogy to other systems (Umland et al., 2001; Cussac et al., 2002a,b), measurement of G_s protein activation by human dopamine D1 receptors in the present cell line is particularly sensitive for differentiation of full from partial agonists.

Besides the control of G_s proteins, indirect approaches suggest that dopamine D_1 receptors can also couple to $G_{\alpha o}$ in, for example, a rat pituitary GH_4C_1 cell line (see Sidhu, 1998 for review). Although potential cross talk between dopamine D1 receptors and endogenously expressed dopamine D2 receptors in the GH_4C_1 cell line may explain these results, a dopamine D1-like receptor has also been implicated in the control of phospholipase C activity in brain via $G_{\alpha q}$ proteins (Undies et al., 1994; Friedman et al., 1997; Jin et al., 2001). In our hands, functional antibody-capture assays performed with selective antibodies against $G_{\alpha o}$ and $G_{\alpha q}$ (Cussac et al., 2002a) showed no stimulation of these G proteins by human dopamine D1 receptors in L-cells (data not shown). These results suggest, together with other studies, that the coupling of "classical" dopamine D1 receptors is mainly restricted to the G_s protein family in recombinant cell systems (Dearry et al., 1990; Zhou et al., 1990 and present results) as well as in intact tissue (Zhuang et al., 2000; Corvol et al., 2001).

The prototypical neuroleptic, haloperidol, which behaves as a low potency antagonist at dopamine D1 receptors as compared to its potent blockade of dopamine D2 receptors (Brunello et al., 1995), abolished activation of G_s by dopamine under the present conditions. Clozapine possesses, in contrast to haloperidol, an equilibrated profile of binding at dopamine D2 and dopamine D1 receptors, and is considered as the prototypical "atypical" antipsychotic, in exhibiting good activity against positive and deficit symptoms at doses which do not provoke a marked extrapyramidal syndrome (Brunello et al., 1995; Millan, 2000; Strange, 2001). Nevertheless, the efficacy of clozapine at dopamine D1 receptors is still the subject of debate (see Ahlenius, 1999 for review). Agonist properties of clozapine at dopamine D1 receptors in vivo have been indicated by its induction of hypothermia and locomotor activation in dopamine-depleted rats, effects blocked by the dopamine D1 receptor antagonist, SCH23390 (Oerther and Ahlenius, 2000; Jackson et al., 1995). However, this interpretation has been challenged inasmuch as other studies suggest that the engagement by clozapine of postsynaptic dopamine D1 receptors located in the prefrontal cortex is indirectly mediated by an increase in dopamine release, reflecting its interaction with serotonergic receptors (Ichikawa and Meltzer, 1999; Millan, 2000; Chen and Yang, 2002). Moreover, in corroboration of in vitro studies showing that clozapine and other antipsychotics exhibit antagonist or inverse agonist properties at the level of adenylyl cyclase in various cell lines transfected with dopamine D1 receptors (Andersen and Braestrup, 1986; Kozell and Neve, 1997; Cai et al., 1999; Martin et al., 2001), the present results of the direct coupling of dopamine D1 receptors to G_s proteins similarly suggest that clozapine behaves as an antagonist (Fig. 3).

In the present experimental system, no inverse agonist properties of clozapine (or haloperidol) were detected even upon lowering the concentrations of NaCl and guanosine diphosphate in the [^{35}S]GTPγS binding assay (not shown), a manipulation which has been demonstrated to increase the efficacies of inverse agonists at 5-HT_{1A} and CC chemokine 3 receptors (Newman-Tancredi et al., 2002; Wan et al., 2002; see Kenakin, 2001). These results may reflect an absence of pre-coupling of human dopamine D1 receptors to G_s and/or the nature of the adenylyl cyclase expressed in L-cells. Indeed, in PC12 cells, inverse agonist properties of clozapine and haloperidol measured at the adenylyl cyclase level were correlated with a strong decrease of pre-existing dopamine D1 receptor-$G_{\alpha s}$ coupling (Cai et al., 1999). Furthermore, potential constitutive activity at dopamine D1 receptors has not, as yet been, fully evaluated in cerebral tissue and additional experiments are needed to clarify the actions of antipsychotic drugs at dopamine D1 receptors in brain tissue.

In this light, it should be noted that the antibody used in this study recognized $G_{\alpha olf}$ as well as $G_{\alpha s}$ subunits, these G proteins being predominantly expressed in striatum and cortex, respectively (Zhuang et al., 2000; Hervé et al., 2001). Thus, employing the present, novel approach, it would be interesting to assess the impact of clozapine and other antipsychotics at dopamine D1 receptors coupled to $G_{\alpha olf}$ and $G_{\alpha s}$ in different brain structures.

Taken together with previous work, although weak, direct agonist actions at dopamine D1 receptors of clozapine may be revealed under particular physiological conditions such as in dopamine-depleted rats (Jackson et al., 1995), the present results suggest that clozapine behaves predominantly as an antagonist at dopamine D1 receptors As such, clozapine resembles haloperidol. However, in contrast to the comparatively high potency of haloperidol for dopamine D2 versus dopamine D1 receptors, clozapine shows similar affinity for these sites. Moreover, clozapine interacts with a broad spectrum of receptors (and, in particular, shows pronounced affinity for 5-HT_{1A} and 5-HT_{2C} receptors), actions participating in the elevation of dopamine release in the frontal cortex and, by consequence its indirect

positive actions at dopamine D1 receptors (see Millan, 2000). Correspondingly, the precise significance of antagonist, inverse agonist or partial agonist properties of antipsychotics at dopamine D1 receptors to their therapeutic efficacy in schizophrenia remains to be further elucidated.

To summarize, the present study demonstrates that human dopamine D1 receptors couple to G_s in L-cells and that this innovative, antibody capture assay should permit efficient screening of ligand efficacy and potency directly at the G_s protein level. Furthermore, we have recently found that the technology employed herein can easily be adapted to the study of native populations of dopamine D1 receptors in cerebral tissue (Cussac D. et al., unpublished observation). Thus, this functional approach of G_s protein activation should prove of use for evaluation of drug actions at discrete cerebral populations of dopamine D1 and other G protein coupled receptors implicated in, for example, schizophrenia and Parkinson's disease.

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