ELSEVIER

European Journal of Pharmacology 382 (1999) 119–127

ejp

www.elsevier.nl/locate/ejphar

Enhanced striatal dopamine D_2 receptor-induced [^{35}S]GTPγS binding after haloperidol treatment

Muriel Geurts, Emmanuel Hermans *, Jean-Marie Maloteaux

Laboratoire de pharmacologie, U.C.L. 54.10, Université catholique de Louvain, Avenue Hippocrate 54, B-1200 Brussels, Belgium

Received 17 May 1999; received in revised form 28 July 1999; accepted 3 August 1999

Abstract

Dopamine receptor-G protein coupling and dopamine D_2 receptor density were assessed in rats treated for 3 weeks with either haloperidol (2 mg/kg; i.p.) or vehicle. After 3 days of withdrawal, agonist-induced guanosine 5′-*O*-(γ-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) and [^{3}H]spiperone binding were determined in striatal homogenates. Maximal [^{3}H]spiperone binding was increased (24.8%, $P < 0.01$) following haloperidol treatment. The efficacy of dopamine and the dopamine D_2 receptor agonist *R*(−)-10,11-dihydroxy-*N*-*n*-propylnorapomorphine (NPA) to induce [^{35}S]GTPγS binding were found to be increased by 24.1% ($P < 0.01$) and 44.6% ($P < 0.001$), respectively. When measured in the presence of a saturating concentration of a dopamine D_2 receptor antagonist, the response to dopamine was not significantly affected by haloperidol treatment. In addition, the measurement of haloperidol-induced catalepsy confirmed that the efficient dopamine receptor blockade was followed by a progressive development of dopaminergic supersensitivity. Taken together, these results indicate that a functional pool of dopamine D_2 receptors is increased after prolonged haloperidol administration.

Keywords: Haloperidol; [^{35}S]GTPγS, agonist-induced; Dopamine D_2 receptor density; G-protein coupling; Catalepsy

1. Introduction

Neuronal plasticity represents an important part of the compensatory processes by which the central nervous system adapts to pathological insult, long-term exposure to drugs or neuronal loss (Pedigo, 1994). Plasticity is a remarkable feature of dopamine receptors and can be categorised according to three main aspects of receptor function: receptor density, sensitivity to receptor agonists and interaction between receptor subtypes. Changes in dopamine receptor function or density can be elicited either by selective destruction of dopamine-containing neurons or by chronic administration of pharmacological agents that alter interaction of dopamine with its receptors (LaHoste and Marshall, 1993).

Neuroleptics, such as haloperidol, are used for the treatment of psychosis but their administration often results in the development of severe extrapyramidal side effects (Tarsy, 1989). Among these, tardive dyskinesia were thought to be the consequence of dopaminergic supersensitivity (Klawans, 1973). Supports for this hypothesis came first from behavioural experiments using rodents treated with neuroleptics for a few days to several weeks (Sayers et al., 1975). More recent reports have shown that chronic treatment with typical neuroleptics resulted in behavioural (Marin and Chase, 1993; Schröder et al., 1994), neurochemical (Cubeddu et al., 1983) and electrophysiological (Vogelsang and Piercey, 1985) supersensitivity to dopamine receptor agonists.

The hypothesis that increased striatal dopamine D_2 receptor density was the cause of the supersensitivity to dopamine receptor agonists had received wide acceptance, probably in part because it was the most studied molecular target in this field (review by Sibley and Neve, 1997). However, several lines of evidence have suggested that behavioural supersensitivity to dopamine receptor agonists and dopamine D_2 receptor up-regulation could be dissociated (Dewey and Fibiger, 1983; LaHoste and Marshall, 1993; Marin and Chase, 1993). A discrepancy between the density of dopamine D_2 receptors and dopamine D_2 receptor-mediated behaviours has also been demonstrated by the use of antisense oligodeoxynucleotides (Qin et al., 1995). This observation could be explained by the existence of a

* Corresponding author. Tel.: +32-2-764-5317; fax: +32-2-764-5460; e-mail: emmanuel.hermans@farl.ucl.ac.be

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00569-5

high proportion of non-functional or 'spare' dopamine receptors. Therefore, the increase in receptor density is not sufficient to explain supersensitivity to dopamine receptor agonists. It has even been suggested that the change in dopamine receptor expression was only a consequence to more profound modulations concerning, for example, G proteins (Costain et al., 1997). Indeed, coupling of receptors with G proteins constitutes the very first biochemical event in the signalling pathway following binding of the agonist to the receptor and this should be a crucial step for regulation processes.

Useful methods have been introduced which allow the study of the functional coupling between receptors and G proteins, such as determination of agonist-induced guanosine 5′-*O*-(γ-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding (Lazareno, 1997) or GTPase activity (Odagaki and Fuxe, 1995). A pharmacological characterization of dopamine receptor agonist-induced [^{35}S]GTPγS binding on rat striatal membranes was previously established (Rinken et al., 1999; Geurts et al., 1999). In the present work, the effect of prolonged haloperidol administration on both dopamine D_2 receptor density ([^{3}H]spiperone binding) and coupling to G proteins (agonist-induced [^{35}S]GTPγS binding) was evaluated. In addition, haloperidol-induced catalepsy was measured, as a behavioural marker of neuroleptic treatment. This phenomenon, defined as the long-term maintenance of an animal in an abnormal posture, is attributed to the blockade of dopamine receptors within the striatum and is closely linked to the induction of extrapyramidal side effects in humans (Hacksell et al., 1995).

2. Materials and methods

2.1. Chemicals

[^{35}S]GTPγS (spec. act. 1000 Ci mmol^{-1}) and [^{3}H]spiperone (spec. act. 105 Ci mmol^{-1}) were purchased from Amersham Belgium (Gent, Belgium). Atropine sulfate, carbamylcholine chloride (carbachol), dopamine hydrochloride, GDP, 5′-guanylylimidodiphosphate (Gpp(NH)p), and haloperidol were purchased from Sigma (St. Louis, MO, USA). Domperidone, ketanserin tartrate, *R*(−)-10,11-dihydroxy-*N*-*n*-propylnorapomorphine HCl (NPA), and L(+)-tartaric acid were obtained from Research Biochemicals (Natick, MA, USA).

2.2. Animals and treatment

Male Wistar rats, weighing 250–300 g, were kept on a 12 h on/off lighting schedule. Food and water were accessible ad libitum. The animals were injected once daily with either vehicle (0.25 % (w/v) L(+)-tartaric acid) or haloperidol (2 mg/kg; i.p.) for 21 days. Striatal dopamine D_2 receptor density and functional coupling with G proteins were evaluated in striatal homogenates, after a 3-day washout period. Experimental protocols were approved by the local ethical committee and meet the guidelines of the responsible governmental agency (Administration de la Santé Animale et de la Qualité des Produits Animaux, Services Vétérinaires du Ministère, Brussels).

2.3. Preparation of striatal membranes

The day of the binding assay, animals were killed by decapitation, the whole brain removed from the skull. The striata were dissected out on ice and immediately homogenized, by the use of a Teflon/glass homogenizer, in 50 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 600 g for 10 min, and the supernatant obtained was centrifuged for 10 min at 49,000 $\times g$. The membranes were washed, resuspended in 100 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) and centrifuged for 10 min at 49,000 $\times g$ twice. The membranes were resuspended in 20 volumes of ice-cold buffer containing 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, and 150 mM NaCl. Protein concentration was determined using Coomassie dye reagent (Bradford, 1976).

2.4. [^{35}S]GTPγS binding assay

This binding experiment was performed at 30°C in plastic tubes containing 20 μg protein resuspended in a final volume of 1 ml. Binding buffer contained 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 150 mM NaCl, 10 μM GDP, 1 mM dithiothreitol and 0.1% sodium metabisulfite. The binding was initiated by the addition of 0.1 nM [^{35}S]GTPγS. Non specific binding was measured in the presence of 0.1 mM Gpp(NH)p. Incubation was performed for 60 min (dopamine; dopamine + domperidone; carbachol) or 15 min (NPA) and was terminated by addition of 3 ml ice-cold washing buffer (50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 150 mM NaCl). The suspension was immediately filtered through GF/B glass fibre filters (Whatman, England) and washed twice with washing buffer. Filters were immersed in 5 ml Aqualuma (Lumac, Groningen, Netherlands) before determination of radioactivity by liquid scintillation counting.

2.5. [^{3}H]spiperone binding assay

Striatal membranes (40 μg protein) were incubated with increasing concentrations of [^{3}H]spiperone in a final volume of 1 ml. Binding buffer contained 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, and 150 mM NaCl. Ketanserin (0.1 μM) was added in order to occlude the binding of the radiolabelled ligand to 5-HT_2 receptors. Non specific binding was determined in the presence of domperidone (1 μM). Incubation was performed for 60 min at 30°C and was terminated as indicated for the [^{35}S]GTPγS assay.

2.6. Catalepsy testing

Haloperidol-induced catalepsy was measured using the horizontal bar test (Neal-Beliveau et al., 1993). Thus, 30 min after the injection of haloperidol or vehicle, rats were placed with their forepaws over a horizontal bar (1.1 cm diameter, 8 cm above the bench surface). The time until the rat brought both forepaws down to the table (descent latency) was recorded, with a maximum cut-off time of 300 s. A cataleptic animal maintains this position for a period of time depending on the degree of catalepsy. To avoid 'false–negative' results, the animals were given five trials on the horizontal bar and only the greater duration of immobility was considered. In the single testing procedure, rats were tested only on the 21st day of the treatment. In the repeated testing schedule, rats were tested on days 1, 5, 10, 15 and 20 of the treatment (30 min after injection) and on the day of the binding assay (following the 3-day washout period).

2.7. Data analyses

Data from the [^{35}S]GTPγS and [^{3}H]spiperone binding experiments were analysed by non-linear regression using the curve fitting program GraphPad Prism (GraphPad Software, San Diego, CA, USA). Values from vehicle- and haloperidol-treated rats were compared using either unpaired Student's *t*-test (B_{max}, pK_d, pEC$_{50}$ and E_{max}) or two-way analysis of variance ANOVA (saturation and concentration–response curves).

3. Results

3.1. [^{3}H]spiperone binding

The binding of increasing concentrations of [^{3}H]spiperone was measured on striatal membranes from haloperidol- and vehicle-treated rats (Fig. 1). Saturation curves corresponding to the haloperidol-treated group were found significantly different from controls ($P < 0.001$, two-way ANOVA). Whereas the dissociation constant (K_d) of [^{3}H]spiperone was not significantly affected by the haloperidol treatment, the maximal density of binding sites (B_{max}) from haloperidol-treated rats was increased by 24.8% ($P < 0.01$) as compared to the control value (Table 1).

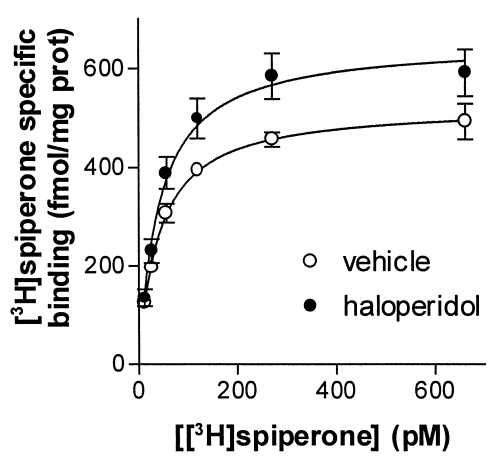

Fig. 1. Effect of 21 days of haloperidol administration on dopamine D_2 receptor density. Results are expressed as the specific binding of [^{3}H]spiperone (fmol/mg protein) and are mean ± S.E.M. of five independent experiments, performed in triplicate. Saturation curves corresponding to the haloperidol-treated group were found significantly different from controls ($P < 0.001$, two-way ANOVA).

Table 1
Pharmacological parameters from [^{3}H]spiperone and [^{35}S]GTPγS binding assays

B_{max} and K_d values were determined in [^{3}H]spiperone binding experiments (Fig. 1). E_{max} and pEC$_{50}$ values were derived from concentration–effect curves of dopamine- or NPA-induced [^{35}S]GTPγS binding (Fig. 2). B_{max} (expressed as fmol of [^{3}H]spiperone specific binding/μg protein), K_d (expressed as pM of free [^{3}H]spiperone), E_{max} (expressed as percentage of stimulation above basal binding) and pEC$_{50}$ values are mean ± S.E.M. of n independent experiments. Parameters measured on striatal membranes from haloperidol- and vehicle-treated rats were compared using unpaired Student's *t*-test. The null hypothesis was rejected at the 0.05 level.

	B_{max} (fmol/μg protein) or E_{max} (% above basal)	K_d (pM) or pEC$_{50}$
[^{3}H]spiperone binding		
Vehicle ($n = 5$)	0.525 ± 0.018	40.99 ± 5.16
Haloperidol ($n = 5$)	0.655 ± 0.033[a]	42.00 ± 8.01
Dopamine-induced [^{35}S]GTPγS binding		
Vehicle ($n = 9$)	57.20 ± 2.14	5.363 ± 0.092
Haloperidol ($n = 9$)	70.98 ± 3.56[a]	5.429 ± 0.133
NPA-induced [^{35}S]GTPγS binding		
Vehicle ($n = 6$)	29.09 ± 1.61	8.638 ± 0.112
Haloperidol ($n = 6$)	42.07 ± 2.15[b]	8.794 ± 0.106

[a] $P < 0.01$.
[b] $P < 0.001$.

3.2. [^{35}S]GTPγS binding

The functional coupling of dopamine receptors to G proteins was assessed by the determination of agonist-induced [^{35}S]GTPγS specific binding. Basal [^{35}S]GTPγS specific binding was not significantly affected by haloperidol treatment. Thus, mean ± S.E.M. (dpm), at the two different incubation times, were 10,340 ± 936, $n = 9$ (60 min) and 3781 ± 511, $n = 6$ (15 min) for the vehicle-treated rats and 9884 ± 970, $n = 9$ (60 min) and 3407 ± 396, $n = 6$ (15 min) for the haloperidol group. The effect of increasing concentrations of dopamine and NPA on the [^{35}S]GTPγS specific binding was determined on striatal membranes from vehicle- and haloperidol-treated rats (Fig. 2). The pharmacological parameters deduced from these curves are indicated in Table 1. It must be indicated that the terms 'potency' and 'efficacy' in the present report are referring to the EC$_{50}$ and E_{max} values, respectively. Efficacies of the agonists, but not potencies were significantly increased after chronic haloperidol administration, by

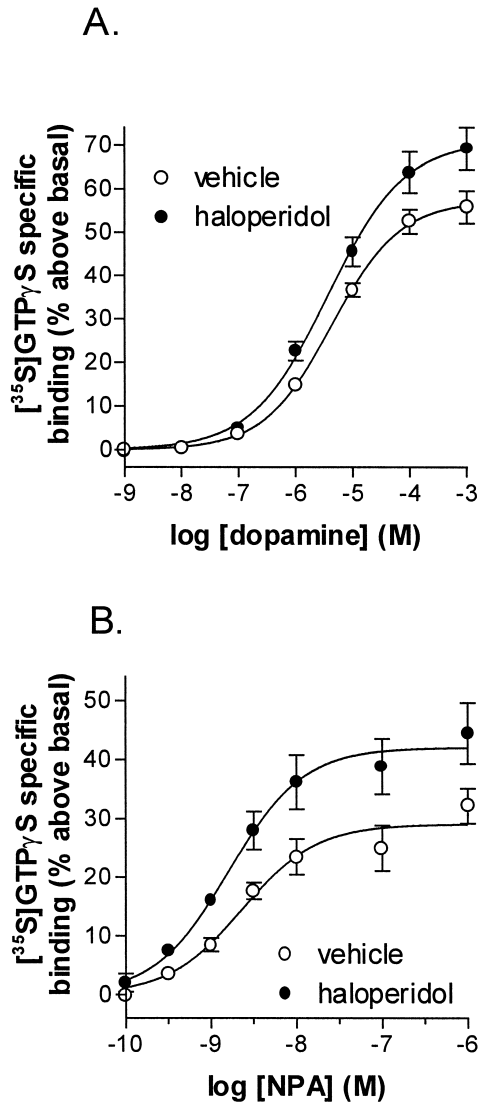

Fig. 2. Effect of 21 days-haloperidol treatment on dopamine- and dopamine D_2 receptor agonist-induced [^{35}S]GTPγS binding. Increasing concentrations of dopamine (A) or NPA (B) were used to stimulate the binding of [^{35}S]GTPγS on striatal membranes from haloperidol- or vehicle-treated rats. The data are expressed as percentage of stimulation above basal binding and are mean ± S.E.M. of six (NPA) or nine (dopamine) independent experiments, performed in triplicate. Concentration–response curves for both dopamine and NPA measured on striatal membranes from haloperidol-treated rats were found significantly different from controls ($P < 0.001$, two-way ANOVA).

24.1% for dopamine ($P < 0.01$) and 44.6% for NPA ($P < 0.001$). When the responses to these agonists were expressed as absolute values, such as dpm or fmol/mg protein of agonist-induced [^{35}S]GTPγS binding, statistical analysis also revealed a significant increase in the maximal response after haloperidol treatment. However, the amplitude of the increase was lower when the data were expressed as absolute values, than as percentages of basal binding. Thus, the maximal effect of NPA (above basal [^{35}S]GTPγS binding) was 1036 ± 58 dpm/20 μg protein (23 fmol/mg protein) and 1336 ± 47 dpm/20 μg protein (30 fmol/mg protein), in vehicle- and haloperidol-groups, respectively. This corresponded to a 28.9% ($P < 0.01$) increase in haloperidol-treated rats compared with controls.

In order to assess the involvement of dopamine D_1 receptors, we measured the response to dopamine in the presence of the potent dopamine D_2 receptor antagonist, domperidone. As shown in Fig. 3A, domperidone was found to competitively inhibit the dopamine (100 μM)-induced [^{35}S]GTPγS specific binding. Maximal inhibition

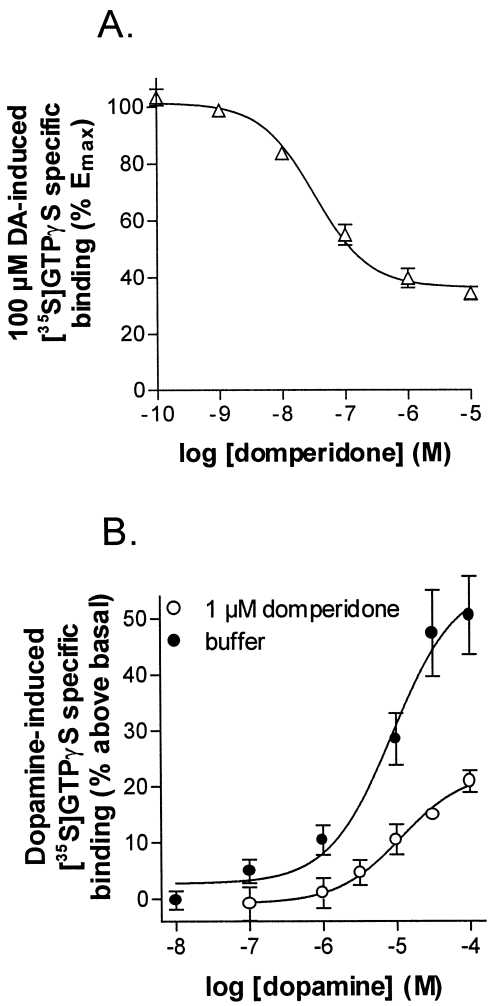

Fig. 3. Dopamine-stimulated [^{35}S]GTPγS binding to striatal membranes, in the presence of a potent and selective dopamine D_2 receptor antagonist. (A) Effect of increasing concentrations of domperidone on the stimulation by 100 μM dopamine (DA) of basal [^{35}S]GTPγS specific binding. The data are expressed as percentage of the response obtained with dopamine alone and are mean ± S.E.M. of three independent experiments, performed in triplicate. Maximal inhibition is achieved at 1–10 μM domperidone and the remaining stimulation is 36.45 ± 2.04% of the maximal response to dopamine. (B) Effect of increasing concentrations of dopamine on [^{35}S]GTPγS specific binding, measured in the absence or in the presence of 1 μM domperidone. The data are expressed as percentage of stimulation above basal binding and are mean ± S.E.M. of three independent experiments, performed in triplicate. The pEC_{50} (EC_{50}) values for dopamine-induced [^{35}S]GTPγS binding are 5.048 ± 0.212 (8.95 μM) and 4.940 ± 0.220 (11.5 μM), in the absence and in the presence of the dopamine D_2 receptor antagonist, respectively.

(~60 %) was achieved with 1 μM domperidone. The pIC_{50} value for domperidone as determined in these conditions was 7.48 ± 0.10 (IC_{50}, 33 nM). A concentration–response curve for dopamine was measured in the presence of 1 μM domperidone (Fig. 3B). In these conditions, the potency of dopamine was in the range of 10 μM. At each of the three concentrations of dopamine tested (10, 50 and 100 μM), the 'dopamine + domperidone'-induced [^{35}S]GTPγS binding was not found to be significantly affected by haloperidol treatment (Table 2).

As a control, the stimulation of [^{35}S]GTPγS binding via another neurotransmitter receptor system was also measured. In the same experimental conditions, the muscarinic cholinergic receptor agonist carbachol dose-dependently stimulated [^{35}S]GTPγS binding to striatal membranes (not shown). The efficacy of this agonist (106.3 ± 3.3%) was much higher than the maximal response obtained with dopamine and the pEC_{50} value was 5.02 ± 0.07 (mean ± S.E.M., $n = 3$). The response to carbachol (up to 1 mM) was completely inhibited by 1 μM atropine. The carbachol (10 μM)-induced [^{35}S]GTPγS specific binding was not significantly changed after chronic haloperidol treatment. Thus, the mean values ± S.E.M. ($n = 7$) for vehicle- and haloperidol-treated rats were 53.3 ± 5.0 and 50.6 ± 4.3% above basal [^{35}S]GTPγS binding, respectively.

3.3. Catalepsy

Haloperidol-induced catalepsy or cataleptic-like behaviour in control rats was assessed using the horizontal bar method (Fig. 4). Using the repeated testing protocol, the descent latencies of haloperidol-treated rats were significantly higher than control values during the entire time course of the treatment. These values were stable up to the last days of injection for haloperidol-treated rats, whereas vehicle-treated rats tended to stay on the horizontal bar only at the end of the treatment. After the 3-day washout period, the catalepsy of haloperidol-treated rats, measured by repeated testing, had disappeared. Moreover, descent latencies for these rats tended to be lower than control values and haloperidol-treated rats were found hyperreactive as compared to the control group, at this stage of the experiment. When the rats were tested for catalepsy only on the 21st day of the treatment, descent latencies were lower than in the repeated testing protocol for both vehicle- and haloperidol-treated rats. In addition, descent latencies of haloperidol-treated rats were lower than on the first day of injection, when rats were tested using this single assay schedule. This decrease was, however, not significant, probably due to the sample size. Using the single testing procedure, values for vehicle-treated rats were not changed between the 1st and the 21st day.

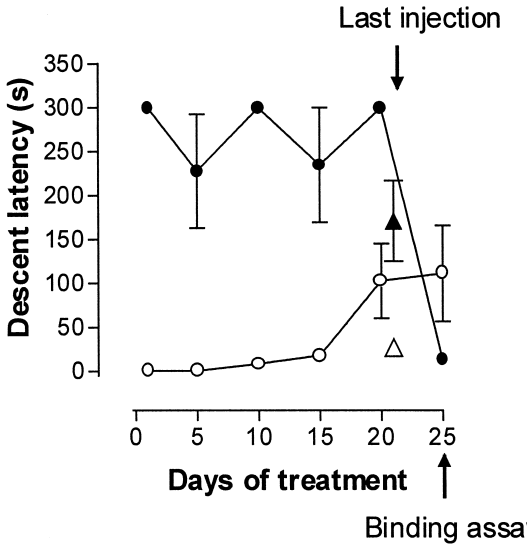

Fig. 4. Effect of haloperidol administration on rat behavior. Catalepsy was assessed using the horizontal bar test, as described in Materials and methods. In the repeated testing procedure (circles), rats were assessed for catalepsy several times during the treatment (30 min after injection on days 1, 5, 10, 15, 20) and on the day of the binding assay (after the 3-day washout period). In the single testing assay (triangles), rats were tested for catalepsy only on the 21st day of the treatment, 30 min after injection. Closed symbols correspond to haloperidol-treated rats and open symbols to vehicle-treated rats. Descent latency was defined as the longest time among five trials until the rat brought both forepaws down to the table, with a maximum of 300 s. The data are mean ± S.E.M. of four (repeated testing) or seven (single testing) independent experiments.

Table 2
Lack of effect of haloperidol administration on 'dopamine + domperidone'-induced [^{35}S]GTPγS binding
The stimulation of [^{35}S]GTPγS specific binding by three different concentrations of dopamine was measured in the presence of a fixed concentration of domperidone (1 μM), on striatal membranes from vehicle- and haloperidol-treated rats. The data are expressed as percentage of stimulation above basal binding and are mean ± S.E.M. of *n* independent experiments, performed in triplicate. Data from vehicle- and haloperidol-treated animals were compared using a Student's *t*-test for unpaired samples. The null hypothesis was rejected at the 0.05 level. No significant difference was found between the two groups.

Dopamine concentration (μM)	Vehicle	Haloperidol	*n*
10	6.64 ± 1.20	9.30 ± 1.10	7
50	15.55 ± 1.31	17.05 ± 2.28	9
100	23.29 ± 1.77	24.29 ± 0.92	7

4. Discussion

Dopamine D_2 receptor density and dopamine receptor-G protein coupling were assessed in striatal homogenates of rats chronically treated with either vehicle or haloperidol. In the same model, haloperidol-induced catalepsy was evaluated in comparison with the behaviour of vehicle-treated rats and the effect of repeated testing on this behaviour was assessed.

An increase in the specific binding of [^{3}H]spiperone to striatal membranes of rats chronically treated with haloperidol was observed, as compared to controls. This

change in B_{max} — but not in K_d — is consistent with previous reports showing a similar increase in dopamine D_2 receptor density after chronic haloperidol administration (Schettini et al., 1992; Schröder et al., 1994). The fact that the apparent affinity state of dopamine D_2 receptors (K_d) was not affected by haloperidol treatment suggests that the washout period and the washing procedure were sufficient to allow the accurate determination of [^{3}H]spiperone binding without a possible contamination by remaining haloperidol.

Consistently with this fact, haloperidol-induced catalepsy returned to control levels, after the 3-day washout period, showing the absence of dopamine D_2 receptor blockade. At this time point, descent latencies were even found lower than control values. Similar observations were reported in a previous study (Karolewicz et al., 1996), where catalepsy was found to disappear within 24 h of withdrawal and rats found hyperresponsive to touch. Using the repeated testing procedure, a high degree of catalepsy was measured during the entire time course of the haloperidol treatment and a sensitization was observed for control rats, as they displayed a cataleptic-like behaviour at the end of the treatment. Therefore, when using a repeated testing procedure, equilibrium between factors inducing sensitization and factors producing tolerance must occur in the case of haloperidol-treated rats (Hillegaart et al., 1987). When the single testing procedure was used, both the haloperidol- and vehicle-treated rats were less inclined to immobility than when measured with the repeated testing procedure. In these conditions, haloperidol-induced catalepsy was found lower on the 21st day than on the 1st day of treatment, showing that tolerance was occurring. It has been proposed that the behavioral tolerance to haloperidol (occuring during the treatment) as well as the behavioral hyperreactivity of rats (occurring after withdrawal) were the consequence of dopaminergic supersensitivity (Bernardi et al., 1981), while sensitization was related to experience and training (Iwata et al., 1989).

Dopamine receptor agonist-induced [^{35}S]GTPγS binding allowed the evaluation of the functional state of upregulated dopamine D_2 receptors. Basal [^{35}S]GTPγS binding was not significantly changed after haloperidol treatment. Although review of the recent literature revealed some conflicting results, most data show a lack of modulation of G protein expression after chronic neuroleptic treatment (Schettini et al., 1992; See et al., 1993; Meller and Bohmaker, 1996). The dopamine-induced [^{35}S]GTPγS binding was found to be significantly increased after repeated haloperidol administration. Such enhanced response in haloperidol-treated rats was more obvious when the dopamine D_2 receptor agonist NPA was used. Furthermore, the absolute increases in the responses to dopamine and NPA following haloperidol treatment were very similar (13.78 and 12.98%, respectively). This is consistent with the fact that the entire increase in the maximal stimulation of [^{35}S]GTPγS binding by dopamine can be related to dopamine D_{2-} rather than D_1 receptors, as suggested by the lack of effect on the response to dopamine measured in the presence of the dopamine D_2 receptor antagonist, domperidone. This also assumes that NPA is a full, specific dopamine D_2 receptor agonist, at least for the response that was measured in the present study. In cultured cells expressing dopamine D_2 receptors, NPA has been reported to be a full agonist for the stimulation of [^{35}S]GTPγS binding (Gardner and Strange, 1998). In striatal membranes, where other receptors are expressed, it is less easy to differentiate between selective and partial agonism, as compared to the endogenous neurotransmitter. When comparing competition for [^{3}H]spiperone and [^{3}H]SCH 23390 binding in vitro, NPA was found to be more than 100-fold selective for dopamine D_2 versus D_1 binding sites (Andersen and Jansen, 1990). However, in the same study, high concentrations of NPA were found to stimulate adenylyl cyclase activity in rat striatal homogenates (EC_{50}, 2400 nM; efficacy, 66% of the dopamine response), raising the question of its selectivity for dopamine D_{2-} versus D_1 receptors, at least at high concentrations. Very similar results were obtained in a previous report (Arnt et al., 1988). In our hand, the dopamine D_1 receptor antagonist SCH 23390 (20 nM) did not inhibit the stimulation of [^{35}S]GTPγS binding induced by 10^{-10} to 10^{-5} M NPA, whereas this concentration of SCH 23390 was sufficient to partially antagonise the dopamine (100 μM)-induced [^{35}S]GTPγS binding (Geurts et al., 1999). Moreover, the stimulation of [^{35}S]GTPγS binding by a nearly maximal concentration of NPA (0.1 μM) was completely inhibited by 1 μM of the specific dopamine D_2 receptor antagonist, domperidone. This inhibition was likely to be mediated by D_2 dopamine receptors as the pK_i value derived from the entire inhibition curve was consistent with the reported affinity of domperidone for these receptors (Geurts et al., 1999). Concerning the intrinsic activity of NPA revealed in the present study, the maximal effect obtained with this agonist represented 58% of the maximal response to dopamine. The maximal response to other dopamine D_2 receptor agonists was obtained with pergolide (63% of dopamine response) and this was consistent with the magnitude of the domperidone-mediated inhibition of the maximal response to dopamine (Geurts et al., 1999). Interestingly, very similar results were obtained by Odagaki and Fuxe (1995), who measured agonist-induced activation of high-affinity GTPase activity in rat striatal membranes. In their study, the maximal responses to NPA and pergolide were 55 and 60% (of dopamine response), respectively.

Altogether these results suggest that NPA behaves as a full, specific dopamine D_2 receptor agonist in the experimental conditions used in the present work and that the dopamine D_2 receptor-mediated activation of G proteins became supersensitive following prolonged haloperidol administration. These findings are consistent with a previous study showing an increase in the activity of the dopamine

D_2 receptor–G_i protein system after prolonged haloperidol administration. It was reported that haloperidol treatment (1 mg/kg/day for 21 days with a 3-day washout period) caused a 15% reduction in the GTP-induced adenylyl cyclase activity, without any change in the basal activity of this enzyme (Schettini et al., 1992). In the same study, the stimulatory effect of 10 μM dopamine (in the presence of 1–10 μM GTP) was shifted to an inhibitory one, also revealing an increased activation of G_i. Other groups reported evidence for supersensitive biochemical events mediated by striatal dopamine D_2 receptors following chronic administration of neuroleptics, such as inhibition of dopamine or acetylcholine release (Cubeddu et al., 1983). Several studies using dopamine to modulate adenylyl cyclase activity after prolonged neuroleptic treatment yielded conflicting results (reviewed by Baker and Greenshaw, 1989), showing either a decrease, an increase or no change in cAMP production. These apparent discrepancies can be explained by the fact that dopamine acts via dopamine D_1 and D_2 receptors to regulate in an opposite fashion the production of cAMP. Using specific dopamine D_1 and D_2 receptor agonists to stimulate or inhibit adenylyl cyclase activity, respectively, Memo et al. (1987) clearly demonstrated that both dopamine D_1 and D_2 receptors-mediated modulation of cAMP production became supersensitive after prolonged administration of haloperidol, without any change in basal adenylyl cyclase activity. As indicated above, no significant change in dopamine-induced [^{35}S]GTPγS binding was detected in the present study, when measured in the presence of the potent dopamine D_2 receptor antagonist, domperidone. This 'dopamine + domperidone'-induced [^{35}S]GTPγS binding was thought to be mainly due to the stimulation of dopamine D_1 receptors (Geurts et al., 1999). At the dosage used in the present study, haloperidol was not thought to occupy a high proportion of striatal dopamine D_1 receptors. Indeed, using an elegant method of quantitative autoradiography, Schotte et al. (1996) measured ex vivo the dopamine receptor occupancy of haloperidol, 2 h after its s.c. administration to rats. The reported ED_{50} values for dopamine D_1 and D_2 receptors in caudate-putamen were >10 mg/kg and 0.14 mg/kg, respectively. The occupancy of dopamine D_1 receptors at this high dosage (10 mg/kg) was only 25–49% of the total binding. Therefore, it is likely that haloperidol treatment in the present study did not significantly change functional parameters related to dopamine D_1 receptors. Consistently, several reports showed increases in dopamine D_2 receptor expression without any significant change in dopamine D_1 receptor density after chronic haloperidol administration (Schettini et al., 1992; LaHoste and Marshall, 1993; Marin and Chase, 1993).

There is considerable biochemical, behavioural and clinical evidence showing a cholinergic–dopaminergic interaction in the striatum (Di Chiara et al., 1994). In addition to an increase in the density of striatal dopamine D_2 receptors, it has been postulated that tardive dyskinesia may be related to subsensitivity of muscarinic cholinergic receptors (Klawans, 1973). In the present study, no significant change was found in the carbachol-induced activation of G proteins. In a previous report, the repeated administration (14 days) of the 'typical' neuroleptic fluphenazine resulted in a non-significant, 5% decrease in the density of muscarinic receptors in the striatum, whereas a 30% up-regulation of dopamine D_2 receptors was measured (Boyson et al., 1988). Other groups reported either no change or a decrease in muscarinic receptor density or function (See et al., 1990; Li et al., 1992).

An interesting finding of the present study is the observation that chronic haloperidol treatment resulted in an increased efficacy of full agonists, without alteration in their potency. The question of what are the effects of varying G protein and receptor expression levels on these pharmacological parameters is fundamental and has not yet been clearly answered (Keen, 1991). Following the assumptions made to test models of receptor-mediated activation of G proteins, changing the receptor:G protein stoichiometry could have different consequences on the shape of the concentration–response curves of agonists. Thus, in the case of a higher level of receptors relative to G proteins, a further increase in receptor reserve is likely to result in an increased potency of full agonists, without any change of their efficacy. To enhance the maximal response of full agonists, an increase in G protein levels is therefore required. Such observations have been reported with different G protein-coupled receptors expressed at high levels in cultured cells (Watts et al., 1995; Newman-Tancredi et al., 1997; Hermans et al., 1999). Furthermore, the number of G proteins has been reported to exceed the expression of receptors in tissues (Van Vliet et al., 1993). In particular, whereas the levels of striatal dopamine D_2 receptors were $\sim$0.2–0.5 pmol/mg protein (Schettini et al., 1992; Schröder et al., 1994), as reported in the present study, the levels of striatal G_{i1}, G_{i2} and G_o proteins were found to be $\sim$12- to 450-fold higher (Asano et al., 1990). Based on this biochemical finding, it can be postulated that an increase in receptor density would lead to an increased maximal activation of G proteins in striatal membranes. However, the evaluation of different classical and newer models of agonism for G protein-coupled receptors led to the conclusion that the theoretical assumption of a higher level of receptors relative to G proteins was correct (Keen, 1991). This apparent discrepancy could be explained by the fact that the expression levels of receptors and G proteins did not reflect their true availability for each other (Van Vliet et al., 1993 and references herein). These molecules could lack a free access to each other and exist in different compartments, such as the plasmalemmal caveolae (Shaul and Anderson, 1998). An overall increase of the amount of G proteins in the neuronal membrane may represent no change in certain compartments but a dramatic increase in others or may represent an increase in

non-functional molecules. Recently, several mechanisms have been proposed to modulate the activity of G proteins, such as covalent modifications or interaction with regulating proteins (Yamane and Fung, 1993; Dohlman and Thorner, 1997). Consistently, it has been reported that small changes in levels of G proteins could lead to profound functional consequences (Nestler et al., 1990).

In the present report, it was shown that the efficacy of NPA to stimulate [^{35}S]GTPγS binding, but not its potency was increased following haloperidol treatment. If the assumption that receptors are in relative excess to G proteins is correct, at least at the functional level, this suggest that the population of functionally coupled rather than spare dopamine D_2 receptors was increased following haloperidol administration. Such an increase in the pool of functional dopamine D_2 receptors may explain the discrepancies between the small changes in receptor levels and the robust enhancement of biochemical, electrophysiological or behavioural responses observed following chronic haloperidol treatment.

It is particularly important to consider the regulation of dopamine receptors at the level of their interaction with G proteins because regional changes in receptor reserve could lead to important clinical applications, such as regional specificity of drugs. It was postulated that changes in receptor reserves could be a general mechanism for the modifications of the responses induced by agonists at regulated receptors (Meller et al., 1987). It could be further hypothesised that regional differences in such regulation could explain regional selectivity of drugs. In particular, antipsychotic drugs have been shown to act as inverse agonists rather than as antagonists, as previously assumed (Strange, 1999). Depending on receptor reserve and intrinsic activity, these molecules could behave either as antagonists or as inverse agonists. Indeed, there is abundant evidence showing that a single ligand could display differences in intrinsic activity depending on the preparation used to study receptor pharmacology (Meller et al., 1987; Kenakin, 1996). In addition, regional differences in receptor reserve were detected among central dopaminergic systems, particularly within the pathways that are implicated in the side or therapeutical effects of neuroleptics, i.e. the nigrostriatal and mesolimbic pathways (Cox and Waszczak, 1990). Moreover, during regulation processes, these receptor reserves are likely to be modified, raising the hypothesis of regional modifications of the pharmacological profile of drugs acting on these pathways during chronic treatment (Enz et al., 1990). The stimulation of [^{35}S]GTPγS binding offers an assay that allows the assessment of the relationship between receptors and G proteins in regulation processes and this could be a fundamental step for future investigations in central dopamine pharmacology. In particular, comparing the effects of antipsychotic drugs using this functional binding assay could give new clues to the mechanisms leading to the atypical comportment of some of these molecules.

Acknowledgements

The authors thank A. Lebbe and H. Lenaert for their excellent technical assistance. This work was supported by the National Fund for Scientific Research (F.N.R.S., Belgium, Convention FRSM 3.453.997F), by a Grant of Ministry of Scientific Policy (Belgium) (ARC 95/00-188), and by the Belgian Queen Elisabeth Medical Foundation. M. Geurts and E. Hermans are Research Assistant and Research Associate of the National Fund for Scientific Research.

References

Andersen, P.H., Jansen, J.A., 1990. Dopamine receptor agonists: selectivity and dopamine D1 receptor efficacy. Eur. J. Pharmacol. Mol. Pharmacol. 188, 335–347.

Arnt, J., Bøgesø, K.P., Hyttel, J., Meier, E., 1988. Relative dopamine D_1 and D_2 receptor affinity and efficacy determine whether dopamine agonists induce hyperactivity or oral stereotypy in rats. Pharmacol. Toxicol. 62, 121–130.

Asano, T., Shinohara, H., Morishita, R., Kato, K., 1990. Immunochemical and immunohistochemical localization of the G protein G_{i1} in rat central nervous tissues. J. Biochem. 108, 988–994.

Baker, G.B., Greenshaw, A.J., 1989. Effects of long-term administration of antidepressants and neuroleptics on receptors in the central nervous system. Cell. Mol. Neurobiol. 9, 1–44.

Bernardi, M.M., De Souza, H., Palermo-Neto, J., 1981. Effects of single and long-term haloperidol administration on open field behavior of rats. Psychopharmacology 73, 171–175.

Boyson, S.J., McGonigle, P., Luthin, G.R., Wolfe, B.B., Molinoff, P.B., 1988. Effects of chronic administration of neuroleptic and anticholinergic agents on densities of D2 dopamine and muscarinic cholinergic receptors in rat striatum. J. Pharmacol. Exp. Ther. 244, 987–993.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Costain, W.J., Gupta, S.K., Johnson, R.L., Mishra, R.K., 1997. Modulation of G proteins in rat striatum by neuroleptic drugs and a peptidomimetic analog of Pro–Leu–Gly–NH_2. In: Mishra, R.K., Baker, G.B., Boulton, A.A. (Eds.), Neuromethods, Vol. 31: G protein Methods and Protocols. Humana Press, Totowa, NJ, pp. 119–138.

Cox, R.F., Waszczak, B.L., 1990. Irreversible receptor inactivation reveals differences in dopamine receptor reserve between A9 and A10 dopamine systems: an electrophysiological analysis. Brain Res. 534, 273–282.

Cubeddu, L.X., Hoffmann, I.S., James, M.K., Niedzwiecki, D.M., 1983. Changes in the sensitivity to apomorphine of dopamine receptors modulating dopamine and acetylcholine release after chronic treatment with bromocriptine or haloperidol. J. Pharmacol. Exp. Ther. 226, 680–685.

Dewey, K.J., Fibiger, H.C., 1983. The effects of dose and duration of chronic pimozide administration on dopamine receptor supersensitivity. Naunyn-Schmiedeberg's Arch. Pharmacol. 322, 261–270.

Di Chiara, G., Morelli, M., Consolo, S., 1994. Modulatory functions of neurotransmitters in the striatum: Ach/dopamine/NMDA interactions. Trends Neurosci. 17, 228–233.

Dohlman, H.G., Thorner, J., 1997. RGS proteins and signaling by heterotrimeric G proteins. J. Biol. Chem. 272, 3871–3874.

Enz, A., Goldstein, M., Meller, E., 1990. Dopamine agonist-induced elevation of striatal acetylcholine: relationship between receptor occupancy and response in normal and denervated rat striatum. Mol. Pharmacol. 37, 560–565.

Gardner, B., Strange, P.G., 1998. Agonist action at $D_{2(long)}$ dopamine receptors: ligand binding and functional assays. Br. J. Pharmacol. 124, 978–984.

Geurts, M., Hermans, E., Maloteaux, J.-M., 1999. Assessment of striatal D1 and D2 dopamine receptor-G protein coupling by agonist-induced [^{35}S]GTPγS binding. Life Sci. 65, 1633–1645.

Hacksell, U., Jackson, D.M., Mohell, N., 1995. Does the dopamine receptor subtype selectivity of antipsychotic agents provide useful leads for the development of novel therapeutic agents? Pharmacol. Toxicol. 76, 320–324.

Hermans, E., Challiss, R.A.J., Nahorski, S.R., 1999. Effects of varying the expression level of recombinant human mGlu1α receptors on the pharmacological properties of agonists and antagonists. Br. J. Pharmacol. 126, 873–882.

Hillegaart, V., Ahlenius, S., Magnusson, O., Fowler, C.J., 1987. Repeated testing of rats markedly enhances the duration of effects induced by haloperidol on treadmill locomotion, catalepsy, and a conditioned avoidance response. Pharmacol. Biochem. Behav. 27, 159–164.

Iwata, S., Izumi, K., Shimizu, T., Fukuda, T., 1989. Effects of repeated testing on the incidence of haloperidol-induced catalepsy in mice. Pharmacol. Biochem. Behav. 33, 705–707.

Karolewicz, B., Antkiewicz-Michaluk, L., Michaluk, J., Vetulani, J., 1996. Different effects of chronic administration of haloperidol and pimozide on dopamine metabolism in the rat brain. Eur. J. Pharmacol. 313, 181–186.

Keen, M., 1991. Testing models of agonism for G protein-coupled receptors. Trends Pharmacol. Sci. 12, 371–374.

Kenakin, T., 1996. The classification of seven transmembrane receptors in recombinant expression systems. Pharmacol. Rev. 48, 413–463.

Klawans, H.L. Jr., 1973. The pharmacology of tardive dyskinesias. Am. J. Psychiatry 130, 82–86.

LaHoste, G.J., Marshall, J.F., 1993. New concepts in dopamine receptor plasticity. Ann. N.Y. Acad. Sci. 702, 183–196.

Lazareno, S., 1997. Measurement of agonist-stimulated [^{35}S]GTPγS binding to cell membranes. In: Challiss, R.A.J. (Ed.), Methods in Molecular Biology, Vol. 83, Receptor Signal Transduction Protocols. Humana Press, Totowa, NJ, pp. 107–116.

Li, R., Wing, L.L., Kirch, D.G., Wyatt, R.J., Chuang, D.M., 1992. Effects of chronic nicotine and haloperidol administration on muscarinic receptor-mediated phosphoinositide turnover in rat brain slices. Psychopharmacology 109, 248–250.

Marin, C., Chase, T.N., 1993. Dopamine D_1 receptor stimulation but not dopamine D_2 receptor stimulation attenuates haloperidol-induced behavioral supersensitivity and receptor up-regulation. Eur. J. Pharmacol. 231, 191–196.

Meller, E., Bohmaker, K., 1996. Chronic treatment with antipsychotic drugs does not alter G protein alpha or beta subunit levels in rat brain. Neuropharmacology 35, 1785–1791.

Meller, E., Bohmaker, K., Namba, Y., Friedhoff, A.J., Goldstein, M., 1987. Relationship between receptor occupancy and response at striatal dopamine autoreceptors. Mol. Pharmacol. 31, 592–598.

Memo, M., Pizzi, M., Missale, C., Carruba, M.O., Spano, P.F., 1987. Modification of the function of D1 and D2 dopamine receptors in striatum and nucleus accumbens of rats chronically treated with haloperidol. Neuropharmacology 26, 477–480.

Neal-Beliveau, B.S., Joyce, J.N., Lucki, I., 1993. Serotoninergic involvement in haloperidol-induced catalepsy. J. Pharmacol. Exp. Ther. 265, 207–217.

Nestler, E.J., Terwilliger, R.Z., Walker, J.R., Sevarino, K.A., Duman, R.S., 1990. Chronic cocaine treatment decreases levels of the G protein subunits $G_{i\alpha}$ and $G_{o\alpha}$ in discrete regions of rat brain. J. Neurochem. 55, 1079–1082.

Newman-Tancredi, A., Conte, C., Chaput, C., Verrièle, L., Millan, M.J., 1997. Agonist and inverse agonist efficacy at human recombinant serotonin 5-HT_{1A} receptors as a function of receptor: G-protein stoichiometry. Neuropharmacology 36, 451–459.

Odagaki, Y., Fuxe, K., 1995. Functional coupling of dopamine D_2 and muscarinic cholinergic receptors to their respective G proteins assessed by agonist-induced activation of high-affinity GTPase activity in rat striatal membranes. Biochem. Pharmacol. 50, 325–335.

Pedigo, N.W. Jr., 1994. Neurotransmitter receptor plasticity in aging. Life Sci. 55, 1985–1991.

Qin, Z.-H., Zhou, L.W., Zhang, S.-P., Wang, Y., Weiss, B., 1995. D_2 dopamine receptor antisense oligodeoxynucleotide inhibits the synthesis of a functional pool of D_2 dopamine receptors. Mol. Pharmacol. 48, 730–737.

Rinken, A., Finnman, U.-B., Fuxe, K., 1999. Pharmacological characterization of dopamine-stimulated [^{35}S]-guanosine 5′-(γ-thiotriphosphate) ([^{35}S]GTPγS) binding in rat striatal membranes. Biochem. Pharmacol. 57, 155–162.

Sayers, A.C., Burki, H.R., Ruch, W., Asper, H., 1975. Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. Psychopharmacologia 41, 97–104.

Schettini, G., Ventra, C., Florio, T., Grimaldi, M., Meucci, O., Marino, A., 1992. Modulation by GTP of basal and agonist-stimulated striatal adenylate cyclase activity following chronic blockade of D1 and D2 dopamine receptors: involvement of G proteins in the development of receptor supersensitivity. J. Neurochem. 59, 1667–1674.

Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., Van Gompel, P., Lesage, A.S., De Loore, K., Leysen, J.E., 1996. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology 124, 57–73.

Schröder, U., Schröder, H., Augustin, W., Sabel, B.A., 1994. Haloperidol-induced behavioral supersensitivity is increased by monosialoganglioside treatment in rats without affecting spiroperidol binding. J. Pharmacol. Exp. Ther. 271, 1193–1196.

See, R.E., Toga, A.W., Ellison, G., 1990. Autoradiographic analysis of regional alterations in brain receptors following chronic administration and withdrawal of typical and atypical neuroleptics in rats. J. Neural Transm. Gen. Sect. 82, 93–109.

See, R.E., Striplin, C., Kalivas, P.W., 1993. Chronic haloperidol does not alter G protein alpha-subunit levels in rats. Brain Res. Mol. Brain Res. 19, 219–221.

Shaul, P.W., Anderson, R.G., 1998. Role of plasmalemmal caveolae in signal transduction. Am. J. Physiol. 275, L843–L851.

Sibley, D.R., Neve, K.A., 1997. Regulation of dopamine receptor function and expression. In: Neve, K.A., Neve, R.L. (Eds.), The Dopamine Receptors. Humana Press, Totowa, NJ, pp. 383–424.

Strange, P.G., 1999. Agonism and inverse agonism at dopamine D_2-like receptors. Clin. Exp. Pharmacol. Physiol. 26, S3–S9, suppl.

Tarsy, D., 1989. Neuroleptic-induced movement disorders. In: Quinn, N.P., Jenner, P.G. (Eds.), Disorders of Movement. Clinical, Pharmacological and Physiological Aspects. Academic Press, London, GB, pp. 361–393.

Van Vliet, B.J., Van Rijswijk, A.L.C.T., Wardeh, G., Mulder, A.H., Schoffelmeer, A.N.M., 1993. Adaptative changes in the number of G_s-and G_i-proteins underlie adenylyl cyclase sensitization in morphine-treated rat striatal neurons. Eur. J. Pharmacol. Mol. Pharmacol. 245, 23–29.

Vogelsang, G.D., Piercey, M.F., 1985. The supersensitivity of dopaminergic neurons to apomorphine in rats following chronic haloperidol. Eur. J. Pharmacol. 110, 267–269.

Watts, V.J., Lawler, C.P., Gonzales, A.J., Zhou, Q.Y., Civelli, O., Nichols, D.E., Mailman, R.B., 1995. Spare receptors and intrinsic activity: studies with D_1 dopamine receptor agonists. Synapse 21, 177–187.

Yamane, H.K., Fung, B.K.K., 1993. Covalent modifications of G-proteins. Annu. Rev. Pharmacol. Toxicol. 32, 201–241.