

# SSR181507, A Dopamine D<sub>2</sub> Receptor Antagonist and 5-HT<sub>1A</sub> Receptor Agonist. I: Neurochemical and Electrophysiological Profile

Yves Clautre<sup>\*1</sup>, Danielle De Peretti<sup>1</sup>, Philippe Brun<sup>3</sup>, Christiane Gueudet<sup>2</sup>, Nathalie Allouard<sup>1</sup>, Richard Alonso<sup>2</sup>, Joëlle Lourdelet<sup>1</sup>, André Oblin<sup>1</sup>, Gabrielle Damoiseau<sup>1</sup>, Dominique Françon<sup>1</sup>, Marie-Françoise Suaud-Chagny<sup>3</sup>, Régis Steinberg<sup>2</sup>, Mireille Sevrin<sup>1</sup>, Hans Schoemaker<sup>1</sup>, Pascal George<sup>1</sup>, Philippe Soubrié<sup>2</sup> and Bernard Scatton<sup>1</sup>

<sup>1</sup>Sanofi-Synthelabo Recherche, Discovery Research, Bagneux, France; <sup>2</sup>Sanofi-Synthelabo Recherche, Discovery Research, Montpellier, France; <sup>3</sup>INSERM U512, Faculté de Pharmacie, Lyon, France

SSR181507 ((3-exo)-8-benzoyl-N-[[[(2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl]methyl]-8-azabicyclo[3.2.1]octane-3-methanamine monohydrochloride) is a novel tropanemethanamine benzodioxane derivative that possesses high and selective affinities for D<sub>2</sub>-like and 5-HT<sub>1A</sub> receptors (K<sub>i</sub> = 0.8, 0.2, and 0.2 nM for human D<sub>2</sub>, D<sub>3</sub>, and 5-HT<sub>1A</sub>, respectively). *In vivo*, SSR181507 inhibited [<sup>3</sup>H]raclopride binding to D<sub>2</sub> receptors in the rat (ID<sub>50</sub> = 0.9 and 1 mg/kg, i.p. in limbic system and striatum, respectively). It displayed D<sub>2</sub> antagonist and 5-HT<sub>1A</sub> agonist properties in the same concentration range *in vitro* (IC<sub>50</sub> = 5.3 nM and EC<sub>50</sub> = 2.3 nM, respectively, in the GTPγS model) and in the same dose range *in vivo* (ED<sub>50</sub> = 1.6 and 0.7 mg/kg, i.p. on striatal DA and 5-HT synthesis, respectively, and 0.03–0.3 mg/kg, i.v. on dorsal raphe nucleus firing rate). It selectively enhanced Fos immunoreactivity in mesocorticolimbic areas as compared to the striatum. This regional selectivity was confirmed in electrophysiological studies where SSR181507, given acutely (0.1–3 mg/kg, i.p.) or chronically (3 mg/kg, i.p., o.d., 22 days), increased or decreased, respectively, the number of spontaneous active DA cells in the ventral tegmental area, but not in the substantia nigra. Moreover, SSR181507 increased both basal and phasic DA efflux (as assessed by microdialysis and electrochemistry) in the medial prefrontal cortex and nucleus accumbens, but not in the striatum. This study shows that the combination of D<sub>2</sub> receptor antagonism and 5-HT<sub>1A</sub> agonism, in the same dose range, confers on SSR181507 a unique neurochemical and electrophysiological profile and suggests the potential of this compound for the treatment of the main dimensions of schizophrenia. *Neuropsychopharmacology* (2003) 28, 2064–2076, advance online publication, 6 August 2003; doi:10.1038/sj.npp.1300262

**Keywords:** 5-HT<sub>1A</sub> agonist; D<sub>2</sub> antagonist; atypical antipsychotic; mesocorticolimbic selectivity

**Note:** This paper is the first of two companion studies published in *Neuropsychopharmacology*. The second paper's citation is *SSR181507, A Dopamine D<sub>2</sub> Receptor Antagonist and 5-HT<sub>1A</sub> Receptor Agonist. II: Behavioral Profile Predictive of an Atypical Antipsychotic Activity* (2003) 28, 1889–1902. doi: 1038/sj.npp.1300261. These two papers should be read together.

## INTRODUCTION

Schizophrenia is a devastating psychiatric disorder characterized by a diversity of symptoms: the so-called positive symptoms (such as delusions and hallucinations), negative symptoms (such as blunted affect and social withdrawal), as well as cognitive-attentional deficits. While classical antipsychotics do show efficacy against the positive symptoms of schizophrenia, negative symptoms and cognitive defects

are clearly less responsive to therapeutic intervention. The main limit to their clinical use, however, resides in their side effects, such as extrapyramidal signs (EPS), neuroendocrine disturbances, sexual dysfunction, orthostatic hypotension, weight gain, and excessive sedation. A second generation of antipsychotics, commonly referred to as 'atypical antipsychotics', is characterized by a modest progress in effect on positive symptoms of schizophrenia, but by an improved separation of efficacy against the positive symptoms and the induction of EPS, and by some efficiency on the cognitive symptoms of schizophrenia (Meltzer and McGurk, 1999). Conceptually, their putative mechanisms are related to a limbic selectivity characterized by a selective blockade of limbic dopaminergic neurotransmission. The predominant profile of these atypical antipsychotics appears to be blockade of both 5-HT<sub>2A</sub> and DA D<sub>2</sub> receptors (multi-receptor concept). Indeed, although activity at 5-HT<sub>2A</sub>

\*Correspondence: Dr Y Clautre, Sanofi-Synthelabo Recherche, Discovery Research, 31 Ave Paul Vaillant-Couturier, 92220 Bagneux, France, Tel: +33 1 45 36 25 55, Fax: +33 1 45 36 20 59, E-mail: Yves.Clautre@Sanofi-Synthelabo.com

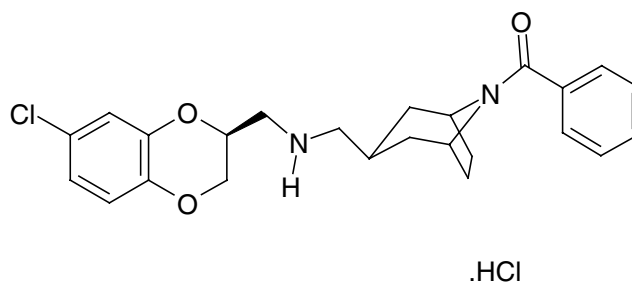
Received 19 November 2002; revised 28 May 2003; accepted 28 May 2003

Online publication: 5 June 2003 at <http://www.acnp.org/citations/Npp060503020417/default.pdf>

receptors is not essential for antipsychotic efficacy, it has been proposed that the atypical clinical profile of several antipsychotics could be related to their ratio of affinities for 5-HT<sub>2A</sub> and DA D<sub>2</sub> receptors (Meltzer, 1999; Scatton and Sanger, 2000). Some of them also interact with additional receptors such as  $\alpha$ -adrenergic receptor subtypes, histamine H<sub>1</sub>, or muscarinic receptors (Miyamoto *et al.*, 2000). The multiplicity of pharmacological targets for these atypical antipsychotics not only fuels the debate about the therapeutically relevant mechanism for such drugs, but also leads to a side-effect profile, besides EPS, that remains open for improvement. That highly selective DA D<sub>2</sub> and D<sub>3</sub> receptor blockade is sufficient to reveal limbic selectivity is demonstrated by amisulpride, which shows atypical antipsychotic activity and also an improved efficacy against the primary negative symptoms of schizophrenia (Boyer *et al.*, 1995).

Although atypical antipsychotics have been a major advance in the treatment of schizophrenia, there is still a need for improvement, as a significant proportion of patients remain refractory to treatment, and negative and cognitive symptoms are little improved by current treatment. Recent preclinical and clinical data have suggested that combining antagonist activity at DA D<sub>2</sub> receptors with agonist activity at 5-HT<sub>1A</sub> receptors might offer a promising alternative to schizophrenia treatment (see Meltzer and McGurk, 1999; Millan, 2000; Ichikawa *et al.*, 2001; Bantick *et al.*, 2001 for reviews).

Autoradiographic studies in post-mortem schizophrenic patients have shown that 5-HT<sub>1A</sub> binding site densities are increased in the dorsolateral prefrontal cortex and orbital frontal cortex (Burnet *et al.*, 1996; Simpson *et al.*, 1996) and PET studies recently revealed an increase in cortical 5-HT<sub>1A</sub> receptor binding in schizophrenia (Kasper *et al.*, 2002), thus suggesting that the 5-HT<sub>1A</sub> receptor may indeed be implicated in this disease or its manifestations. Furthermore, 5-HT<sub>1A</sub> agonists attenuate certain DA D<sub>2</sub> receptor-mediated motor side effects: 8-OH-DPAT and ipsapirone, two prototypical 5-HT<sub>1A</sub> receptor agonists, prevent or reduce haloperidol-induced catalepsy in rodents (Wadenberg and Ahlenius, 1991). In clinical studies, signs of Parkinsonism were improved by the addition of buspirone (Goff *et al.*, 1991) to neuroleptic-treated schizophrenic patients. Thus, DA D<sub>2</sub> receptor antagonists with high affinity and agonist properties at the 5-HT<sub>1A</sub> receptor should have a low incidence of EPS. Moreover, recent work with tandospirone (Sumiyoshi *et al.*, 2000, 2001) have suggested the usefulness of 5-HT<sub>1A</sub> agonists for improving cognition in patients with schizophrenia. It has also been proposed that the ability of atypical antipsychotics to alleviate the negative and cognitive symptoms of schizophrenia is related to the fact that they enhance DA release in the medial prefrontal cortex (Kapur and Remington, 1996). As selective 5-HT<sub>1A</sub> agonists increase DA release in this structure (Arborelius *et al.*, 1993), this property may lead to efficacy against the negative-cognitive symptomatology in schizophrenia. Finally, since stress and anxiety are often associated with schizophrenic episodes (Lieberman and Corrigan, 1992) and because up to 75% of first diagnosed schizophrenics are depressed (Koreen *et al.*, 1993), relief of these symptoms is also a desirable property. Along with anxiolysis (Borison *et al.*, 1990), antidepressant activity has



**Figure 1** Chemical structure of SSR181507.

also been associated with 5-HT<sub>1A</sub> agonism (Robinson *et al.*, 1989), thus suggesting that compounds with this activity should have additional advantages on schizophrenia-associated mood disorders.

We have recently synthesized a compound, SSR181507 (Figure 1), which combines DA D<sub>2</sub> antagonism and 5-HT<sub>1A</sub> agonism in the same concentration and dose range. In the present study, we report on the binding profile of this compound and its intrinsic and functional activity at these two receptors, on its electrophysiological (firing of serotonergic cell bodies of dorsal raphe nucleus (DRN), number of active cells in DA cell bodies) and neurochemical (Fos expression, basal and evoked DA efflux) profile in support of the above-mentioned hypothesis. A companion paper (Depoortere *et al.*, 2003) provides further psychopharmacological evidence to this end.

## MATERIALS AND METHODS

### Animals

Unless otherwise indicated, adult male Sprague–Dawley rats (OFA from Iffa Credo, Les Oncins, France or COBS from Charles River, St Aubin-les-Elbeuf, France) and Dunkin Hartley guinea-pigs (Iffa Credo) were used. All experiments were performed in accordance with the 'Guide and Care and Use of Laboratory Animals' (National Institutes of Health) and were approved by the in-house Animal Ethics Committee.

### Material and Drugs

SSR181507 ((3-exo)-8-benzoyl-N-[[[(2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl]methyl]-8-azabicyclo[3.2.1]octane-3-methanamine monohydrochloride), WAY100635, amisulpride, clozapine, olanzapine, haloperidol, eliprodil, paroxetine, NAN-190, and 8-OH-DPAT were synthesized by the Chemistry Department of Sanofi-Synthelabo Recherche. Other chemicals (apomorphine, pargyline) were obtained commercially at the highest purity available.

Drugs were administered by the i.p. route, except when indicated otherwise; control groups received an equal volume of the corresponding vehicle. Doses refer to the free base equivalent. Pargyline, clozapine, and haloperidol were dissolved in saline (0.9% NaCl). SSR181507 and WAY100635 were suspended with Tween 80 (0.1%) in saline vehicle.

### Radioligand-Binding Studies *In Vitro*

Studies of radioligand binding to animal receptors were performed essentially as described by the authors indicated: the DA D<sub>2</sub> receptor in rat striatum (0.3 nM [<sup>3</sup>H]spiperone, Briley and Langer, 1978), the DA D<sub>3</sub> receptor in bovine caudate nucleus (0.8 nM [<sup>3</sup>H]7-OH-DPAT in the presence of 0.2 μM eliprodil, Schoemaker, 1993), the serotonin 5-HT<sub>1A</sub> receptor in rat hippocampus (1 nM [<sup>3</sup>H]8-OH-DPAT in the presence of 3 μM paroxetine, Sanger and Schoemaker, 1992; Schoemaker and Langer, 1986). Assay conditions and references for competition binding studies at human receptor subtypes are summarized in Table 2. Results were evaluated as the drug concentration required to inhibit 50% of specific radioligand binding and converted to K<sub>I</sub> values as described by Cheng and Prusoff (1973).

### Radioligand Binding Studies *In Vivo*

[<sup>3</sup>H]raclopride selectively recognizes DA D<sub>2</sub>-like receptors *in vitro* and was used to label these receptors *in vivo* (Köhler *et al*, 1985). [<sup>3</sup>H]raclopride (60–87 Ci/mmol, NEN Life Science Products, Boston, MA, USA) was injected (9 μCi/200 μl) into the tail vein of rats, 45 min before being killed. Test drugs or vehicle were administered in a final volume of 550 μl, either 45 min (SSR181507) or 75 min (haloperidol, amisulpride) before [<sup>3</sup>H]raclopride. The striatum and the limbic system (nucleus accumbens, septum, and olfactory tubercle) were dissected by hand, and tissue radioactivity was measured after overnight digestion in 0.5 ml Soluene. Nonspecific binding for the fitting of the competition curves was defined in the presence of haloperidol (1 mg/kg, i.p.).

### [<sup>35</sup>S]GTPγS Binding at Human D<sub>2</sub> and 5-HT<sub>1A</sub> Receptors

Functional activity at D<sub>2</sub> and 5-HT<sub>1A</sub> receptors was evaluated using [<sup>35</sup>S]GTPγS binding. Assays were carried out essentially as described by Newman-Tancredi *et al* (1999) with some minor modifications. Briefly, membranes (7–11 μg protein) from CHO cells recombinantly expressing the human D<sub>2</sub> (2 pmol/mg membrane protein, Receptor Biology, Baltimore, MD, USA) or 5-HT<sub>1A</sub> receptor (1.2 pmol/mg membrane protein, CRM-0.35, NEN Life Science Products) were incubated at room temperature (75 and 40 min, respectively) with agonists and/or antagonists and 0.1 nM [<sup>35</sup>S]GTPγS (1065 Ci/mmol; Amersham Pharmacia Biotech, England) in a final volume of 100 μl of 20 mM HEPES buffer (pH 7.4), containing 3 mM MgSO<sub>4</sub>, 3 μM GDP, 1 mM dithiothreitol, and 150 mM NaCl. Nonspecific binding was determined with 20 μM unlabeled GTPγS. Results were expressed as EC<sub>50</sub> (concentration of the compound producing 50% effect) and IC<sub>50</sub> (concentration of the compound producing 50% inhibition of the dopamine response) and were obtained by nonlinear regression analysis.

### *In Vitro* Dopamine Release

The modulation of electrically evoked [<sup>3</sup>H]dopamine release from slices of rat striatum was performed essentially as described by Schoemaker *et al* (1997). Briefly, slices were

incubated with 0.1 μM [<sup>3</sup>H]dopamine for 30 min at 37°C in Krebs buffer (mM: NaCl, 118; KCl, 4.7; CaCl<sub>2</sub>, 1.3; MgCl<sub>2</sub>, 1.2; NaH<sub>2</sub>PO<sub>4</sub>, 1.0; NaHCO<sub>3</sub>, 25.0; glucose, 11.1; EDTA, 0.004, equilibrated with 5% CO<sub>2</sub>/95% O<sub>2</sub>), washed, and superfused with Krebs buffer for 90 min at a flow rate of 0.7 ml/min. After this period, fractions (5 ml) were collected until the end of the experiment. Two 2-min periods of electrical field stimulation (rectangular pulses of 2 ms and 16 mA; 3 Hz) were applied to each slice 110 and 160 min after the beginning of superfusion. Apomorphine was added to the superfusion buffer starting 20 min before the second stimulation period. When the interaction between SSR181507 and apomorphine was studied, SSR181507 was present in the superfusion buffer 20 min before the first stimulation period. The stimulation-evoked [<sup>3</sup>H]dopamine overflow (S<sub>1</sub> and S<sub>2</sub>, respectively) was calculated with respect to the spontaneous outflow (sp1 and sp2, respectively) in the fractions immediately before the stimulation. Apparent pA<sub>2</sub> values were determined as pA<sub>2</sub> = log([E′]/[E] − 1) − log[B], where [E′] and [E] are the concentrations of the agonist producing the half-maximal effect in the presence and the absence of the antagonist, respectively, and [B] the concentration of the antagonist (Furchgott, 1972).

### Measurement of Dopamine and Serotonin Synthesis Rates

The rate of DA and serotonin (5-HT) synthesis was estimated by measuring the accumulation of dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP), respectively, after administration of 100 mg/kg, i.p. of the aromatic L-amino acid decarboxylase inhibitor NSD-1015 (Schoemaker *et al*, 1997). At 1 h after administration of SSR181507 (0.3–10 mg/kg, i.p.) and 30 min after the administration of NSD-1015, rats were killed by decapitation and different brain structures (striatum, hippocampus, limbic system (nucleus accumbens, septum, and olfactory tubercle), cortex) were dissected on ice. Brain structures were then weighed, homogenized in 20 volumes of 0.1 N HClO<sub>4</sub> and centrifuged at 10 000 g for 10 min. L-DOPA and 5-HTP levels were measured in the supernatant by HPLC with electrochemical detection as described previously (Schoemaker *et al*, 1997).

Identical procedures were used in rats chronically treated with SSR181507 (3 or 10 mg/kg, i.p. b.i.d. for 20 days). In this case, SSR181507 was administered acutely at the dose of 3 mg/kg, i.p., 72 h after the last injection of the repeated treatment of vehicle control or SSR181507.

### *In Vivo* Recording of Dorsal Raphe Neuronal Firing

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and were mounted in a stereotaxic frame after femoral vein cannulation. Additional doses of chloral hydrate were administered to maintain surgical anesthesia throughout the experiment. A burr hole was drilled into the skull over the DRN and tungsten electrodes were lowered (0.5–1 mm anterior to lambda, on the midline, 4.5–5.5 mm under the cortical surface, according to the atlas of Paxinos and Watson (1998)) for recording spontaneous cell unit activity. Action potentials were amplified and counted by 10-s

periods with a spike discriminator connected to a computer driven by the software (Haigler and Aghajanian, 1974). SSR181507 (0.01–0.3 mg/kg, i.v.) was injected and its effect expressed as percentage of baseline firing rate. For antagonist studies, vehicle or WAY100635 (0.1 mg/kg, i.v.) was administered 5 min later.

### ***In Vivo* Recording of the Number of Spontaneously Active DA Cells**

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and were mounted in a stereotaxic frame after cannulation of the lateral vein of the tail in order to allow both the i.v. injection of drugs and the continuous infusion of chloral hydrate (120 mg/kg/h). A burr hole was drilled into the skull above the area to be recorded. The micropipettes (glass tubing of 1.5 mm outer diameter that was heated, pulled, and broken under microscopic control to obtain a tip of 2.5 µm diameter) were filled with 0.9% saline and stereotaxically aimed at the vicinity of DA cells in the substantia nigra (SN: A9) or the ventral tegmental area (VTA: A10). The spikes recorded from the micropipette were amplified, filtered (0.1–2 kHz), and visualized on an oscilloscope.

In all, 12 electrode tracks were defined on a 12-box, 240 000 µm<sup>2</sup> grid within the A9 or A10 area, according to the following coordinates: A9 (5.8–5.2 mm anterior to bregma, 1.8–2.2 mm lateral to bregma, 6.5–8.0 mm under the cortical surface), A10 (6.0–5.4 mm anterior to bregma, 0.4–0.8 mm lateral to bregma, 7.0–8.5 mm under the cortical surface). The number of spontaneously active DA cells was counted for each track until the grid was completed. The DA cells were identified and the number of spontaneously firing neurons per electrode track was determined according to Grace and Bunney (1983). The effects of SSR181507 on population response were investigated in an acute and a repeated treatment procedure (3 mg/kg, i.p. once daily for a period of 22 days). On the day of the experiment, SSR181507 or vehicle was administered 45–60 min before initiation of the first electrode track. WAY100635 (0.1 mg/kg, i.v.) or apomorphine (0.063 mg/kg, i.v.) was administered after completion of the last electrode track, and cell counting was restarted for an additional five tracks, according to White and Wang (1983).

### ***In Vivo* Measurement of Basal Dopamine Efflux**

Basal DA efflux was monitored in the medial prefrontal cortex, nucleus accumbens, or striatum by microdialysis. Adult male Sprague–Dawley rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and guide cannulae were stereotaxically implanted onto the dura mater above the medial prefrontal cortex (3.2 mm anterior to bregma, 0.6 mm lateral to bregma), the nucleus accumbens (1.7 mm anterior to bregma, 1.0 mm lateral to bregma), and the striatum (0.7 mm anterior to bregma, 3.0 mm lateral to bregma) (Paxinos and Watson, 1998). At least 5 days after surgery, microdialysis probes (Carnegie Medicine, Stockholm, Sweden), 250 µm in diameter with an exposed membrane length of 3 mm (medial prefrontal cortex) and 2 mm (nucleus accumbens and striatum), were positioned within the guide cannulae (vertical coordinates: 4.5, 8.0, and 8.0 mm, respectively, under the cortical surface) and

perfused with artificial cerebrospinal fluid (mM: NaCl, 147; KCl, 4; CaCl<sub>2</sub>, 1.2; MgCl<sub>2</sub>, 1.0) using a CMA/100 pump (Carnegie Medicine) at a flow rate of 2 µl/min. Dialysate samples were collected every 20 min and analyzed using HPLC with electrochemical detection. The average concentration of five stable fractions immediately preceding drug administration was defined as the 100% control value. The area under the curve was expressed relative to these values and was calculated for 120 min after drug administration.

### ***In Vivo* Measurement of Evoked Catecholamine Efflux**

Rats were anesthetized with urethane (1.15–1.4 g/kg, i.p.), treated with pargyline (75 mg/kg, s.c.), and placed in a stereotaxic frame. Evoked catecholamine (CA) efflux was monitored in the nucleus accumbens or medial prefrontal cortex by electrochemically treated carbon fiber electrodes combined with differential pulse amperometry (DPA). The sensitivity of the treated electrode for CA vs metabolites (<10 vs 100 nM) and the use of pargyline to prevent CA catabolism allow a selective measurement of DA levels in the nucleus accumbens (Suaud-Chagny *et al.*, 1992). In the medial prefrontal cortex, DA is probably the main source of the CA signal variations induced by the electrical stimulation of the dopaminergic pathway, although a participation of noradrenaline to the evoked CA efflux measured in the medial prefrontal cortex cannot be excluded.

Carbon fiber electrodes (250 µm long and 8 µm in diameter) were electrochemically treated in phosphate-buffered saline (PBS) as previously described (Brun *et al.*, 1995). Electrodes were then implanted in the nucleus accumbens or the medial prefrontal cortex at the following coordinates: 2.0–2.2 mm anterior to bregma, 1.3 mm lateral to bregma and from 6.5 to 7.0 mm below the cortical surface and 2.7–3.0 mm anterior to bregma, 0.5–0.7 mm lateral to bregma and from 4.0 to 4.5 mm below the cortical surface, respectively (Paxinos and Watson, 1998). They were connected to a voltammetric recorder (Biopulse; Radiometer Analytical, France). Electrically evoked changes in CA efflux were monitored every second using DPA with the final potential adjusted at +80 mV vs the Ag/AgCl reference electrode (Suaud-Chagny *et al.*, 1992; Brun *et al.*, 1995). All results were expressed as percentages of the mean of the three effects recorded before drug injection.

### **Electrical Stimulation of the Dopaminergic Pathway**

Electrical stimulation of the ascending dopaminergic pathway was used to evoke short-lasting increases in the extracellular DA concentration. A bipolar stimulating electrode (SNEX-200, Rhodes Medical Instruments, USA) was positioned in the ascending dopaminergic pathway at the following coordinates: 4.0 mm posterior to bregma and 1.0 mm lateral to bregma. The depth (8.2–8.6 mm below the cortical surface) was adjusted for each experiment so that the response was maximal. Electrical stimulations consisted of square current pulses (300 µA, 0.5 ms) and were applied by an isolated stimulator (DS2, Digitimer, USA) every 10 min and for 10 s at the frequency of 20 Hz (nucleus accumbens) or 40 Hz (medial prefrontal cortex).

## Fos Immunohistochemistry

Experiments were performed as described previously (Alonso *et al*, 1999). Briefly, rats were anesthetized with sodium pentobarbital (75 mg/kg, i.p.) and perfused transcardially with saline, followed by 4% paraformaldehyde. Brains were postfixed overnight and 50  $\mu$ m coronal sections were cut using a vibratome. Immunohistochemistry was performed on free-floating tissue sections according to a standard avidin-biotin-peroxidase procedure using an anti-Fos rabbit polyclonal antibody directed against residues 3–16 of the N-terminal region of the Fos protein (sc-52, Santa Cruz Biotechnologies Inc., CA, USA). Sections were imaged through a Leica DMRX microscope, and the Fos-like immunoreactive signal was quantified with an image analysis system (Samba Technologies, Meylan, France) by counting the number of Fos-positive cells.

## Statistical Analyses

Statistical differences between groups were assessed using ANOVA tests, followed by Dunnett's *post hoc* tests.

In DPA experiments, comparisons between treatments were performed using a one-way ANOVA with repeated measures, followed by a Fisher's PLSD *post hoc* test.

## RESULTS

### *In Vitro* Radioligand-Binding Studies

SSR181507 showed high affinity for animal DA D<sub>2</sub>, DA D<sub>3</sub>, and 5-HT<sub>1A</sub> receptors ( $K_I = 7.5, 3.6,$  and  $4.5$  nM, respectively, Table 1). Clozapine had a lower affinity for these receptors. Olanzapine, as well as amisulpride and haloperidol, had almost a similar affinity for DA D<sub>2</sub> and DA D<sub>3</sub> receptors, but in contrast to SSR181507, they were devoid of significant affinity for the 5-HT<sub>1A</sub> receptor (Table 1). SSR181507 displayed high affinity for cloned human (h) DA D<sub>2</sub>, DA D<sub>3</sub>, and 5-HT<sub>1A</sub> receptor subtypes ( $K_I = 0.8, 0.2,$  and  $0.2$  nM, respectively, Table 2). It had no affinity for hDA D<sub>1</sub> and hDA D<sub>5</sub> receptor subtypes and had only a modest affinity for hDA D<sub>4</sub> receptor, h5-HT<sub>2A</sub>, h5-HT<sub>2C</sub>, h5-HT<sub>6</sub>, and h5-HT<sub>7</sub> receptor subtypes, for h $\alpha_{1D}$  and h $\alpha_{2A}$  adrenoceptors, and for all five human muscarinic receptor subtypes (selectivity ratio > 60-fold, Table 2).

### *In Vivo* Radioligand-Binding Studies

*In vivo* [<sup>3</sup>H]raclopride-binding studies in rats revealed occupancy of DA D<sub>2</sub> receptors by SSR181507 with similar ID<sub>50</sub> values in the limbic system and in the striatum ( $0.9 \pm 0.2$  and  $1.0 \pm 0.2$  mg/kg, i.p., respectively, Table 3). In this test, SSR181507 behaved like haloperidol, which

**Table 1** Affinity of SSR181507 and Antipsychotic Drugs for Animal Receptors *In Vitro*

Receptor	$K_I$ (nM)				
	SSR181507	Clozapine	Olanzapine	Amisulpride	Haloperidol
D <sub>2</sub>	7.5 $\pm$ 0.6	87 $\pm$ 24	12 $\pm$ 2	5.1 $\pm$ 0.6	1.4 $\pm$ 0.2
D <sub>3</sub>	3.6 $\pm$ 0.5	395 $\pm$ 91	39 $\pm$ 3	1.8 $\pm$ 0.9	6.2 $\pm$ 1.6
5-HT <sub>1A</sub>	4.5 $\pm$ 0.7	391 $\pm$ 112	> 1000	> 1000	> 1000

Ligand-binding affinity to animal receptors *in vitro* was determined as described in Materials and methods.  $K_I$  values are the mean  $\pm$  SEM of at least three independent determinations.

**Table 2** Radioligand-Binding Conditions and Affinity of SSR181507 for Human Receptors *In Vitro*

Receptor	Tissue	Radioligand	Conc. (nM)	Reference	SSR181507 $K_I$ (nM)
D <sub>1</sub>	CHO cells	[ <sup>3</sup> H]SCH-23390	1.4	Deary <i>et al</i> (1990)	> 1000
D <sub>5</sub>	CHO cells	[ <sup>3</sup> H]SCH-23390	2.5	Sibley and Monsma (1992)	> 1000
<b>D<sub>2L</sub></b>	CHO cells	[ <sup>3</sup> H]Spiperone	2.0	Grandy <i>et al</i> (1989)	<b>0.84 <math>\pm</math> 0.02</b>
<b>D<sub>3</sub></b>	CHO cells	[ <sup>3</sup> H]Spiperone	2.0	Sokoloff <i>et al</i> (1990)	<b>0.18 <math>\pm</math> 0.06</b>
D <sub>4</sub>	CHO cells	[ <sup>3</sup> H]Spiperone	1.2	Van Tol <i>et al</i> (1991)	825 $\pm$ 197
<b>5-HT<sub>1A</sub></b>	CHO cells	[ <sup>3</sup> H]8-OH-DPAT	1.5	Martin and Humphrey (1994)	<b>0.242 <math>\pm</math> 0.009</b>
5-HT <sub>2A</sub>	CHO-K <sub>1</sub> cells	[ <sup>3</sup> H]Ketanserin	0.5	Bonhaus <i>et al</i> (1995)	66 $\pm$ 14
5-HT <sub>2C</sub>	CHO cells	[ <sup>3</sup> H]mesulergine	0.7	Bonhaus <i>et al</i> (1995)	298 $\pm$ 134
5-HT <sub>6</sub>	Hela cells	[ <sup>3</sup> H]LSD	1.5	Monsma <i>et al</i> (1993)	110 $\pm$ 5
5-HT <sub>7</sub>	HEK-293 cells	[ <sup>3</sup> H]LSD	6.5	Shen <i>et al</i> (1993)	54 $\pm$ 13
$\alpha_{1D}$	HEK-293 cells	[ <sup>3</sup> H]Prazosin	0.6	Kenny <i>et al</i> (1995)	121 $\pm$ 24
$\alpha_{2A}$	Sf9 cells	[ <sup>3</sup> H]Prazosin	0.25	Greengrass and Bremner (1979)	96 $\pm$ 13
M <sub>1</sub>	Sf9 cells	[ <sup>3</sup> H]N-methylscopolamine	0.29	Buckley <i>et al</i> (1989)	1130 $\pm$ 32
M <sub>2</sub>	Sf9 cells	[ <sup>3</sup> H]N-methylscopolamine	0.29	Buckley <i>et al</i> (1989)	1560 $\pm$ 841
M <sub>3</sub>	Sf9 cells	[ <sup>3</sup> H]N-methylscopolamine	0.29	Buckley <i>et al</i> (1989)	818 $\pm$ 309
M <sub>4</sub>	Sf9 cells	[ <sup>3</sup> H]N-methylscopolamine	0.29	Buckley <i>et al</i> (1989)	1230 $\pm$ 145
M <sub>5</sub>	Sf9 cells	[ <sup>3</sup> H]N-methylscopolamine	0.29	Buckley <i>et al</i> (1989)	5440 $\pm$ 168

$K_I$  values are the mean  $\pm$  SEM of at least two independent determinations.

**Table 3** Comparative Potencies of Several Compounds at Inhibiting *In Vivo* [<sup>3</sup>H]Raclopride-Specific Binding in the Rat Striatum and Limbic System

Compound	ID <sub>50</sub> (mg/kg, i.p.)	
	Striatum	Limbic system
SSR181507	1.0 ± 0.2	0.9 ± 0.2
Haloperidol	0.07 ± 0.01	0.11 ± 0.02
Amisulpride	43.6 ± 6.2	17.3 ± 1.9

Compounds were administered either 45 min (SSR181507) or 75 min (haloperidol and amisulpride) before the radioligand. Average specific binding to the striatum and limbic system amounted to 0.83 and 0.17 fmol/mg tissue, respectively. ID<sub>50</sub> values shown as mean ± SEM (n = 6/group, four doses) represent the estimated doses required to produce a half-maximal inhibition of radioligand binding.

showed similar affinity in both brain regions, but different from amisulpride, which was more potent at competing with [<sup>3</sup>H]raclopride binding in the limbic system than in the striatum (Table 3).

### [<sup>35</sup>S]GTPγS Binding to the Human DA D<sub>2</sub> Receptor

Dopamine induced a concentration-dependent increase in [<sup>35</sup>S]GTPγS binding to recombinantly expressed DA D<sub>2</sub> receptors (EC<sub>50</sub> = 3.1 μM). SSR181507, up to 100 nM, failed to stimulate [<sup>35</sup>S]GTPγS binding to the DA D<sub>2</sub> receptor, but antagonized DA (3 μM)-stimulated [<sup>35</sup>S]GTPγS binding with an IC<sub>50</sub> = 5.3 ± 1.0 nM (Figure 2a). Under the same experimental conditions, haloperidol had an IC<sub>50</sub> = 3.9 ± 0.8 nM.

### [<sup>35</sup>S]GTPγS Binding to the Human 5-HT<sub>1A</sub> Receptor

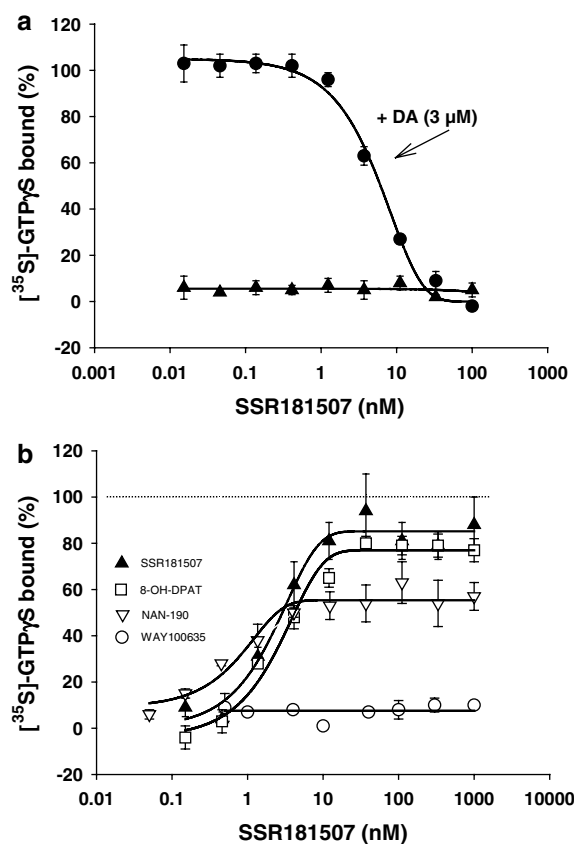
5-HT concentration-dependently increased [<sup>35</sup>S]GTPγS binding to the recombinant 5-HT<sub>1A</sub> receptor with an EC<sub>50</sub> of 7.1 ± 2.6 nM. SSR181507 stimulated [<sup>35</sup>S]GTPγS binding (EC<sub>50</sub> = 2.3 ± 1.2 nM) with a maximal efficacy (E<sub>max</sub>) of 85% as compared to that of 5-HT (defined as 100%). The corresponding values for the full 5-HT<sub>1A</sub> agonist 8-OH-DPAT and the partial 5-HT<sub>1A</sub> agonist NAN-190 were: EC<sub>50</sub> = 2.8 ± 1.5 nM and E<sub>max</sub> = 79%, and EC<sub>50</sub> = 0.8 ± 0.2 nM and E<sub>max</sub> = 56%, respectively. The maximal efficacy of the 5-HT<sub>1A</sub> antagonist WAY100635 was 10% (Figure 2b).

### *In Vitro* Dopamine Release

The electrically evoked [<sup>3</sup>H]dopamine release from slices of rat striatum is subject to an inhibitory modulation mediated by DA D<sub>2</sub>-like terminal autoreceptors as shown by the inhibitory effect of the DA agonist, apomorphine (IC<sub>50</sub> = 23 nM). SSR181507 (30 and 100 nM) produced a rightward shift of the apomorphine dose-response curve with an apparent pA<sub>2</sub> = 7.94 (Figure 3).

### *In Vivo* Dopamine and Serotonin Synthesis

SSR181507 (0.3–10 mg/kg, i.p.) dose-dependently increased (322% of controls) the synthesis of DA, as measured by the accumulation of L-DOPA, in rat striatum (ED<sub>50</sub> = 1.6 ± 0.1 mg/kg, i.p.). In the same dose range, it decreased the synthesis of 5-HT (maximal effect: 42% of controls at 10 mg/kg, i.p.), as measured by the accumulation

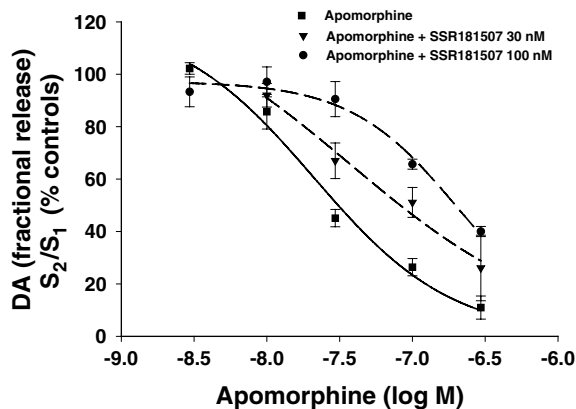


**Figure 2** Effects of SSR181507 (a) alone and on dopamine-stimulated [<sup>35</sup>S]GTPγS binding in CHO cells expressing the human recombinant DA D<sub>2</sub> receptor and (b) on the [<sup>35</sup>S]GTPγS binding in CHO cells expressing the human recombinant 5-HT<sub>1A</sub> receptor. (a) Binding of [<sup>35</sup>S]GTPγS (0.1 nM) to CHO cell membranes was measured after incubation with different concentrations of SSR181507 in the presence of 3 μM GDP (▲) and 3 μM dopamine (●). Data are representative results from four independent experiments. (b) Binding of [<sup>35</sup>S]GTPγS (0.1 nM) to CHO cell membranes was measured after incubation with different concentrations of compounds. Data are representative results from four independent experiments.

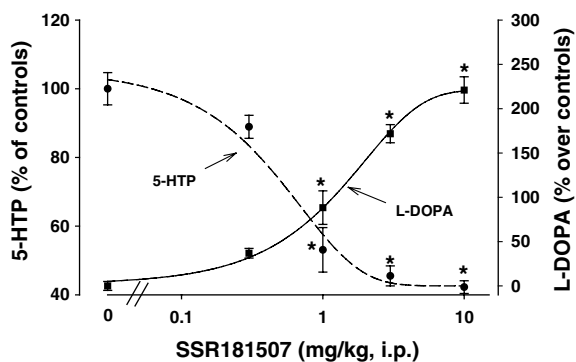
of 5-HTP in this same structure (ED<sub>50</sub> = 0.7 ± 0.3 mg/kg, i.p., Figure 4). Likewise, SSR181507 increased DA synthesis in the limbic system (ED<sub>50</sub> = 1.1 ± 0.1 mg/kg, i.p.) and decreased 5-HT synthesis in the hippocampus and cortex (ED<sub>50</sub> = 4.1 ± 3.1 and 1.1 ± 0.6 mg/kg, i.p., respectively). Under the same experimental conditions (results not shown), the 5-HT<sub>1A</sub> agonist 8-OH-DPAT (0.3 mg/kg, i.p.) decreased striatal 5-HT synthesis (47% of controls) without modifying DA synthesis (96% of controls) and the selective DA D<sub>2</sub> antagonist amisulpride (100 mg/kg, i.p.) only affected striatal DA synthesis (363% of controls vs 98% of controls for 5-HT synthesis). After repeated treatment (20 days at 3 or 10 mg/kg, i.p., b.i.d., followed by 72 h of withdrawal), SSR181507 (3 mg/kg, i.p.) induced changes in DA and 5-HT synthesis similar to those observed in animals who received vehicle chronically (Figure 5).

### Number of Spontaneously Active DA Cells

In the rat, acute administration of SSR181507 (0.1–3 mg/kg, i.p.) increased the number of spontaneously active DA cells



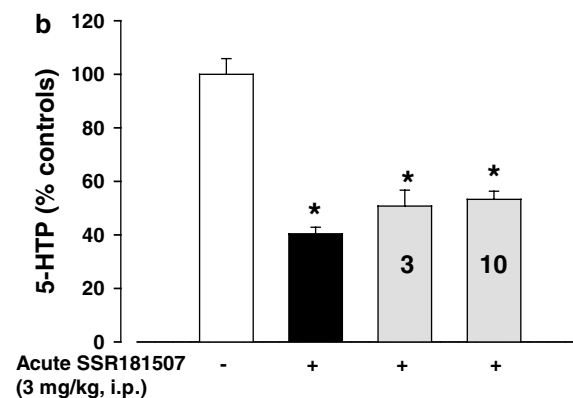
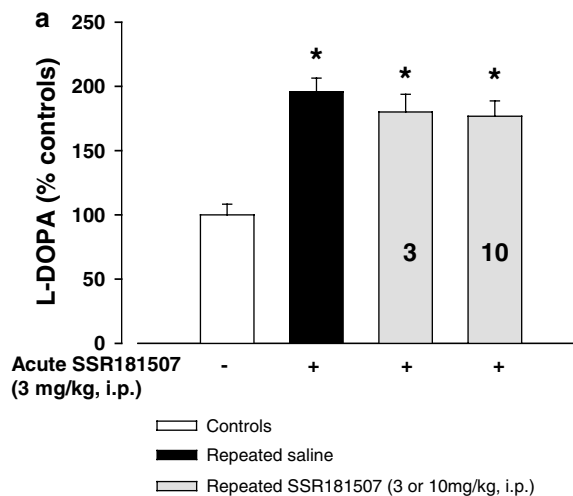
**Figure 3** Effect of SSR181507 on the apomorphine-induced inhibition of electrically evoked [<sup>3</sup>H]dopamine release from the rat striatum *in vitro*. The effect of two concentrations of SSR181507 on electrically evoked [<sup>3</sup>H]dopamine release was studied using slices from rat striatum. Slices were initially stimulated in the absence of apomorphine and SSR181507 and 40 min thereafter in their presence. Apparent pA<sub>2</sub> values were determined for each concentration of SSR181507 (30 and 100 nM) according to Furchgott (1972) as detailed in Materials and methods (average pA<sub>2</sub> = 7.94). Data shown are means with SEM of 3–13 replicates for each point on the curves.



**Figure 4** Effect of SSR181507 on DA and 5-HT synthesis in the rat striatum. Rats received NSD-1015 (100 mg/kg, i.p.) 30 min after the administration of SSR181507 (0.3–10 mg/kg, i.p.) or its vehicle control, and were killed 30 min thereafter. Results are expressed as percentage over the control group for L-DOPA and of the control group for 5-HTP and are the mean with SEM of data obtained from six animals per group. ED<sub>50</sub> values represent the estimated doses required to produce a half-maximal effect. (■) L-DOPA (ED<sub>50</sub> = 1.6 mg/kg; control value: 1008 ± 50 ng/g), (●) 5-HTP (ED<sub>50</sub> = 0.7 mg/kg; control value: 219 ± 10 ng/g). L-DOPA, F(4,25) = 47.7; 5-HTP, F(4,25) = 34.2, \*p < 0.05 compared with the respective control group (Dunnett's test following ANOVA).

per track in the A10 area but, up to 10 mg/kg, i.p., failed to affect this parameter in the A9 area. In this latter area, when WAY100635 (0.1 mg/kg, i.v.) was administered with an inactive dose of SSR181507 (3 mg/kg, i.p.), the number of active DA cells was increased (Figure 6a).

Following repeated administration (22 days, 3 mg/kg, i.p., o.d.), SSR181507 decreased the number of spontaneously active DA cells in the A10, but not in the A9 area, as compared to controls. The effect of the drug in the A10 area was reversed by an acute injection of apomorphine (63 μg/kg, i.v.), thus demonstrating the induction of depolarization block. The involvement of the 5-HT<sub>1A</sub> agonist property in

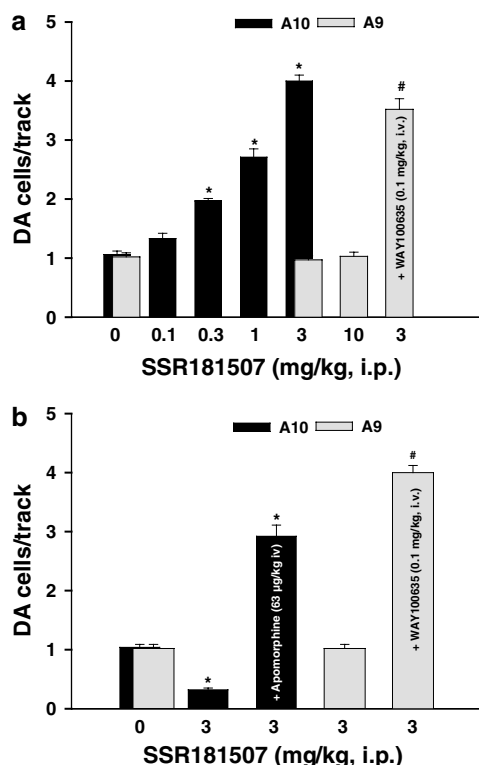


**Figure 5** Effect of repeated treatment with SSR181507 on DA and 5-HT synthesis in the rat striatum. Rats received NSD-1015 (100 mg/kg, i.p.) 30 min after the administration of SSR181507 or its vehicle control and were killed 30 min thereafter. SSR181507 was administered at a dose of 3 mg/kg, i.p. 72 h after the last chronic injection of vehicle control or SSR181507 (3 or 10 mg/kg, i.p., b.i.d. for 20 days). Results are expressed as percentage of the respective control group and are means with SEM of data obtained from eight animals per group. (a) L-DOPA (control value: 964 ± 80 ng/g), (b) 5-HTP (control value: 268 ± 17 ng/g); L-DOPA, F(3,27) = 14.2, 5-HTP, F(3,27) = 37.1, \*p < 0.05 compared with the respective control group (Dunnett's test following ANOVA).

this selective regional effect was demonstrated by the fact that the injection of WAY100635 (0.1 mg/kg, i.v.) with an inactive dose of SSR181507 (3 mg/kg/day, i.p.), induced an increase in the number of spontaneously active DA cells in the A9 area (Figure 6b).

#### Basal DA Efflux Measured by *In Vivo* Microdialysis

SSR181507 (3 mg/kg, i.p.) markedly increased extracellular DA levels in the nucleus accumbens (164 ± 11% of controls), but was devoid of effect in the striatum (Figure 7a). At 1 mg/kg, i.p., it also increased extracellular DA levels in the medial prefrontal cortex (153 ± 10% of controls, Figure 7b). Under similar experimental conditions, clozapine (10 mg/kg, i.p.) increased this parameter by 173 ± 25 and 164 ± 32% of controls in the nucleus accumbens and striatum, respectively (results not shown), and by 188 ± 14% of controls in the medial prefrontal cortex (Figure 7b).

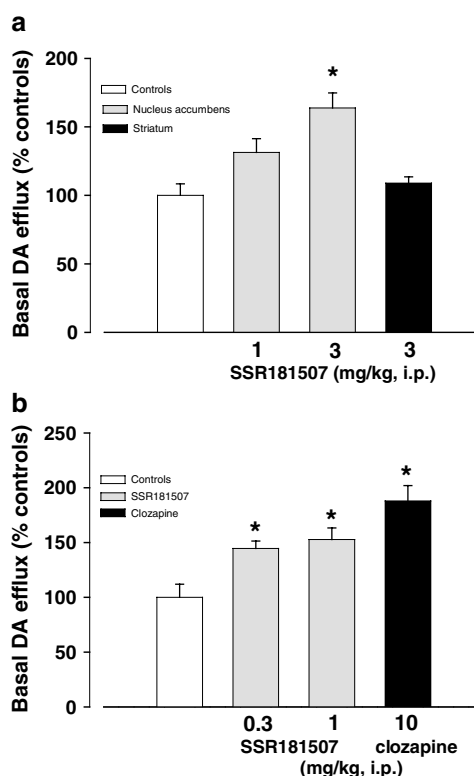


**Figure 6** Effect of acute and repeated treatment with SSR181507 on the number of spontaneously active DA cells in the A9 and A10 areas. The number of spontaneously active DA cells in the VTA (A10) and the SN (A9) was measured as described in Materials and methods. (a) Acute treatment: SSR181507 (0.1–10 mg/kg, i.p.) or vehicle were administered 45–60 min before initiation of the first electrode track. Results are mean with SEM of four to six animals by group.  $F(4,19) = 140.2$ ,  $*p < 0.05$ , SSR181507 compared with control;  $F(4,27) = 289.0$ ,  $#p < 0.05$ , SSR181507 with WAY100635 compared with SSR181507 (Dunnett's test following ANOVA). (b) Repeated treatment: SSR181507 (3 mg/kg, i.p.) or vehicle were administered once a day for 21 days. On the day of the experiment (24 h after the last chronic injection), SSR181507 or vehicle was administered. Results are means with SEM of four to six animals per group.  $F(5,23) = 30.0$ ,  $*p < 0.05$ , compared with respective control;  $F(2,8) = 402.1$ ,  $#p < 0.05$ , SSR181507 with WAY100635 compared with SSR181507 (Dunnett's test following ANOVA).

### Evoked CA Efflux Measured by *In Vivo* Electrochemistry

Electrical stimulation of the ascending dopaminergic pathway evoked immediate, reversible, and highly reproducible increases in DA efflux in the nucleus accumbens and CA efflux in the medial prefrontal cortex (Suaud-Chagny *et al*, 1992; Brun *et al*, 1995).

In the nucleus accumbens, haloperidol (50  $\mu$ g/kg, s.c.) significantly increased the evoked DA efflux to a maximum of  $114 \pm 21\%$  above predrug values (Figure 8a). Both doses of SSR181507 (1 and 3 mg/kg, i.p.) elicited a large, sustained, and significant increase in the evoked DA efflux (Figure 8a). This increase was larger with the highest dose of SSR181507 (maximum,  $106 \pm 22$  and  $444 \pm 53\%$  above predrug values for the lowest and highest dose of SSR181507, respectively). WAY100635 (1 mg/kg, i.p.) almost completely blocked the response induced by SSR181507 (3 mg/kg, i.p., Figure 8a). In the medial prefrontal cortex, haloperidol (50  $\mu$ g/kg, s.c.) and clozapine (10 mg/kg, s.c.) did not significantly modify the evoked CA efflux (Figure 8b). In contrast, both doses of



**Figure 7** Effect of SSR181507 on basal DA efflux (a) in the nucleus accumbens and striatum and (b) in the medial prefrontal cortex. Compounds were evaluated on basal DA efflux as described in Materials and methods. Results are expressed as percent of area under the curves between 0 and 120 min after compound administration and are means with SEM of data obtained from four to nine rats per group. (a) Nucleus accumbens  $F(2,10) = 9.9$ ,  $*p < 0.05$ , compared with control (Dunnett's test following ANOVA). (b) Medial prefrontal cortex, SSR181507  $F(2,17) = 7.0$ , and clozapine  $F(1,12) = 34.4$ ,  $*p < 0.05$ , compared with control (Dunnett's test following ANOVA).

SSR181507 (1 and 3 mg/kg, i.p.) elicited a significant increase in the evoked CA efflux (Figure 8b). Moreover, as observed in the nucleus accumbens, *post hoc* comparison indicated a significant difference ( $p < 0.05$ ) between both doses of SSR181507 in the medial prefrontal cortex (Figure 8b).

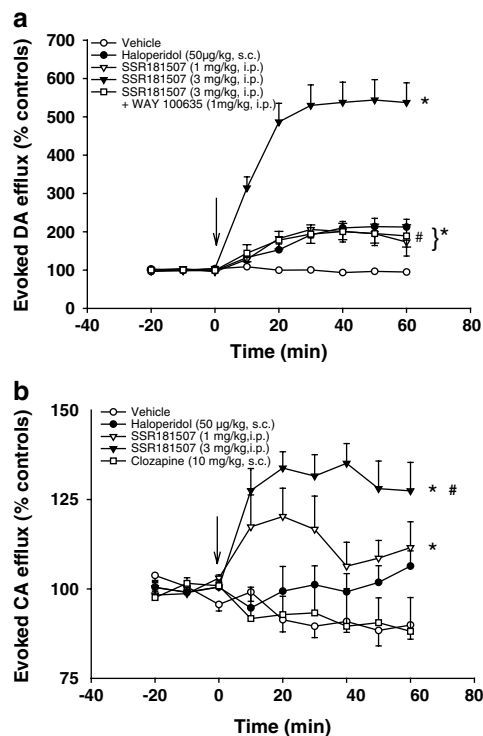
### *In Vivo* Recording of Dorsal Raphe Neuronal Firing

5-HT neurons present a slow (0.5–3 Hz) and regular firing rate and long-duration (0.8–2 ms) biphasic action potentials. SSR181507 induced a marked and dose-dependent inhibition of the neuronal firing rate in the rat DRN (0.03–0.3 mg/kg, i.v., Figure 9a). Treatment with WAY100635 (0.1 mg/kg, i.v.) reversed the SSR181507-induced inhibition of raphe neurons (Figure 9b). When administered alone, WAY100635 (0.1 mg/kg, i.v.) had no effect (not shown).

### Fos Immunoreactivity

In the rat, SSR181507 (1–10 mg/kg, i.p.), like clozapine (20 mg/kg, i.p.), selectively increased the number of Fos-positive cells in mesocorticolimbic areas (prefrontal cortex, nucleus accumbens shell, dorsomedial caudate putamen



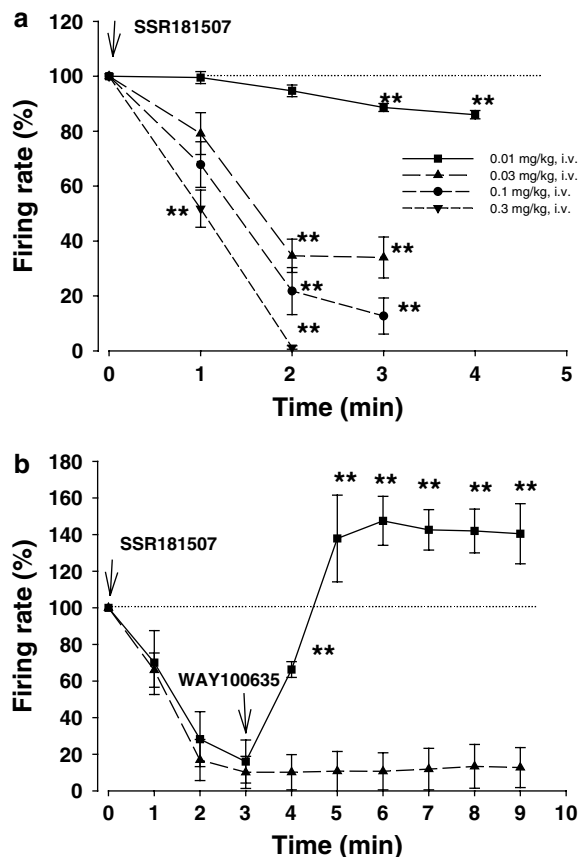


**Figure 8** Effect of SSR181507 on phasic DA efflux (a) in the nucleus accumbens and (b) in the medial prefrontal cortex. (a) Effect of the administration (arrow) of vehicle, haloperidol (50 µg/kg, s.c.), and SSR181507 (1 and 3 mg/kg, i.p.) on the electrically evoked DA efflux recorded by DPA in the nucleus accumbens. When present, WAY100635 was given 20 min before SSR181507. Stimulations of the medial forebrain bundle (20 Hz, 10 s) were repeated every 10 min. The amplitude of each effect is expressed as a percentage of the mean of the three responses to stimulation recorded before drug injection. Data represent means with SEM from four to seven experiments. \* $p < 0.05$  compared with vehicle-treated animals and # $p < 0.05$  compared with SSR181507 (3 mg/kg, i.p.) group (Fisher's PLSD test following ANOVA). (b) Effect of the administration (arrow) of vehicle, haloperidol (50 µg/kg, s.c.), SSR181507 (1 and 3 mg/kg, i.p.), and clozapine (10 mg/kg, s.c.) on the electrically evoked CA efflux recorded by DPA in the medial prefrontal cortex. The amplitude of each effect is expressed as a percentage of the mean of the three responses to stimulation recorded before drug injection. Data represent means with SEM from four to seven experiments. \* $p < 0.05$  compared with vehicle-treated animals and # $p < 0.05$  compared with a low dose of SSR181507 (1.0 mg/kg, i.p.)-treated animals (Fisher's PLSD test following ANOVA).

(CPu)) and did not alter or weakly affected this parameter in motor-related structures (nucleus accumbens core, dorsolateral CPu) (Figure 10). In contrast, haloperidol (0.5 mg/kg, i.p.) increased the number of Fos-positive cells to a similar extent in all these limbic and motor-related brain regions and failed to affect this parameter in the prefrontal cortex (Figure 10).

## DISCUSSION

We took advantage of the discovery of SSR181507 to test whether the combination in the same molecule of selective DA D<sub>2</sub> receptor antagonism and 5-HT<sub>1A</sub> agonist activity would result in a pharmacological and neurochemical profile characteristic of atypical antipsychotics. The present report and its companion paper dealing with the psycho-



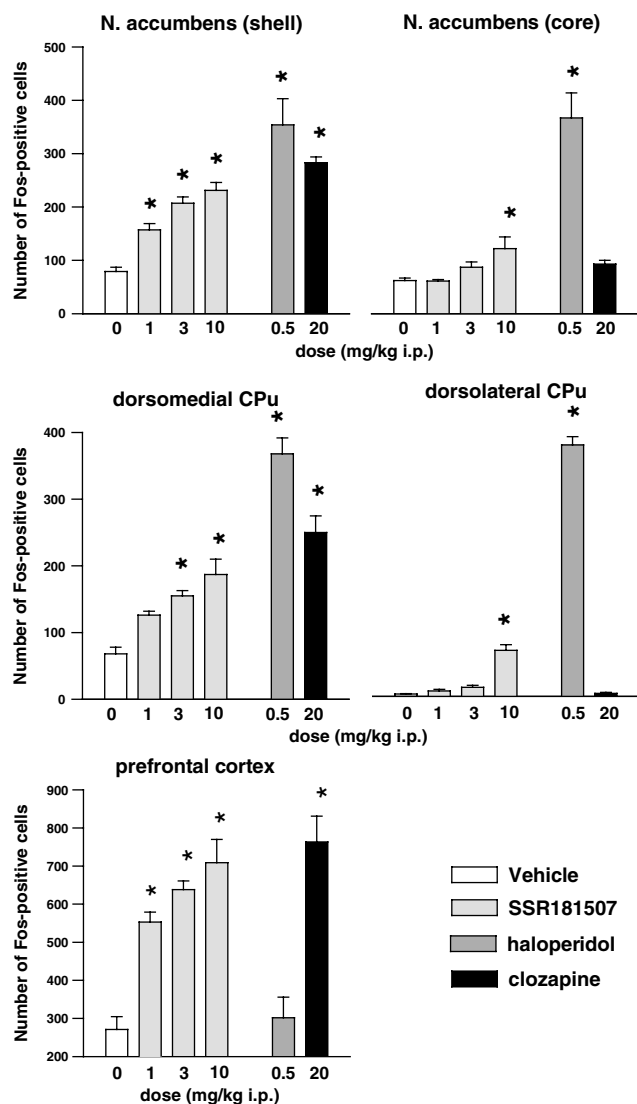
**Figure 9** Effect of SSR181507 on DRN firing in the rat brain. (a) Decrease in firing rate, expressed in percent of baseline activity, is plotted against the dose of SSR181507. Each curve is the mean with SEM of three to four independent determinations. SSR181507 0.01 mg/kg, i.v.,  $F(4,12) = 17.8$ ; 0.03 mg/kg, i.v.,  $F(3,6) = 51.7$ ; 0.1 mg/kg, i.v.,  $F(3,6) = 22.1$ ; 0.3 mg/kg, i.v.,  $F(2,4) = 156.5$ ; \* $p < 0.05$ , \*\* $p < 0.01$ , SSR181507 compared with control (Dunnett's test following ANOVA). (b) Antagonism by WAY100635 (0.1 mg/kg, i.v.) of the inhibition by SSR181507 (0.1 mg/kg, i.v.) of dorsal raphe cell firing. Points represent the mean  $\pm$  SEM for  $n = 3-4$  cells. (▲, SSR181507 alone; ■, SSR181507 with WAY100635).  $F(1,5) = 60.4$ , \*\* $p < 0.01$ , SSR181507 and WAY100635 compared with SSR181507 alone (Dunnett's test following ANOVA).

pharmacological profile of SSR181507 (Depoortere et al, 2003) provide strong evidence that this dual mechanism of action results in a drug profile compatible with an original, atypical antipsychotic activity.

## Selectivity for DA D<sub>2</sub> and 5-HT<sub>1A</sub> receptors

SSR181507 has a similar and high-nanomolar affinity for DA D<sub>2</sub> and D<sub>3</sub> receptors *in vitro*. It does not bind to DA D<sub>1</sub> and DA D<sub>5</sub> subtypes and in contrast to almost all atypical antipsychotics (clozapine, risperidone, olanzapine, ziprasidone), it has no appreciable affinity for the DA D<sub>4</sub> subtype (Miyamoto et al, 2000).

It also has a high affinity and selectivity for the 5-HT<sub>1A</sub> receptor subtype. Some atypical antipsychotics such as ziprasidone, iloperidone, and aripiprazole also display significant affinity for 5-HT<sub>1A</sub> receptor but in contrast to SSR181507, they also bind to other 5-HT receptor subtypes,



**Figure 10** Number of Fos-positive neurons after administration of vehicle, SSR181507 (1–10 mg/kg, i.p.), haloperidol (0.5 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.). Results were evaluated as described in Materials and methods and are mean with SEM of four to six animals per group. CPU: caudate putamen. \* $p < 0.05$  compared with vehicle-treated rats (Dunnett's test following ANOVA).

for example, the 5-HT<sub>2A</sub> subtype (Richelson and Souder, 2000; Jordan *et al.*, 2002).

SSR181507 also has no noticeable affinity for  $\alpha$ -adrenergic, muscarinic M<sub>1</sub>, and histamine H<sub>1</sub> receptors (that are thought to be involved in the autonomic and sedative side effects of antipsychotics).

Thus, *in vitro*, SSR181507 shows a unique profile in the sense that it selectively binds to the DA D<sub>2</sub> and 5-HT<sub>1A</sub> receptor subtypes with similar nanomolar affinity.

Our findings *in vivo* confirmed this binding profile. Thus, SSR181507 inhibits the *in vivo* binding of [<sup>3</sup>H]raclopride to DA D<sub>2</sub> receptors in the rat striatum and limbic system with a similar high potency. Moreover, SSR181507 fails to inhibit (ID<sub>50</sub> > 30 mg/kg, i.p.) the *in vivo* binding of [<sup>3</sup>H]spiperone to the 5-HT<sub>2</sub> receptor of the mouse frontal cortex and the *in vivo* binding of [<sup>3</sup>H]RS-79948 to the  $\alpha_2$  receptor in the mouse hypothalamus (results not shown).

## Intrinsic Activity at DA D<sub>2</sub> and 5-HT<sub>1A</sub> Receptors

The functional activity of SSR181507 at DA D<sub>2</sub> and 5-HT<sub>1A</sub> receptors was evaluated in the [<sup>35</sup>S]GTP $\gamma$ S-binding model. In this *in vitro* model, SSR181507 behaved as a potent antagonist at the DA D<sub>2</sub> receptor and had the profile of a full agonist or of a high-efficacy partial agonist at the 5-HT<sub>1A</sub> receptor. In contrast, ziprasidone, clozapine, and aripiprazole behave as partial agonists at cloned 5-HT<sub>1A</sub> receptors (Newman-Tancredi *et al.*, 1998; Jordan *et al.*, 2002). SSR181507 also antagonized rat presynaptic DA D<sub>2</sub> receptors, as demonstrated by the rightward shift by this drug of the concentration–response curve of DA agonist-induced decrease of [<sup>3</sup>H]dopamine release in rat striatal slices.

That SSR181507 blocks rat DA D<sub>2</sub> receptors *in vivo* was suggested by the fact that this drug increased striatal and limbic DA synthesis. As would be expected for a 5-HT<sub>1A</sub> agonist, SSR181507, in the same dose range, inhibited the synthesis of 5-HT in several rat brain areas, and its maximal effect was similar to that of 8-OH-DPAT, thus confirming that it behaves as a full 5-HT<sub>1A</sub> agonist on the serotonergic transmission. Electrophysiological data showing that SSR181507 decreased the firing rate of 5-HT neurons in the dorsal raphe are also compatible with activation of 5-HT<sub>1A</sub> receptors by this drug. This effect is mediated by selective activation of 5-HT<sub>1A</sub> receptors as revealed by its sensitivity to the 5-HT<sub>1A</sub> antagonist WAY100635. In this model, the total inhibition of firing rate at the dose of 0.3 mg/kg, i.v. is also in agreement with a full 5-HT<sub>1A</sub> agonist profile. However, it is known that the intrinsic activity is dependent on the experimental model. In the *in vitro* GTP $\gamma$ S model, it is highly dependent on the density of recombinant 5-HT<sub>1A</sub> receptors and in the two preceding *in vivo* models, it reflects the activity at presynaptic 5-HT<sub>1A</sub> receptors (Newman-Tancredi *et al.*, 1998). Interestingly, in an integrated functional model in rats involving 5-HT<sub>1A</sub> postsynaptic receptors (serotonergic syndrome), SSR181507 has the profile of a partial 5-HT<sub>1A</sub> agonist (Depoortere *et al.*, 2003). The effects of SSR181507 on DA and 5-HT synthesis were of long duration (at least 6 h, results not shown) and persisted after repeated treatment for 20 days, demonstrating that there is no development of tolerance.

## Mesocorticolimbic Selectivity and Atypical Antipsychotic Profile

The combination of DA D<sub>2</sub> receptor antagonism and 5-HT<sub>1A</sub> agonist effect was expected to confer on SSR181507 selectivity for mesocortical and mesolimbic dopaminergic activity, resulting in a pharmacological profile compatible with atypical antipsychotic properties (see Introduction).

Electrophysiological studies confirmed the limbic selectivity of SSR181507. Thus, acute treatment with SSR181507 resulted in an increased number of spontaneously active DA neurons in the VTA, but not in the SN. Repeated treatment with SSR181507 reduced the number of spontaneously active DA neurons in the VTA without modifying it in the SN. In this latter model, SSR181507 behaved like olanzapine, sertindole, and risperidone, which decrease the number of spontaneously active DA cells in the mesolimbic system only, but differed from quetiapine and ziprasidone

that also affect the nigrostriatal system (Skarsfeldt, 1995). This model of depolarization inactivation of DA neurons is generally considered to be of predictive value of the nature of antipsychotic compounds (Jones-Humble *et al*, 1996). The regional selectivity of SSR181507 could be due to its agonist activity at 5-HT<sub>1A</sub> receptors. Indeed, the capacity of SSR181507 to affect selectively the number of spontaneously active DA cells in the VTA disappeared in the presence of WAY100635. Moreover, the fact that this limbic selectivity was still present after a chronic treatment with SSR181507 confirms the lack of desensitization of 5-HT<sub>1A</sub> receptors.

The regional effect of antipsychotics on DA release has also been shown to highlight their typical or atypical profile (Nomikos *et al*, 1994; Volonté *et al*, 1997). The effect of SSR181507 on basal and evoked DA efflux in rat brain areas also supports the mesocorticolimbic selectivity of this compound. Thus, SSR181507 enhanced basal DA efflux in the nucleus accumbens and medial prefrontal cortex, but not in the striatum. A similar regional selectivity was previously found for the atypical antipsychotics ziprasidone, clozapine, olanzapine, and risperidone, while haloperidol increased DA efflux more markedly in the striatum than in the nucleus accumbens, and was inactive in the prefrontal cortex (Kuroki *et al*, 1999; Rollema *et al*, 2000). The limbic selectivity of SSR181507 is likely related to 5-HT<sub>1A</sub> receptor activation, as its cortical response is antagonized (results not shown) by a 5-HT<sub>1A</sub> antagonist (SL88.0338-08). Moreover 5-HT<sub>1A</sub> agonists are known to increase cortical DA efflux without affecting striatal DA efflux (Arborelius *et al*, 1993; Rollema *et al*, 2000). Ichikawa *et al* (2001) recently hypothesized that simultaneous blockade of 5-HT<sub>2A</sub> and DA D<sub>2</sub> receptors causes an increase in cortical DA efflux by facilitating 5-HT<sub>1A</sub> agonist activity by endogenous 5-HT. It may thus be suggested that, by directly activating 5-HT<sub>1A</sub> receptors, SSR181507 is functionally equivalent to atypical antipsychotics that indirectly achieve this effect.

The 5-HT<sub>1A</sub> agonist activity of SSR181507 may also account for its original neurochemical profile on evoked DA efflux, as its effect in the nucleus accumbens was antagonized by WAY100635. In the medial prefrontal cortex, in contrast to clozapine and haloperidol, SSR181507 dose-dependently increased evoked CA efflux. The site(s) at which 5-HT<sub>1A</sub> receptors control DA neuron activity is still a matter of debate. A direct effect on DA cell bodies seems to be excluded as neither 5-HT<sub>1A</sub> receptor protein nor mRNA have been detected in the VTA and SN (Miquel *et al*, 1991). Moreover, since DA efflux was evoked by electrical stimulation of the ascending dopaminergic pathway, activation of 5-HT<sub>1A</sub> autoreceptors present on DRN neurons afferent to the VTA cannot be invoked. As suggested by Sakaue *et al* (2000), 5-HT/DA interactions could take place directly in the medial prefrontal cortex, via the activation of 5-HT<sub>1A</sub> receptors located on postsynaptic neurons.

A disruption in the function of the prefrontal cortex ('hypofrontality') is thought to play a major role in the negative symptoms and cognitive deficit of schizophrenia. The robust increase in basal and evoked DA output in the medial prefrontal cortex induced by SSR181507 suggests that this drug may improve negative symptoms (Weinberger and

Lipska, 1995) and also cognitive-attentional symptoms of the pathology (Meltzer and McGurk, 1999).

We have shown that SSR181507 has a high affinity for the DA D<sub>3</sub> receptor subtype. However, complementary experiments have revealed that SSR181507 behaves as a partial agonist at this receptor (result not shown), thus suggesting that its antipsychotic profile can hardly be explained by an interaction with this receptor. Moreover, disappointing results regarding the behavioral phenotype of DA D<sub>3</sub> receptor knockout mice, and the lack of consistency in the behavioral effects of the different putative DA D<sub>3</sub> receptor ligands, have cast some doubt about the *in vivo* function of this receptor, and one step beyond, about its possible relevance in schizophrenia (Depoortere *et al*, 2002).

The immunohistochemistry of the Fos protein has been shown to be useful in mapping functional pathways in the central nervous system and especially in identifying brain areas that are targets for antipsychotics. Moreover, the ability of these drugs to increase Fos protein expression in the striatal complex has been considered useful in discriminating between typical and atypical antipsychotic compounds (Deutch *et al*, 1992; Robertson and Fibiger, 1992). In contrast to haloperidol, but like most atypical antipsychotics, SSR181507 did not affect Fos immunoreactivity in the regions implicated in the control of extrapyramidal motor function. However, like clozapine, it markedly enhanced the expression of Fos protein in mesocorticolimbic regions that are involved in the control of affective and motivational behaviors. In this respect, SSR181507 clearly behaves as an atypical antipsychotic. It is noticeable, however, that among atypical antipsychotics that similarly increase Fos expression in limbic areas, only some of them also enhance this expression in the medial prefrontal cortex (Deutch *et al*, 1992; Semba *et al*, 1996). Interestingly, it has been shown (Tremblay *et al*, 1998) that the 5-HT<sub>1A</sub> agonist 8-OH-DPAT is able to convert the 'typical' pattern of haloperidol on c-fos expression into a pattern resembling that of clozapine, suggesting that the original profile of Fos protein expression of SSR181507 may be due to its dual 5-HT<sub>1A</sub> agonist and DA D<sub>2</sub> antagonist properties.

## CONCLUSION

SSR181507 has a unique neurochemical and electrophysiological profile that is linked to its dual properties (DA D<sub>2</sub> antagonist and 5-HT<sub>1A</sub> agonist) expressed in the same dose range. Several advantages may be expected from such a profile and SSR181507 should have the features of an atypical antipsychotic compound. It should have efficacy against positive, and also negative and cognitive symptoms of schizophrenia and should lack the liability of extrapyramidal and other significant side effects. In addition, it should be efficacious on schizophrenia-associated mood and anxiety disorders. A companion paper dealing with the psychopharmacological profile of SSR181507 (Depoortere *et al*, 2003) adds further weight to these hypotheses.

## ACKNOWLEDGEMENTS

We thank N Brunel, G Danielou, and M Vinay for technical assistance.

## REFERENCES

- Alonso R, Voutsinos B, Fournier M, Labie C, Steinberg R, Souilhac J *et al* (1999). Blockade of cannabinoid receptors by SR141716 selectively increases fos expression in rat mesocorticolimbic areas via reduced dopamine D<sub>2</sub> function. *Neuroscience* **91**: 607–620.
- Arborelius LE, Nomikos GG, Hacksell U, Svenson TH (1993). (R)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex. *Acta Physiol Scand* **148**: 465–466.
- Bantick RA, Deakin JF, Grasby PM (2001). The 5-HT<sub>1A</sub> receptor in schizophrenia: a promising target for novel atypical neuroleptics? *J Psychopharmacol* **15**: 37–46.
- Bonhaus DW, Bach C, De Souza A, Salazar FHR, Matsuoka BD, Zuppan P *et al* (1995). The pharmacology and distribution of human 5-hydroxytryptamine<sub>2B</sub> (5-HT<sub>2B</sub>) receptor gene products: comparison with 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. *Br J Pharmacol* **115**: 622–628.
- Borison RL, Albrecht JW, Diamond BL (1990). Efficacy and safety of a putative anxiolytic agent: ipsapirone. *Psychopharmacol Bull* **26**: 207–210.
- Boyer P, Lecrubier Y, Puech AJ, Dewailly J, Aubin F (1995). Treatment of negative symptoms in schizophrenia with amisulpride. *Br J Psychiatry* **166**: 68–72.
- Briley M, Langer SZ (1978). Two binding sites for <sup>3</sup>H-spiroperidol on rat striatal membranes. *Eur J Pharmacol* **50**: 283–284.
- Brun P, Steinberg R, Le Fur G, Soubrié P (1995). Blockade of neurotensin receptor by SR 48692 potentiates the facilitatory effect of haloperidol on the evoked *in vivo* dopamine release in the rat nucleus accumbens. *J Neurochem* **64**: 2073–2079.
- Buckley NJ, Bonner TI, Buckley CM, Brann MR (1989). Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol Pharmacol* **35**: 469–476.
- Burnet PWJ, Eastwood SL, Harrison PJ (1996). 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor mRNAs and binding site densities are differentially altered in schizophrenia. *Neuropsychopharmacology* **15**: 442–455.
- Cheng Y, Prusoff WH (1973). Relationship between the inhibition constant (K<sub>i</sub>) and the concentration of the inhibitor which causes 50% inhibition (IC<sub>50</sub>) of an enzymatic reaction. *Biochem Pharmacol* **22**: 3099–3108.
- Dearry A, Gringrich JA, Falardeau P, Fremereau RT, Bates MD, Caron MG (1990). Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature* **347**: 72–76.
- Depoortere R, Boulay D, Perrault G, Bergis O, Decobert M, Françon D *et al* SSR181507, a dopamine D<sub>2</sub> receptor antagonist and 5-HT<sub>1A</sub> receptor agonist. II. Behavioral profile predictive of an atypical antipsychotic activity. *Neuropsychopharmacology*, 2003.
- Depoortere R, Boulay D, Perrault G, Sanger DJ (2002). Behavioral pharmacology of dopamine D<sub>2</sub> and D<sub>3</sub> receptors: use of the knock-out mice approach. In: Di Chiara G (ed) *Dopamine in the CNS II*. Springer: Heidelberg, pp 239–264.
- Deutch AY, Lee MC, Iadarola MJ (1992). Regionally specific effects of atypical antipsychotic drugs on striatal fos expression: the nucleus accumbens shell as a locus of antipsychotic action. *Mol Cell Neurosci* **3**: 332–341.
- Furchgott RF (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Blaschko H, Muscholl E (eds) *Handbook of Experimental Pharmacology* Vol. XXXIII Springer: Berlin, Heidelberg, New York, pp 283–335.
- Goff D, Midha K, Brotman A, McCormick S, Waites M, Amico E (1991). An open trial of buspirone added to neuroleptics in schizophrenic patients. *J Clin Psychopharmacol* **11**: 193–197.
- Grace AA, Bunney BS (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons 1. Identification and characterization. *Neuroscience* **10**: 301–315.
- Grandy DK, Marchionni MA, Makam H, Stofko RE, Alfano M, Frothingham L *et al* (1989). Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proc Natl Acad Sci USA* **86**: 9762–9766.
- Greengrass P, Bremner R (1979). Binding characteristics of 3H-prazosin to rat brain alpha-adrenergic receptors. *Eur J Pharmacol* **55**: 323–326.
- Haigler HJ, Aghajanian GK (1974). Lysergic acid diethylamide and serotonin: a comparison of effects on serotonergic neurons and neurons receiving a serotonergic inputs. *J Pharmacol Exp Ther* **188**: 688–699.
- Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O’Laughlin IA, Meltzer HY (2001). 5-HT<sub>2A</sub> and D2 receptor blockade increases cortical DA release via 5-HT<sub>1A</sub> receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J Neurochem* **76**: 1521–1531.
- Jones-Humble SA, Durcan MJ, Lyerly D, Norton RM, Tang FLM, Russell AV *et al* (1996). Preclinical neurochemical and electrophysiological profile of 1192U90 a potential antipsychotic. *Neuropsychopharmacology* **15**: 217–230.
- Jordan S, Koprivica V, Chen R, Tottori K, Kikuchi T, Altar CA (2002). The antipsychotic aripiprazole is a potent, partial agonist at the human 5-HT<sub>1A</sub> receptor. *Eur J Pharmacol* **441**: 137–140.
- Kapur S, Remington G (1996). Serotonin–dopamine interaction and its relevance to schizophrenia. *Am J Psychiatry* **153**: 466–476.
- Kasper S, Tauscher J, Willeit M, Stamenkovic M, Neumeister A, Kufferle B *et al* (2002). Receptor and transporter imaging studies in schizophrenia, depression, bulimia and Tourette’s disorder: implication for psychopharmacology. *World J Biol Psychiatry* **3**: 133–146.
- Kenny BA, Chalmers DH, Philpott PC, Naylor AM (1995). Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br J Pharmacol* **115**: 981–986.
- Köhler C, Hall H, Ogren SO, Gawell L (1985). Specific *in vitro* and *in vivo* binding of 3H-raclopride, a potent substituted benzamide drug with high affinity for dopamine D-2 receptors in the rat brain. *Biochem Pharmacol* **34**: 2251–2259.
- Koreen AR, Siris SG, Chakos M, Alvir J, Mayerhoff D, Lieberman J (1993). Depression in first-episode schizophrenia. *Am J Psychiatry* **150**: 1643–1648.
- Kuroki T, Meltzer HY, Ichikawa J (1999). Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J Pharmacol Exp Ther* **288**: 774–781.
- Lieberman RP, Corrigan PW (1992). Is schizophrenia a neurological disorder? *J Neuropsychiatry Clin Neurosci* **4**: 119–124.
- Martin GR, Humphrey PA (1994). Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacology* **33**: 261–273.
- Meltzer HY (1999). The role of serotonin in antipsychotic drug action. *Neuropharmacology* **21**: 1065–1155.
- Meltzer HY, McGurk SR (1999). The effects of clozapine, risperidone and olanzapine on cognitive function in schizophrenia. *Schizophr Bull* **25**: 233–255.
- Millan MJ (2000). Improving the treatment of schizophrenia: focus on serotonin (5-HT)<sub>1A</sub> receptors. *J Pharmacol Exp Ther* **295**: 853–861.
- Miquel MC, Doucet E, Boni C, El Mestikawy S, Mathiessen L, David G *et al* (1991). Central serotonin<sub>1A</sub> receptors: respective distributions of encoding mRNA, receptor protein and binding sites by *in situ* hybridization histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. *Neurochem Int* **19**: 453–465.
- Miyamoto S, Duncan G, Mailman R, Lieberman J (2000). Developing novel antipsychotic drugs: strategies and goals. *Curr Opin CPNS Invest Drugs* **2**: 25–39.

- Monsma FJ, Shen Y, Ward RP, Hamblin MW, Sibley DR (1993). Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol Pharmacol* **43**: 320–327.
- Newman-Tancredi A, Cussac D, Audinot V, Pasteau V, Cavaudan S, Millan MJ (1999). G protein activation by human dopamine D<sub>3</sub> receptors in high-expressing Chinese hamster ovary cells: a guanosine-5'-O-(3-[<sup>32</sup>S]thio)-triphosphate binding and antibody study. *Mol Pharmacol* **55**: 564–574.
- Newman-Tancredi A, Gavaudan S, Conte C, Chaput C, Touzard M, Verrielle L et al (1998). Agonist and antagonist actions of antipsychotic agents at 5-HT<sub>1A</sub> receptors. *Eur J Pharmacol* **355**: 245–256.
- Nomikos GG, Iurlo M, Andersson JL, Kimura K, Svensson TH (1994). Systemic administration of amperozide, a new atypical antipsychotic drug, preferentially increases dopamine release in the rat medial prefrontal cortex. *Psychopharmacology* **115**: 147–156.
- Paxinos G, Watson C (1998). *The Rat Brain in Stereotaxic Coordinates*. Academic Press: Sydney, Australia.
- Richelson E, Souder T (2000). Binding of antipsychotic drugs to human brain receptors. Focus on newer generation compounds. *Life Sci* **68**: 29–39.
- Robertson GS, Fibiger HC (1992). Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. *Neuroscience* **46**: 315–328.
- Robinson DS, Alms DR, Shrotriya RC, Messina M, Wickramarante P (1989). Serotonergic anxiolytics and treatment of depression. *Psychopathology* **22**: 27–36.
- Rollema H, Lu Yi, Schmidt AW, Sprouse JS, Zorn SH (2000). 5-HT<sub>1A</sub> receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. *Biol Psychiatry* **48**: 229–237.
- Sakaue M, Somboonthum P, Nishihara B, Koyama Y, Hashimoto H, Baba A et al (2000). Postsynaptic 5-hydroxytryptamine (1A) receptor activation increases *in vivo* dopamine release in rat prefrontal cortex. *Br J Pharmacol* **129**: 1028–1034.
- Sanger DJ, Schoemaker H (1992). Discriminative properties of 8-OH-DPAT. Relationship to affinity for 5-HT<sub>1A</sub> receptors. *Psychopharmacology* **108**: 85–92.
- Scatton B, Sanger DJ (2000). Pharmacological and molecular targets in the search for novel antipsychotics. *Behav Pharmacol* **11**: 242–256.
- Schoemaker H (1993). [<sup>3</sup>H]7-OH-DPAT labels both dopamine D<sub>3</sub> receptors and  $\sigma$  sites in the bovine caudate nucleus. *Eur J Pharmacol* **242**: R1–R2.
- Schoemaker H, Claustre Y, Fage D, Rouquier L, Chergui O, Curet O et al (1997). Neurochemical characteristics of amisulpride, an atypical dopamine D<sub>2</sub>/D<sub>3</sub> receptor antagonist with both pre-synaptic and limbic selectivity. *J Pharmacol Exp Ther* **280**: 83–97.
- Schoemaker H, Langer SZ (1986). [<sup>3</sup>H]8-OH-DPAT labels the serotonin transporter in the rat striatum. *Eur J Pharmacol* **124**: 371–373.
- Semba J, Sakai M, Miyoshi R, Mataga N, Fukamauch F, Kito S (1996). Differential expression of c-fos mRNA in rat prefrontal cortex, striatum, n. accumbens and lateral septum after typical and atypical antipsychotics: an *in situ* hybridization study. *Neurochem Int* **29**: 435–442.
- Shen Y, Monsma FJ, Metcalf MA, Jose PA, Hamblin MW, Sibley D (1993). Molecular cloning and expression of 5-hydroxytryptamine 7 serotonin receptor subtype. *J Biol Chem* **268**: 18200–18204.
- Sibley DR, Monsma FJ (1992). Molecular biology of dopamine receptors. *Trends Pharmacol Sci* **13**: 61–69.
- Simpson MDC, Lubman DJ, Slater P, Deakin JFW (1996). Autoradiography with [<sup>3</sup>H]8-OH-DPAT reveals increases in 5-HT<sub>1A</sub> receptors in ventral prefrontal cortex in schizophrenia. *Biol Psychiatry* **39**: 919–928.
- Skarsfeldt T (1995). Differential effects of repeated administration of novel antipsychotic drugs on the activity of midbrain dopamine neurons in the rat. *Eur J Pharmacol* **281**: 289–294.
- Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990). Molecular cloning and characterization of a novel dopamine receptor (D<sub>3</sub>) as a target for neuroleptics. *Nature* **347**: 146–151.
- Suaud-Chagny MF, Brun P, Buda M, Gonon F (1992). Fast *in vivo* monitoring of electrically evoked dopamine release by differential pulse amperometry with untreated carbon fibre electrodes. *J Neurosci Methods* **45**: 183–190.
- Sumiyoshi T, Matsui M, Nohara S, Yamashita I, Kurachi M, Sumiyoshi C et al (2001). Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *Am J Psychiatry* **158**: 1722–1725.
- Sumiyoshi T, Matsui M, Yamashita I, Nohara S, Uehara T, Kurachi M et al (2000). Effect of adjunctive treatment with serotonin-1A agonist tandospirone on memory functions in schizophrenia. *J Clin Psychopharmacol* **20**: 386–388.
- Tremblay PO, Gervais J, Rouillard C (1998). Modification of haloperidol-induced pattern of c-fos expression by serotonin agonists. *Eur J Neurosci* **10**: 3546–3555.
- Van Tol HHM, Bunzow JR, Guan HG, Sunahara RK, Seeman P, Niznik HB et al (1991). Cloning of the gene for a human dopamine D<sub>4</sub> receptor with high affinity for the antipsychotic clozapine. *Nature* **350**: 610–614.
- Volonté M, Monferini E, Cerutti M, Fodritto F, Borsini F (1997). BIMG 80, a novel potential antipsychotic drug: evidence for multireceptor actions and preferential release of dopamine in prefrontal cortex. *J Neurochem* **59**: 182–190.
- Wadenberg ML, Ahlenius S (1991). Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. *J Neural Transm [Gen Sect]* **83**: 43–53.
- Weinberger DR, Lipska BK (1995). Cortical maldevelopment, antipsychotic drugs and schizophrenia: a search for common ground. *Schizophr Rev* **16**: 87–110.
- White FJ, Wang RY (1983). Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopamine neurons. *Science* **221**: 1054–1057.