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SSR181507, A Dopamine D_2 Receptor Antagonist and 5-HT_{1A} Receptor Agonist. II: Behavioral Profile Predictive of an Atypical Antipsychotic Activity

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SSR181507 ((3-exo)-8-benzoyl-*N*-(((2*S*)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 that displays antagonist activity at dopamine D_2 receptors and agonist activity at 5-HT_{1A} receptors. SSR181507 antagonized apomorphine-induced climbing in mice and stereotypies in rats (ED_{50} of 2 and 3.4 mg/kg i.p., respectively) and blocked D-amphetamine-induced hyperlocomotion in rats at lower doses (0.3–1 mg/kg i.p.). At 1–10 mg/kg, it was found to disrupt active avoidance in mice. SSR181507 did not induce catalepsy in rats (MED > 60 mg/kg i.p.) and antagonized (3–10 mg/kg i.p.) haloperidol-induced catalepsy. SSR181507 was also active in two models sensitive to antidepressant/anxiolytic drugs: in a guinea-pig pup/mother separation test, it decreased (1–3 mg/kg i.p.) the time spent vocalizing during the separation episode, and in a lithium-induced taste aversion procedure in rats, it partially reversed (3 mg/kg i.p.) the decrease of intake of a saccharin solution. Furthermore, SSR181507 increased (3 mg/kg i.p.) the latency time to paradoxical sleep in rats, an effect commonly observed with antidepressants. Coadministration of the selective 5-HT_{1A} blocker SL88.0338 produced catalepsy and antagonized the effects of SSR181507 in the depression/anxiety tests, confirming the view that activation of 5-HT_{1A} receptors confers an atypical profile on SSR181507, and is responsible for its antidepressant/anxiolytic properties. Finally, SSR181507 (1–3 mg/kg) did not affect memory performance in a Morris water maze task in rats. The pharmacological profile of SSR181507 suggests that it should control the symptoms of schizophrenia, in the absence of extrapyramidal signs and cognitive deficits, with the additional benefit of antidepressant/anxiolytic activities.

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INTRODUCTION

Despite the therapeutic revolution that followed the introduction of chlorpromazine in the early 1950s, the first drug to show efficacy against schizophrenia, pharmacological treatment of this devastating condition has been far from optimal. Extrapyramidal signs (EPS), autonomic and endocrine side effects, and poor efficacy against negative symptoms and cognitive disturbances have considerably limited the therapeutic usefulness of the so-called 'typical' antipsychotics.

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Newer ('atypical') antipsychotics offer definite advantages over their predecessors, notably in terms of greatly reduced incidence of EPS. One of the hypotheses (but see Kapur and Remington, 2001, for alternatives) put forward to account for this characteristic is their preferential affinity for the serotonin 2 (5-HT_2) *vs* dopamine (DA) D_2 receptors (Meltzer, 1999). Indeed, early results in animal tests considered to be predictive of occurrence of EPS, suggested that 5-HT_2 receptor antagonists reversed catalepsy produced by haloperidol in rodents (Balsara *et al*, 1979) and diminished parkinsonism and dystonia in monkeys treated for extended periods with haloperidol (Korsgaard *et al*, 1985). However, not all subsequent studies confirmed these early results (see review by Kapur, 1996).

Preclinical data have suggested that agonist activity at another subtype of serotonergic receptor, the 5-HT_{1A} receptor, could offer an alternative approach for the control of EPS associated with blockade of DA D_2 receptors. 8-OH-DPAT, the prototypical 5-HT_{1A} receptor full agonist,

blocked catalepsy produced by haloperidol (Broekkamp *et al*, 1988; Invernizzi *et al*, 1988) and reduced dyskinesia in monkeys chronically treated with DA D_2 blockers (Christoffersen and Meltzer, 1998; Liebman *et al*, 1989). In the clinic, buspirone and tandospirone, two 5-HT_{1A} receptor partial agonists, have been shown to decrease parkinsonian signs or tardive dyskinesia in schizophrenic patients treated with antipsychotics (Goff *et al*, 1991; Moss *et al*, 1993; Yoshida *et al*, 1998). In addition, when added onto antipsychotics, tandospirone improved cognition in patients (Sumiyoshi *et al*, 2000, 2001).

A combination of DA D_2 antagonist and 5-HT_{1A} agonist activities might offer additional advantages. First, schizophrenic patients suffer from a high incidence of depression (Barnes *et al*, 1989). Serotonin is known to be implicated both in the etiology of depression and in the mechanism of action of antidepressants, and the 5-HT_{1A} receptor has become a major target for antidepressant activity (De Vry, 1995). Second, there is a relatively high prevalence of anxiety in schizophrenics, which translates into the prescription of anxiolytics in a substantial percentage of the patients (Buchanan *et al*, 2002). There are strong preclinical data implicating the 5-HT_{1A} receptor in the control of anxiety states (De Vry, 1995), together with the demonstrated efficacy of targeting this receptor in the clinic: buspirone and tandospirone (Tajima, 2001) are marketed as anxiolytics.

Altogether, these considerations have led to the hypothesis that a mixed 5-HT_{1A} agonist/DA D_2 antagonist could represent a new therapeutic approach for the treatment of schizophrenia (Ichikawa *et al*, 2001; and see Millan, 2000; Bantick *et al*, 2001, for reviews). In a companion paper (Claustre *et al*, 2003), we have shown that the novel tropanemethanamide benzodioxane SSR181507 is a potent and selective DA D_2 antagonist and 5-HT_{1A} agonist (K_i: 7.5 and 4.5 nM, for D_2 and 5-HT_{1A} rat receptors, respectively), with no appreciable affinity for a variety of other receptors, in particular 5-HT_{2A}, 5-HT_{2c}, adrenergic α_1 and α_2, histaminergic H_1 and muscarinic M_1. This compound also possesses unique neurochemical and electrophysiological profiles: it diminishes the activity of cerebral serotoninergic pathways and selectively facilitates central dopaminergic transmission in mesocortical as compared to nigro-striatal pathways. Moreover, like other atypical antipsychotics, SSR181507 selectively enhances the expression of Fos protein in mesocorticolimbic regions.

In the present study, we have assessed the behavioral profile of this compound in tests considered to be predictive of antipsychotic activity (apomorphine-induced climbing behavior in mice and stereotypies in rats, D-amphetamine-induced hyperlocomotion in rats and active avoidance in mice) and of occurrence of EPS (catalepsy in rats). Moreover, since activation of 5-HT_{1A} receptors has been associated with antidepressant/anxiolytic activities, we have also evaluated the potential of SSR181507 in tests sensitive to antidepressant/anxiolytic compounds (vocalization in guinea-pig pups separated from their mother, lithium-induced aversion for a saccharin solution in rats, and effects on paradoxical sleep (PS) in rats). The implication of 5-HT_{1A} receptors in these effects was evaluated by coadministration of SSR181507 with SL88.0338, an antagonist with high affinity ($K_i = 2.6$ nM) and selectivity (>100-fold) for the 5-HT_{1A} receptor. Finally, SSR181507 was evaluated in a model of spatial working memory (Morris water maze), as this type of memory is known to be affected by antipsychotics (Skarsfeldt, 1996).

MATERIALS AND METHODS

Animals

Experimental subjects were supplied by Iffa-Credo (Les Oncins, France) or Charles-River (St Aubin-les-Elbeuf, France) unless specified otherwise. Animals were kept in temperature- and humidity-controlled rooms (22°C, 50%) with lights on from 0700 to 1900, with water and food available *ad libitum*. 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.

Drugs

SSR181507 ((3-exo)-8-benzoyl-*N*-(((2*S*)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl)methyl)-8-azabicyclo(3.2.1)octane-3-methanamine monohydrochloride) and SL88.0338 ((4-((3,4-dihydro-5,8-dimethoxy-2(1H)-iso-quinolinyl)methyl)-1-(3-ethoxybenzoyl)-piperidine fumarate) were synthesized by the Chemistry Department of Sanofi-Synthelabo Recherche. Apomorphine hydrochloride, haloperidol, and 8-OH-DPAT were obtained from Sigma (St Louis, MS, USA). D-Amphetamine was obtained from Boyer (Paris, France). For i.p. and s.c. injections, drugs were diluted in saline with a few drops of Tween 80, with the exception of haloperidol (water + a few drops of 10% w/w of ascorbic acid, final pH: 3–4). Doses refer to the weight of the base, except for apomorphine. All drug solutions were prepared fresh daily and injected i.p. (20 ml/kg in mice, 2 or 5 ml/kg in rats, 5 ml/kg in guinea-pig pups), except apomorphine and 8-OH-DPAT (s.c. route, 5 ml/kg in rats, 10 ml/kg in mice). For p.o. administrations (60 min pretest), SSR181507 was suspended in distilled water with a few drops of Tween 80, and volumes were 5 ml/kg in rats.

Antagonism by SSR181507 of Locomotor Hyperactivity Induced by D-Amphetamine in Rats

Male Sprague–Dawley rats (180–220 g) were individually isolated in Perspex boxes and were then pretreated (i.p.) with vehicle or SSR181507 30 min before being injected with D-amphetamine (2 mg/kg i.p.). At 30 min following the D-amphetamine injection, they were placed in actimeters ($38 \times 38 \times 25$ cm high, Apelex, France) equipped with two perpendicular infrared beams 2 cm from the floor. Locomotor activity (number of interrupted infrared beams) was recorded for a period of 20 min after placing the rat in the actimeter. Data were analyzed with a one-way ANOVA, followed by Dunnett's *post hoc* tests.

Antagonism by SSR181507 of Stereotypies Induced by Apomorphine in Rats

Male Sprague–Dawley rats (180–220 g) were first isolated in Plexiglas cages ($25 \times 20 \times 14$ cm high) for 45 min before

being pretreated i.p. with SSR181507 or vehicle. After 30 min, they were injected with apomorphine (0.5 mg/kg s.c.), and were observed for 30 s at 10, 20, and 30 min postapomorphine injection for stereotypies quotation. Stereotypies were scored according to the following scale (adapted from Costall and Naylor, 1973):

0 sleeping
1 awake and behaving normally
2 head bobbing and/or grooming and/or locomotion
3 sniffing
4 licking cage walls
5 gnawing (vacuous chewing)

For each rat, a global score was calculated by averaging the three stereotypy scores obtained at 10-min intervals. Data were analyzed with a Kruskal–Wallis test, followed by Dunn's *post hoc* tests.

Antagonism by SSR181507 of Climbing Behavior and Hypothermia Induced by Apomorphine in Mice

Male CD1 mice (18–24 g) were first isolated in Perspex cages (21 × 9 × 9 cm high) for 45 min. They were then injected i.p. with vehicle or SSR181507 30 min before an injection of apomorphine (1 mg/kg s.c.). Immediately following the injection of apomorphine, mice were placed in a Plexiglas cylinder (15 cm high × 14 cm diameter) lined with mesh. At 15 min post-apomorphine injection, the time spent in a vertical position (2, 3, or 4 paws clinging to the mesh) was recorded for 1 min.

The rectal temperature was measured 30 min post-apomorphine injection using a thermal rectal probe (mouse model, Physitemp Instruments, Clifton, NJ, USA) connected to a digital thermometer (Micra-T, Française d'Instrumentation) with a precision reading of $\pm$ 0.1°C.

Data (time spent climbing and body temperature) were analyzed with one-way ANOVAs followed by Dunnett's *post hoc* tests.

Antagonism by SSR181507 of Active Avoidance Behavior in Mice

Male and female C57BL6J mice (25–30 g) were trained to avoid delivery of a foot-shock in a shuttle box (Med Associates, East Fairfield, VT, USA). Each shuttle box consisted of two compartments (20.5 cm × 16 cm × 24 cm high) separated by a motor-driven guillotine door, with a grid floor connected to a shock scrambler, and a stimulus light (conditioned stimulus: CS) located in each compartment. Following a period of 15 min of isolation in a Perspex box, each mouse was first placed into the right compartment of the shuttle box, with the CS off and the guillotine door lowered. After 2 min of habituation, the CS was turned on and the guillotine door was raised. If the mouse entered the opposite compartment within 15 s, the CS was turned off, the guillotine door was lowered, and an avoidance response was recorded. If the animal failed to move to the opposite compartment within this 15-s period, an electric shock (0.4 mA, sinusoidal 50 Hz current) was applied through the grid floor until the mouse entered the opposite compartment (escape response). If the animal failed to move to the opposite compartment at the end of the shock period (15 s), a response failure was recorded. Following either an avoidance, an escape, or a failure, the next cycle started with presentation of the CS in the side where the animal was present. A 30 s period of rest separated two consecutive cycles, and each session was limited to 40 cycles. All events were controlled and recorded by the Med-Associates software running on a PC.

After acquisition of the active avoidance task (criterion: average number of avoidance trials greater than 36 for three consecutive training sessions), mice were isolated for 15 min and pretreated i.p. with vehicle or SSR181507 and placed in the shuttle box 30 min postinjection. Each mouse was subjected to four i.p. injections (vehicle and three doses of the drug), administered in a pseudo-randomized (counterbalanced) order, with a minimum of 2 days between two consecutive treatments. Data (number of escape responses) were analyzed with a two-way ANOVA for repeated measures, with the treatment as the within factor, and the sex as the between factor, followed by Dunnett's *post hoc* tests.

To study the contribution of the 5-HT_{1A} receptor, a group of mice were pretreated with i.p. injections of vehicle or SL88.0338, 5 min before an injection of 10 mg/kg of SSR181507. The control condition consisted of vehicle/vehicle-treated mice injected at the appropriate times. At 30 min following the last injection, mice were placed in the shuttle boxes. Each mouse was subjected to five i.p. injections (vehicle/vehicle, vehicle/SSR181507, and vehicle plus one of three doses of SL88.0338), administered in a pseudo-randomized (counterbalanced) order, with a minimum of 2 days between two consecutive treatments. Data (number of escape trials) were analyzed with two-way ANOVAs for repeated measures, with the treatment as the within factor, and the sex as the between factor, followed by Dunnett's *post hoc* tests.

Induction by SSR181507 of the Serotonergic Syndrome in Rats, and Antagonism by SSR181507 of the Serotonergic Syndrome Produced by 8-OH-DPAT

Male Sprague–Dawley rats (180–220 g) were first individually isolated in Plexiglas cages for 45 min. They were then injected i.p. with vehicle or SSR181507, and observed for 30 s at 3, 6, 9, 12, and 15 min post-injection for occurrence of forepaw treading according to the following scoring scale:

0 no treading
1 occasional treading
2 frequent treading

For each rat, a global score was calculated by adding the individual scores observed at each of the five observation times.

At 30 min after the first injection, all rats were injected with 8-OH-DPAT (1 mg/kg s.c.), and observed for 30 s at 3, 6, 9, 12, and 15 min post-injection of 8-OH-DPAT for occurrence of forepaw treading using the same scale. Data (global scores) were subjected to a Kruskal–Wallis test, followed by Dunn's *post hoc* tests.

Assessment of the Cataleptogenic Effect of SSR181507

Male Sprague–Dawley rats (230–280 g) were grouped four to a Plexiglas cage and were injected i.p. with vehicle or SSR181507, or with 2 mg/kg haloperidol as a comparator. Catalepsy was assessed by placing the rat in a position where each paw rested on one of four wooden platforms (1.2 cm^2, 2.5 cm high, with 8 cm between contralateral platforms and 13 cm between ipsilateral platforms). Catalepsy was recorded 1, 2, and 4 h post-injection, as the time (in seconds, with a cutoff of 120 s) during which the rat stayed in this position.

Induction of Catalepsy in Rats by Cotreatment with SSR181507 and the 5-HT_{1A} Receptor Antagonist SL88.0338

Male Sprague–Dawley rats (230–280 g) were grouped four to a Plexiglas cage and were pretreated i.p. with vehicle or SL88.0338, followed 30 min later by an i.p. injection of 30 mg/kg of SSR181507. Catalepsy was recorded 1 and 2 h after the second i.p. injection with the method described in the preceding paragraph. Data were subjected to a Kruskal–Wallis test, followed by Dunn's *post hoc* tests.

Antagonism by SSR181507 of Catalepsy Induced by Haloperidol in Rats, and its Reversal by Cotreatment with the 5-HT_{1A} Receptor Antagonist SL88.0338

Male Sprague–Dawley rats (230–280 g) were grouped four to a Plexiglas cages and were pretreated i.p. with 2 mg/kg haloperidol, followed 30 min by an i.p. injection of vehicle or SSR181507. Catalepsy was recorded 1 and 2 h following the last injection.

In order to investigate the role of the 5-HT_{1A} receptor in the antagonism of haloperidol-induced catalepsy by SSR181507, the following procedure was followed: rats were injected i.p. with 2 mg/kg haloperidol. After 30 min, they were given two successive injections of vehicle or SSR181507 (3 mg/kg), and vehicle or SL88.0338 (1 or 3 mg/kg) (see Figure 6b for more details). Catalepsy was recorded 1 and 2 h after the double i.p. treatment with the method described in the preceding paragraph.

For both experiments, data were analyzed with a Kruskal–Wallis test, followed by Dunn's *post hoc* tests.

Antagonism of Vocalization Induced by Separation from the Mother in Guinea-Pig Pups, and its Reversal by Cotreatment with the 5-HT_{1A} Receptor Antagonist SL88.0338

Hartley guinea-pig pups (9-day-old at the start of the experiment, male and female, mothers obtained from Harlan, Horst, The Netherlands) were first subjected to two pretest sessions (Monday and Wednesday): 30 min following an i.p. injection with vehicle, pups were isolated for 5 min in a Macrolon box (30 × 19.5 × 14 cm) placed in a sound-attenuated chamber with a white masking noise and a white light, after which they were returned to their mother. The time spent vocalizing was recorded and pups emitting vocalization for at least 120 s during at least one of the two pretest sessions were retained for the pharmacological test sessions (Friday, then Monday and Thursday of the following week). Pups who spent less than 120 s vocalizing during either the first or second pretest sessions were subjected to a third session on Friday (consequently, these pups, provided they reached the criterion on this third pretest session, had only two pharmacological challenges, on Monday and Thursday of the following week). Hence, each selected pup was subjected to two or three treatments (vehicle and one or two doses of SSR181507) administered i.p. 30 min before a pharmacological test session. The time spent by the pup in vocalization was recorded, with a cutoff of 5 min.

Implication of 5-HT_{1A} receptors in the effects of SSR181507 was assessed by pretreating pups i.p. 60 min presession with vehicle or SL88.0338 (3 or 10 mg/kg). After 30 min, they were injected with 3 mg/kg of SSR181507.

For all experiments, data were analyzed with one-way ANOVAs, followed by Dunnett's tests for *post hoc* comparisons.

Effects of SSR181507, Alone, or in Association with the 5-HT_{1A} Receptor Antagonist SL88.0338, on the Cortical Electroencephalogram (EcoG) in Rats

Male Sprague–Dawley rats (240–260 g) were anesthetized with sodium pentobarbital (75 mg/kg i.p.) and mounted in a stereotaxic apparatus. Cortical electrodes (stainless-steel screws, 0.9 mm diameter) were affixed to the bone over the sensorimotor cortex (1.5 mm lateral to the median suture and 1.5 mm behind the fronto-parietal suture), the visual cortex (1.5 mm lateral to the median suture and 1.5 mm in front of the parieto-occipital suture), and the cerebellum (reference electrode). Cortical electrodes were attached to a connector and fixed with dental cement to the cranium.

After 3 weeks of postoperative recovery, rats were placed in Plexiglas cylinders (60 cm diameter) with free access to food and water. Recording sessions took place from 1100 to 1700 during 3 consecutive days (control day 1, drug day, and control day 3).

EcoG activity in sensorimotor and visual cortices was recorded with the cerebellar electrode as the reference. Three stages were differentiated: wakefulness (W: characterized by low-voltage EcoG activity), slow wave sleep (SWS: characterized by an increase in EcoG activity, that is, high-amplitude slow waves with some bursts of sleep spindles), and PS (characterized by hypersynchronization of the theta rhythm in the visual area). Analysis of the ECoG signal was performed automatically by means of a computerized system discriminating between the various sleep phases using spectral frequency analysis (Coherence, Deltamed, Paris, France). SSR181507 (3 mg/kg, day 2) or vehicle (days 1 and 2) was administered i.p. 15 min before recording; SL88.0338 or vehicle was administered simultaneously with SSR181507. The effects of SSR181507 on the time spent in wakefulness, in SWS, in PS, and the latency time to enter PS were analyzed over a 6-h period and were expressed as the percentage of the control values obtained on day 1. Statistical analysis was carried out using one-way ANOVAs for repeated measures, followed by Dunnett's *post hoc* tests.

Antagonism of Lithium-Induced Aversion Towards a Saccharin Solution in Rats, and its Reversal by Cotreatment with the 5-HT_{1A} Receptor Antagonist SL88.0338

This model described by Ervin and Cooper (1988) requires 10 test days. Rats (Sprague–Dawley, male 200–250 g) were isolated in single cages with food and water *ad libitum*. At 24 h before the start of the experiment, rats were deprived of water. On test day 1, tap water was available for 1 h. On test day 2, tap water was available for 30 min. On test days 3, 4, 5, 6, 7, and 9, tap water was available for 15 min. After the drinking on test day 5, rats were allowed water *ad libitum* for 24 h. This interval between test days 5 and 6 allowed rats to rehydrate. Rats were then deprived of water for 24 h before being offered water on test day 7 (15 min). On test day 8, rats were weighed at 0800 and saccharin (0.25% w/v), instead of tap water, was made available for 15 min at the usual drinking time (1100). At 15 min after the saccharin drinking period, rats received an i.p. injection of vehicle or lithium chloride (30 mg/kg). On test day 10, rats were weighed at 0800 and received an i.p. injection of SSR181507 or vehicle, 30 min before being presented saccharin. The quantity of saccharin drunk during the 15-min period was recorded with a precision of 0.1 ml.

In a separate experiment, rats where subjected to the same protocol as that described above, except for day 10: rats were first injected i.p. with vehicle or SL88.0338, 60 min pretest, followed by an i.p. injection of vehicle or SSR181507, 30 min pretest (see Figure 9b for details of drug and doses combinations).

Data were analyzed by means of a Kruskal–Wallis test, followed by Dunn's *post hoc* tests.

Effects of SSR181507 on the Morris Water Maze Task in Rats

Male Wistar rats (240–260 g) were housed five per cage. The Morris water maze consisted in a gray PVC circular tank (150 cm diameter × 60 cm high), filled (35 cm high) with warm water (28 ± 2°C), to which 5 l of milk were added in order to make the water opaque. A square Plexiglas platform (12 cm side) was placed 2 cm underneath the water level, 10 cm from the wall of the tank, at one of four possible cardinal locations (NW, NE, SW, SE). The behavior and swimming pathways were automatically recorded for each rat via a video tracking system (VIDEOTRACK, View Point, France). The experiment consisted of four daily learning sessions. For each session, four consecutive trials were performed, with a 30-s intertrial interval. The location of the platform was the same for the four trials within a session, but changed in a pseudo-randomized order between consecutive sessions. At the beginning of a trial, the rat was gently placed into the tank, at one of the start points, facing the center of the maze. For each trial within a session, a different start point was used. The time limit to find the platform was set at 120 s. Once on the platform, the rat was left for 30 s (intertrial interval), and then the next trial occurred. The time to reach the platform was recorded with a precision of 0.1 s for each trial.

SSR181507 or vehicle was injected i.p. 30 min before the start of each session. Data (times to reach the platform, in seconds) were analyzed with three-way ANOVAs, with the treatment as the between factor, and the session and trial as the within factors.

RESULTS

Antagonism by SSR181507 of Locomotor Hyperactivity Induced by D-Amphetamine in Rats

With respect to controls, D-amphetamine produced a 120% increase in the number of interrupted infrared beams (from 281 to 617; Figure 1). SSR181507 dose-dependently antagonized this D-amphetamine-induced hypermotility, with the level of motility at 1 mg/kg being close to that obtained in vehicle-injected animals. This was confirmed by the statistical analysis, with Dunnett's *post hoc* tests (following a global effect: $F(4,55) = 8.91$, $P < 0.001$) showing that both the vehicle group and the D-amphetamine + SSR181507 (1 mg/kg) group were significantly different from the D-amphetamine group. The dose of 1 mg/kg that nearly completely antagonized D-amphetamine hyperactivity was below the ED_{50} (1.5 ± 0.3 mg/kg i.p.) for antagonism of spontaneous locomotor activity, suggesting that this antagonism was not due to a nonspecific motor effect.

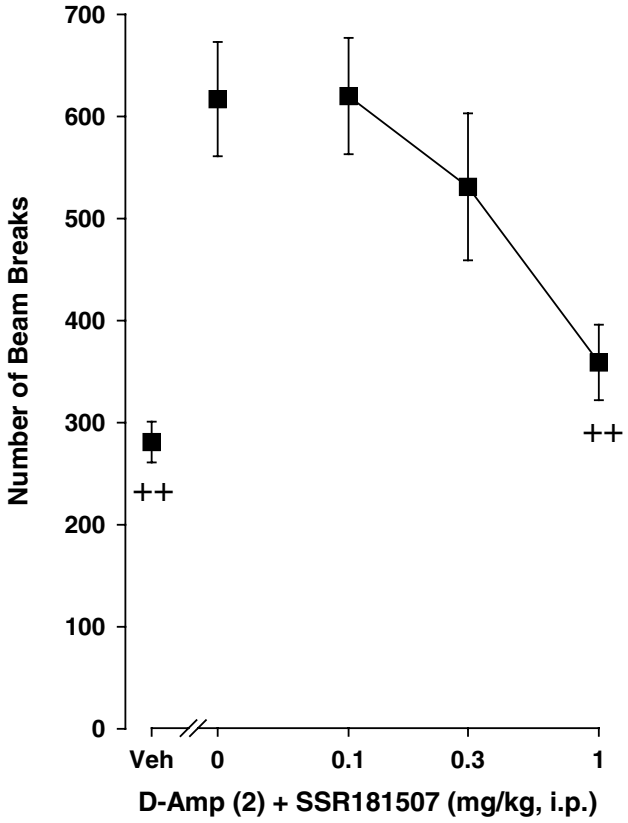

Figure 1 Reversal of D-amphetamine-induced hyperactivity by SSR181507. Each symbol represents the average (± SEM) number of infrared beam interruptions recorded for 20 min, 30 min following an i.p. injection of D-amphetamine (Amp: 2 mg/kg i.p.). SSR181507 or vehicle (Veh) was injected 30 min before D-amphetamine. $^{++}P < 0.01$, compared to the D-amphetamine group. $N = 12$ rats per group.

Antagonism of Stereotypies Induced by Apomorphine in Rats

SSR181507 decreased ($H = 26.78$, df = 4, $P < 0.0001$) the score of stereotypies induced by apomorphine in a dose-dependent way ($ED_{50} = 3.4$ mg/kg i.p., Figure 2a). At 6 mg/kg, SSR181507 reduced the stereotypy score to about 1, which corresponds to 'awake and normally behaving' according to the scale used. SSR181507 was also found to be active following p.o. administration ($ED_{50} = 11.7$ mg/kg), indicating that the compound has good oral bioavailability.

Antagonism of Climbing Behavior and Hypothermia Induced by Apomorphine in Mice

SSR181507 dose-dependently reduced ($F(5,42) = 37.56$, $P < 0.001$) the time spent in a climbing position by mice injected with apomorphine ($ED_{50} = 2$ mg/kg i.p., Figure 2b, square symbols). From the dose of 6 mg/kg, SSR181507 completely blocked the effects of apomorphine, as the time spent climbing was reduced to 0 s.

In the same mice, the hypothermia (drop of core temperature of 4.5°C) produced by apomorphine was significantly potentiated by SSR181507 at 3 mg/kg i.p. (Dunnett's *post hoc* tests with the apomorphine group as the comparator, following a global effect : $F(6,49) = 18.04$, $P < 0.001$). Doses below and above 3 mg/kg were without significant effects (Figure 2b: round symbols). This potentiation at some doses of SSR181507 of apomorphine-induced hypothermia was hypothesized to be due to hypothermic effects of SSR181507 itself. Indeed, in a separate experiment, SSR181507 was found to decrease core temperature significantly (−0.2, −0.9, and −1.0°C with respect to controls, at 1, 3, and 10 mg/kg, 30 min post i.p. injection ($F(4,30) = 8.36$, $P < 0.001$)). Further, this hypothermia was probably due to activity at 5-HT_{1A} receptors, as 3 mg/kg i.p. of the 5-HT_{1A} receptor antagonist SL88.0338 could reverse these drops in core temperature (data not shown).

Effects of SSR181507 on Active Avoidance Behavior in Mice

Two-way ANOVAs showed that there was neither a sex nor a sex × treatment interaction (all F's < 2.02, all P's > 0.05) for the number of escape responses. As a consequence, males and females were pooled, and data were analyzed by means of a one-way ANOVA with treatment as the within factor.

SSR181507 produced a dose-dependent increase ($F(3,33) = 41.39$, $P < 0.001$) in the number of escape

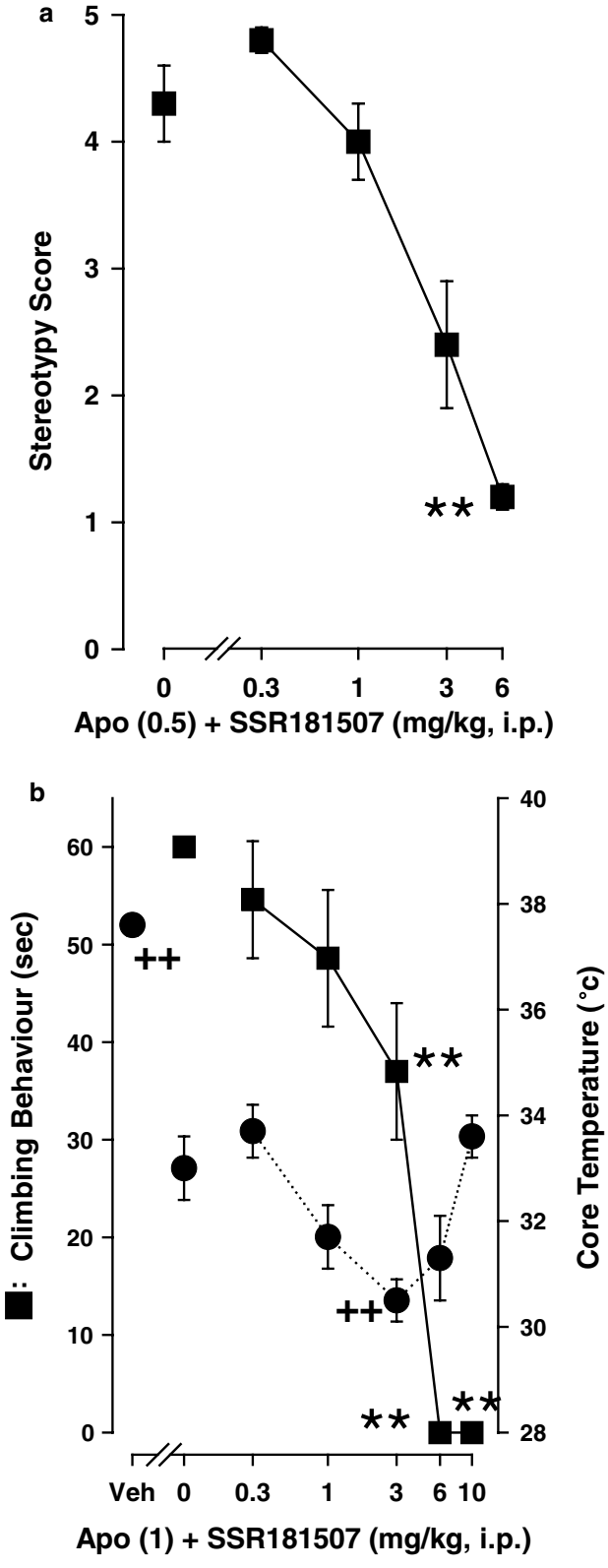

Figure 2 (a) Antagonism by SSR181507 of stereotypies induced by apomorphine in rats. (b) Effects of SSR181507 on climbing behavior and on hypothermia induced by apomorphine in mice. (a) Each symbol represents the average stereotypy score (± SEM) obtained from three scores recorded for 30 s at 10, 20, and 30 min post-apomorphine injection (0.5 mg/kg s.c.). Vehicle or SSR181507 was injected 30 min before the apomorphine challenge. $^{**}P < 0.01$, compared to the vehicle group. $N = 8$ rats per group. (b) Each symbol represents the mean (± SEM) amount of time spent in a climbing position (squares) or the mean core temperature (circles) induced by 1 mg/kg s.c. apomorphine. Mice were injected i.p. with vehicle or SSR181507 30 min before apomorphine. At 15 min post-apomorphine injection, the time spent in the vertical position (two, three, or four paws clinging to the mesh of the observation cylinder) was recorded for 1 min. $^{**}P < 0.01$, compared to the vehicle group, for climbing behavior, $^{++}P < 0.01$, compared to the apomorphine group, for hypothermia. $N = 8$ mice per group.

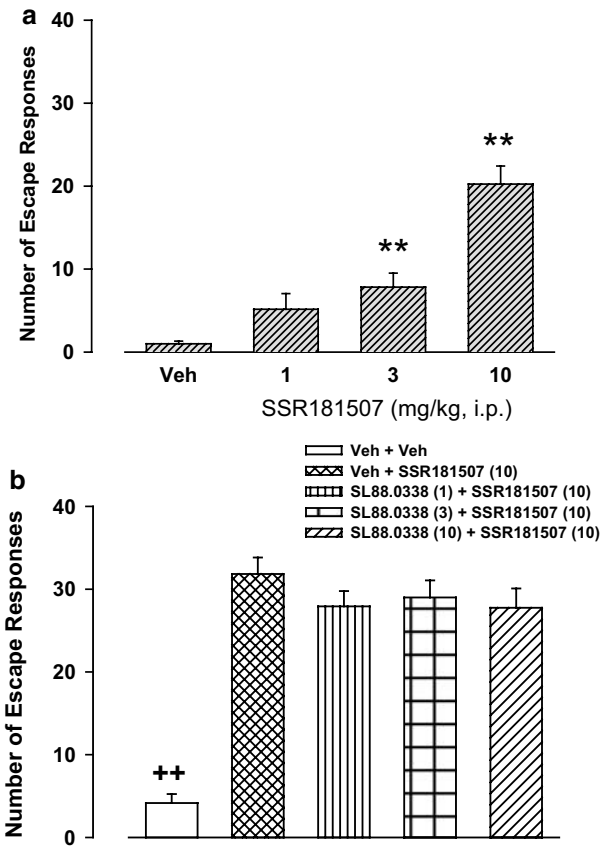

Figure 3 (a) Antagonism by SSR181507 of active avoidance behavior in mice. (b) Lack of effects of pretreatment with the 5-HT_{1A} receptor antagonist SL88.0338 on the antagonism by SSR181507 of active avoidance behavior in mice. (a) Each bar represents the average (+SEM) number of escape responses (out of a maximum of 40) recorded during a single session. $^{**}P<0.01$, compared to the vehicle group. (b) See legend of top panel for details. $^{++}P<0.01$, compared to the vehicle + SSR181507 group. For both panels, $N=12$ mice per group.

responses (Figure 3a), with 10 mg/kg i.p. increasing the average number of escape responses from control values of about 1 to ca 20. SSR181507 appeared to disrupt avoidance behavior selectively, as the increase in the number of escape responses was seen in the virtual absence of escape failures (one mouse had one failure at 10 mg/kg). Furthermore, escape latencies were not significantly affected by the treatment ($F(3,18)=2.23$, $P>0.05$), suggesting that the disruption of avoidance response was not a consequence of a motor side effect of SSR181507 (average escape latencies in seconds, $\pm$ SEM, for vehicle, 1, 3, and 10 mg/kg of SSR181507 were: 0.8 ± 0.3, 1.6 ± 0.5, 1.9 ± 0.7, and 1.1 ± 0.2).

Pretreatment with 1, 3, or 10 mg/kg of the 5-HT_{1A} receptor antagonist SL88.0338 did not modify the ability of 10 mg/kg i.p. of SSR181507 to augment the number of escape responses significantly (Figure 3b). This was supported by Dunnett's *post hoc* tests (following a global effect: $(F(4,44)=58.45$, $P<0.001)$ comparing the group treated with SSR181507 alone *vs* groups cotreated with SSR181507 and various doses of SL88.0338.

Lack of Induction by SSR181507 of the Serotonergic Syndrome in Rats, and Antagonism by SSR181507 of the Serotonergic Syndrome Produced by 8-OH-DPAT

Compared to the 5-HT_{1A} receptor full agonist 8-OH-DPAT, SSR181507 produced minimal forepaw treading (Figure 4a): even at the highest dose tested (30 mg/kg), the score obtained with SSR181507 was less than a third (about 3 *vs* 10) of that observed with the control group injected with 1 mg/kg of the full 5-HT_{1A} receptor agonist 8-OH-DPAT (see square symbol at the upper left corner in Figure 4b).

SSR181507 was able to dose-dependently ($H=20.77$, $df=4$, $P<0.001$) reduce the forepaw treading score recorded with 1 mg/kg 8-OH-DPAT (Figure 4b). The decrease was found to be significant at doses of 10 mg/kg and above.

Lack of Cataleptogenic Effects of SSR181507, but Induction of Catalepsy by Cotreatment with SSR181507 and the 5-HT_{1A} Receptor Antagonist SL88.0338

SSR181507, up to a dose of 60 mg/kg i.p., did not produce notable catalepsy in rats, whether tested 1, 2, or 4 h postinjection (Figure 5a). By contrast, rats treated with 2 mg/kg of haloperidol spent close to the maximum of 120 s in a cataleptic position (Figure 5a, open bars).

However, when rats were pretreated with the 5-HT_{1A} receptor antagonist SL88.0338 (10 and 30 mg/kg i.p.) before an injection of 30 mg/kg of SSR181507, the time spent in a cataleptic position was significantly increased ($H=11.0$, $df=2$, $P<0.01$ and $H=11.75$, $df=2$, $P<0.01$, at 1 and 2 h following the injection of SSR181507, respectively) with respect to a vehicle pretreatment (Figure 5b). By itself, SL88.0338 was found to be devoid of cataleptogenic activity (at 1 and 2 h post treatment, for 10 and 30 mg/kg i.p., times spent in a cataleptic position were comprised between 2.4 ± 1.1 and 6.1 ± 2.3 s, $n=7$–8 rats per dose).

Antagonism by SSR181507 of Catalepsy Induced by Haloperidol in Rats, and its Reversal by Cotreatment with the 5-HT_{1A} Receptor Antagonist SL88.0338

The catalepsy produced by 2 mg/kg of haloperidol was dose-dependently antagonized 1 h ($H=15.33$, $df=3$, $P<0.01$; $ED_{50}=0.9$ mg/kg) and 2 h ($H=15.73$, $df=3$, $P<0.01$; $ED_{50}=1.7$ mg/kg) following an i.p. injection of SSR181507. At 6 mg/kg, SSR181507 reduced the time spent in catalepsy by about 85% (Figure 6a). SSR181507 was also found to be active following p.o. administration ($ED_{50}=2.1$ mg/kg at 2 h).

Post hoc analysis following a global treatment effect ($H=17.03$, $df=3$, $P<0.001$) showed that the antagonism of haloperidol-induced catalepsy seen with 3 mg/kg of SSR181507, at 1 h post treatment, was reversed with 3 mg/kg, but not 1 mg/kg of SL88.0338 (Figure 6b).

Antagonism by SSR181507 of Vocalization Induced by Separation from the Mother in Guinea-Pig Pups, and its Reversal by Pretreatment with the 5-HT_{1A} Receptor Antagonist SL88.0338

Separation of guinea-pig pups from their mother produced vocalization that lasted on average 178 s in the control

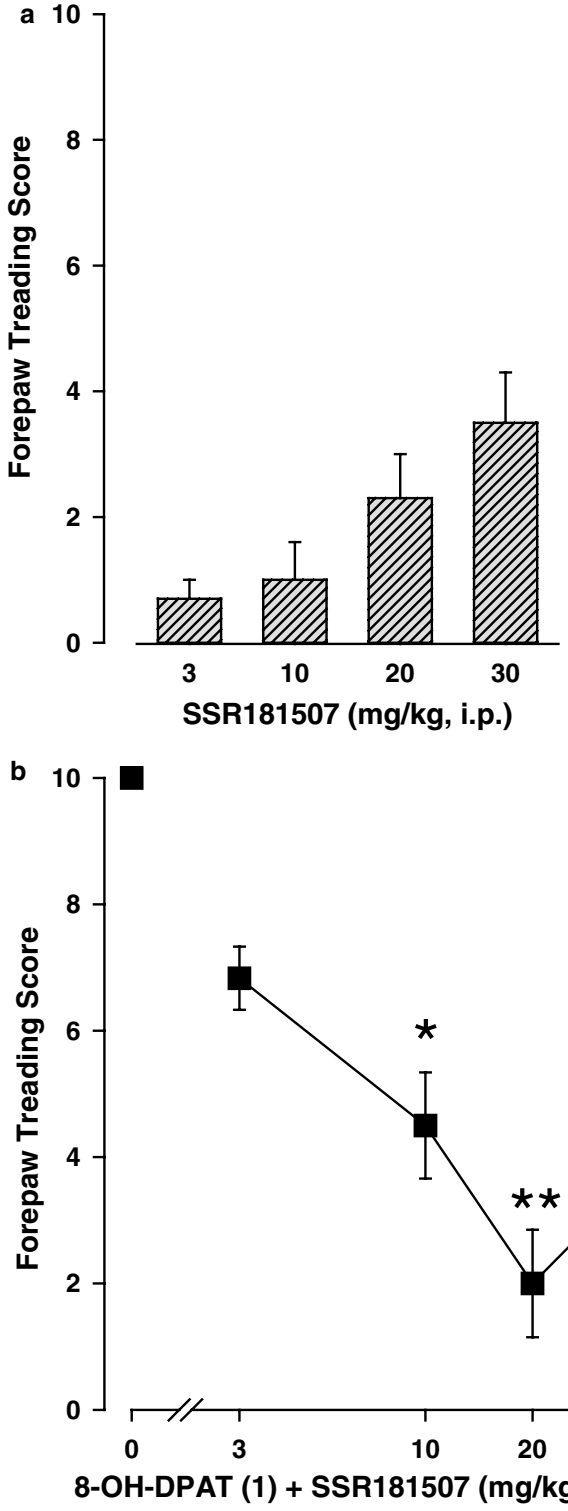

Figure 4 (a) Lack of induction by SSR181507 of the serotonergic syndrome in rats. (b) Antagonism by SSR181507 of the serotonergic syndrome produced by 8-OH-DPAT. (a) Each bar represents the averaged sum (+SEM) of the four scores recorded for 30 s at 3, 6, 9, 12, and 15 min following an i.p. injection with SSR181507. (b) Each symbol represents the averaged sum (±SEM) of four additional scores recorded for 30 s at 3, 6, 9, 12, and 15 min following an s.c. injection of 1 mg/kg of 8-OH-DPAT, given 30 min after the i.p. injection of SSR181507. *$P<0.05$, **$P<0.01$, compared to the 8-OH-DPAT + vehicle group. $N=6$ rats per group.

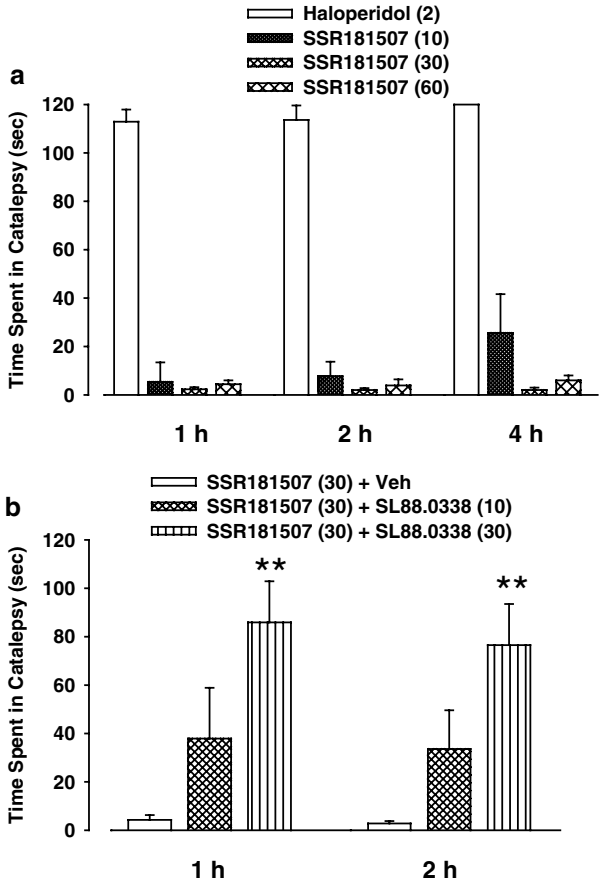

Figure 5 (a) Lack of cataleptogenic activity of SSR181507. (b) Induction of catalepsy by coadministration of SSR181507 and of the 5-HT_{1A} receptor antagonist SL88.0338. For both panels, each bar represents the average (+SEM) time spent by the rat in a cataleptic position (cutoff time: 120 s). (a) Catalepsy reading was taken 1, 2, and 4 h following an i.p. injection of SSR181507 or haloperidol (as a positive control group). (b) Rats were pretreated i.p. with vehicle or SL88.0338, followed 30 min later by an i.p. injection of SSR181507. Catalepsy was recorded 1 and 2 h after the second i.p. injection. **$P<0.01$, compared to the SSR181507 + vehicle group. $N=7$–8 rats per group for both panels.

(vehicle-injected) group. The time spent vocalizing was dose-dependently decreased by pretreatment with SSR181507 ($F(4,64)=13.61$, $P<0.001$), with significant effects observed at doses of 1 and 3 mg/kg (Figure 7a).

In a separate experiment (Figure 7b), pretreatment with 10 mg/kg, but not 3 mg/kg, of SL88.0338 attenuated the decrease in time spent vocalizing consecutive to treatment with 3 mg/kg of SSR181507. By itself, SL88.0338 at 10 mg/kg had no effect on the vocalization time. This was supported by the statistical analysis, which showed with Dunnett's *post hoc* tests (following a global effect: $F(4,53)=18.59$, $P<0.001$), that all groups, except the SSR181507 (3 mg/kg) + SL88.0338 (3 mg/kg) group, were significantly different from the SSR181507 (3 mg/kg) group.

Effects of SSR181507, Alone or in Association with the 5-HT_{1A} Receptor Antagonist SL88.0338, on the Cortical EcoG in Rats

SSR181507, at the dose of 3 mg/kg i.p., had no effect on the time spent in a wakeful state or in SWS (Figure 8a: white

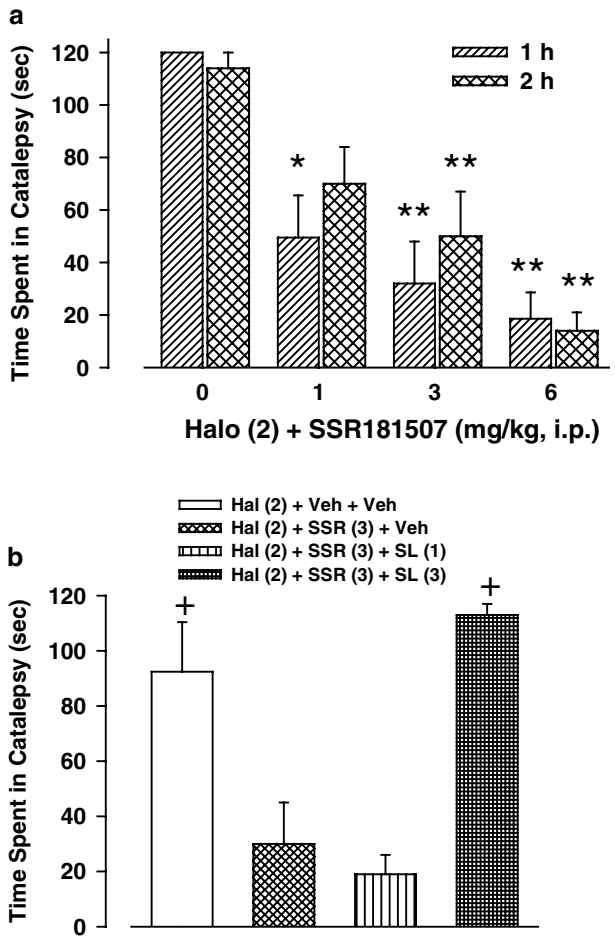

Figure 6 (a) Antagonism by SSR181507 of haloperidol-induced catalepsy. (b) Reversal of the antagonism by SSR181507 of haloperidol-induced catalepsy by pretreatment with the 5-HT_{1A} receptor antagonist SL88.0338. For both panels, each symbol represents the average (+SEM) time spent by the rat in a cataleptic position (cut-off time: 120 s). (a) Injection of haloperidol (2 mg/kg i.p.) was followed 30 min later by an injection of vehicle, SSR181507. Catalepsy was recorded 1 h following the last injection. $^{*}P<0.05$, $^{**}P<0.01$, compared to haloperidol + vehicle. $N=7$–8 rats per group. (b) Injection of haloperidol (2 mg/kg i.p.) was followed 30 min later by a double injection of vehicle, SSR181507, or SL88.0338. Catalepsy was recorded 1 h following the double injection. $^{+}P<0.05$, compared to the haloperidol + SSR181507 + vehicle group. $N=6$ rats per group.

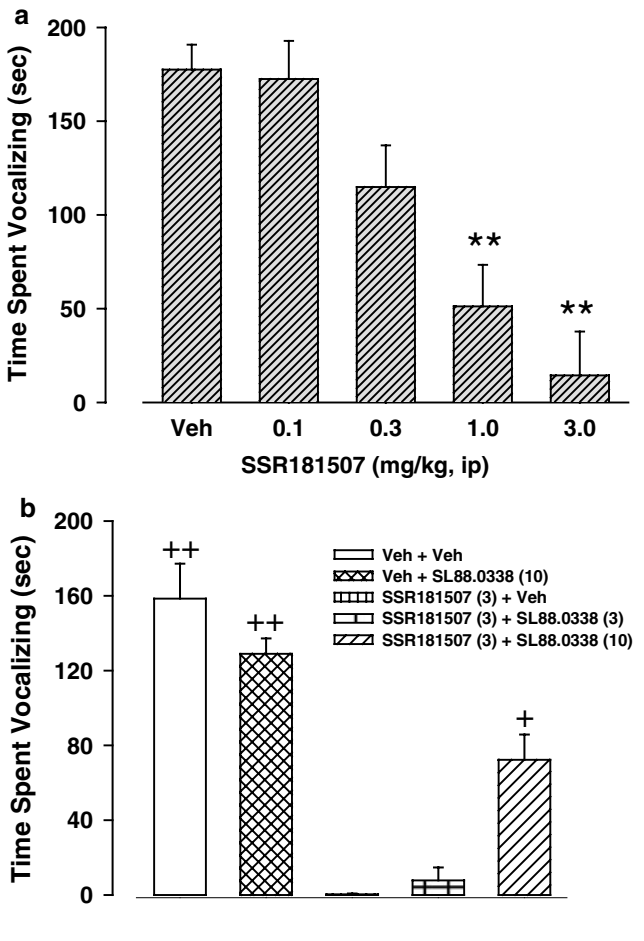

Figure 7 (a) Antagonism by SSR181507 of vocalization induced by separation from the mother in guinea-pig pups. (b) Reversal of the effects of SSR181507 by pretreatment with the 5-HT_{1A} receptor antagonist SL88.0338. Each bar represents the average time (in seconds, +SEM) spent vocalizing by pups during a 5-min separation period. Vehicle or SSR181507 was injected i.p. 30 min pretest, preceded (bottom panel) 30 min earlier by vehicle or SL88.0338 i.p. (a) $^{**}P<0.01$, compared to the vehicle group; $N=9$–28 pups per group. (b) $^{+}P<0.05$, $^{++}P<0.01$ compared to the SSR181507 + vehicle group. $N=7$–22 pups per group.

bars). However, the time spent in the paradoxical state was significantly reduced by 62% ($F(2,10)=19.25$, $P<0.05$). This decrease resulted mainly from a large increase in the latency time to enter PS ($F(2,10)=186.96$, $P<0.001$). The other two parameters were not significantly affected by treatment with SSR181507. When recorded on day 3 (24 h post-SSR181507 challenge), none of the four parameters differed significantly from control values of day 1 (Figure 8a: dark bars). This shows that there was no PS rebound effect.

Coadministration of SL88.0338 (3 mg/kg i.p.), while having no effect on the time spent in wakeful state or in SWS, fully antagonized the effects of 3 mg/kg i.p. of SSR181507 on the latency time to enter, and on the time spent in the PS stage (all F's <3.24, all P's >0.05; Figure 8b: white bars). As for treatment with SSR181507 alone, values for all four parameters of day 3 returned towards control values of day 1 (Figure 8b: dark bars). The other 5-HT_{1A} antagonist Way100635, 3 mg/kg i.p., was found to antagonize the effects of SSR181507, 3 mg/kg i.p., to an extent similar to that found with SL88.0338 (the time spent in paradoxical state was reduced from 62% of control value to 19%).

Antagonism by SSR181507 of Lithium-Induced Aversion Towards a Saccharin Solution in Rats, and its Reversal by Pretreatment with the 5-HT_{1A} Receptor Antagonist SL88.0338

Under control conditions, rats consumed an average of 10 ml of a saccharin solution (Figure 9a); in the lithium-treated group, this consumption dropped to 1.4 ml. This decreased consumption of sweetened solution was partially reversed by pretreatment with SSR181507, with the dose of 3 mg/kg being significantly different from the lithium + vehicle group (Dunn's *post hoc* tests, following a global effect: $H=29.69$, df $=4$, $P<0.001$).

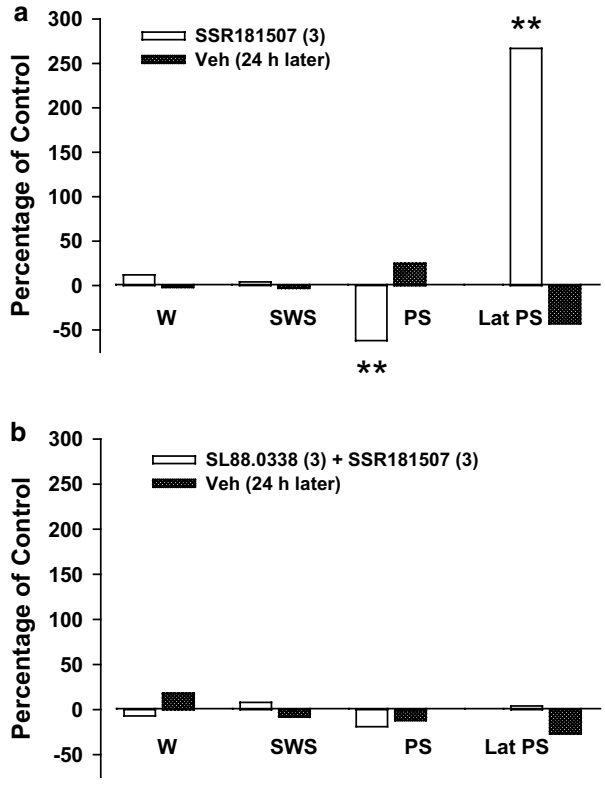

Figure 8 (a) Effects of SSR181507 on the cortical EcoG activity in rats. (b) Reversal of the effects of SSR181507 by pretreatment with the 5-HT_{1A} receptor antagonist SL88.0338. Each white bar represents the average time spent during the drug challenge (day 2) in wakefulness (W), slow wave sleep (SWS), paradoxical sleep (PS) and the latency time to enter paradoxical sleep (Lat PS) as a percentage of control values obtained the day before (day 1). Treatment with SSR181507 alone (a) or in combination with SL88.0338 (b) was administered i.p., 15 min before the 6-h recording session. Each dark bar represents values obtained during day 3, that is, 24 h postdrug challenge. $^{**}P<0.01$ compared to values obtained on day 1. $N=5$–6 rats per group.

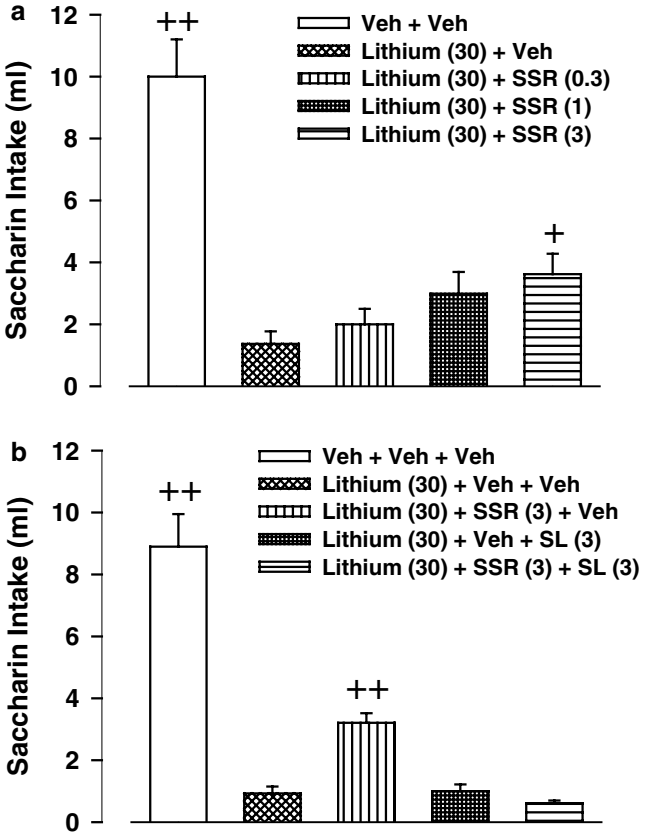

Figure 9 (a) Antagonism by SSR181507 of lithium-induced aversion towards a saccharin solution in rats. (b) Reversal of the effects of SSR181507 by pretreatment with the 5-HT_{1A} receptor antagonist SL88.0338. Each bar represents the average (+SEM) amount of a 0.25% w/v saccharin solution consumed by rats during a 15 min test. Vehicle or SSR181507 was injected i.p. 30 min pretest, preceded (bottom panel) 30 min earlier by vehicle or SL88.0338 i.p. (see the relevant Materials and Methods section for the particulars of the lithium treatment). (a) $^{+}P<0.05$, $^{++}P<0.01$ compared to the lithium + vehicle group. (b) $^{++}P<0.01$ compared to the lithium + vehicle + vehicle group. $N=10$–12 rats per group.

A pretreatment with 3 mg/kg of SL88.0338, while having no effect on the level of sucrose consumption following administration of lithium, fully reversed the effects of 3 mg/kg of SSR181507 (Figure 9b). This was supported by the statistical analysis, which showed that using *post hoc* Dunn's tests following a global effect ($H=41.65$, df = 4, $P<0.001$), only the control group and the lithium + SSR181507 group were significantly different from the lithium group.

Effects of SSR181507 on Spatial Working Memory in the Morris Water Maze Task in Rats

SSR181507, at none of the doses tested, had any effect on the Morris water maze task (Figure 10). The three-way ANOVA revealed that there was a nonsignificant treatment effect, and nonsignificant treatment × session, treatment × trial, and treatment × session × trial interaction effects (all F's < 2.90, all *P*'s > 0.05). Furthermore, there were significant session ($F(3,69)=30.80$; $P<0.0001$) and trial ($F(3,69)=65.02$; $P<0.0001$) effects. All this suggests that SSR181507 affected neither within-session (working memory) nor between-session (procedural memory) performances in this procedure.

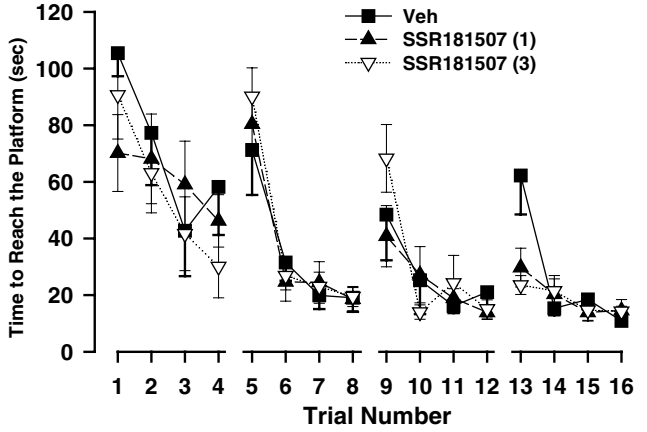

Figure 10 Lack of effects of SSR181507 on the Morris water maze task in rats. Each symbol represents the mean time (± SEM) taken by rats to find the submerged platform for each trial (four trials per daily session, separated by a 30 s intertrial interval). SSR181507 was administered i.p. 30 min presession. $N=9$–10 rats per group.

Table 1 reports on the in-house activity of several reference compounds in comparison with SSR181507 in most of the tests used in this study. These summary results indicate that the different tests were validated with appropriate reference compounds, but due to constraints of space, details of the results obtained with reference compounds are not discussed.

DISCUSSION

The present results highlight the following points: (1) SSR181507 induced behavioral effects consistent with DA D_2 receptor blockade and partial agonist activity at the 5-HT_{1A} receptor, both activities being observed at similar dose ranges; (2) A combination of D_2 receptor antagonism and 5-HT_{1A} receptor agonism confers a particular behavioral profile on SSR181507, characterized by efficacy in models predictive of antipsychotic activity (D_2 receptor antagonism), along with a lack of cataleptogenic activity and impairment in a model of spatial working memory, and with effects suggesting potential antidepressant/anxiolytic properties (5-HT_{1A} receptor agonism).

SSR181507 had clear antagonist activity at DA D_2 receptors in behavioral models: it dose-dependently reduced D-amphetamine hyperactivity in rats, and stereotyped and climbing behaviors produced by apomorphine in rats and mice, respectively. These results are in line with biochemical data presented in the companion paper (Claustre *et al*, 2003): in rats, SSR181507 displaced [^{3}H]raclopride binding and increased the synthesis of DA in limbic and/or striatal regions, in a dose range (0.3–3 mg/kg i.p.) very similar to that at which activity was observed in these behavioral tests. In addition, SSR181507 was found to selectively interfere with active avoidance behavior in mice, a test that has been claimed to be selectively sensitive to antipsychotics (Wadenberg and Hicks, 1999). This finding, in concert with the anti-apomorphine (stereotypies and climbing) and anti-amphetamine activity referred to above, suggests that SSR181507 should control positive symptoms of schizophrenia. The lack of involvement of 5-HT_{1A} receptors in the effects of SSR181507 on active avoidance was suggested by the finding that coadministration of the 5-HT_{1A} receptor blocker SL88.0338 did not modify the activity of SSR181507. This indicates that the DA D_2 receptor antagonist activity is responsible for the effects of SSR181507. This supposition is substantiated by the observation that in mice lacking the gene coding for the DA D_2 receptor (D_2 receptor knock-out mice), SSR181507 was totally devoid of activity in this active avoidance test (manuscript in preparation). This finding differs from observations made by others: 8-OH-DPAT potentiates the effects of haloperidol (Prinssen *et al*, 1996) and raclopride (Wadenberg and Ahlenius, 1991) on active avoidance in rats. The difference in the species used (rats *vs* mice in the present study) might account for these discrepancies. Future studies assessing the effects of combination of SSR181507 and SL88.0338 in rats are warranted to shed light on this possible species confound.

Up to a dose (60 mg/kg) that was 10 and 60 times higher than anti-stereotypy and anti-hypermotility doses, respectively, SSR181507 lacked cataleptogenic activity in rats. At this dose, occupation of DA D_2 receptors in the striatum appears to be maximal (Claustre *et al*, 2003), and should normally translate into catalepsy, thought to occur when striatal occupancy is greater than 80% (Wadenberg *et al*, 2000). However, biochemical/electrophysiological data presented in the companion paper support the limbic selectivity of SSR181507, as shown by its preferential effects on the number of spontaneously active DA cells in A10 *vs* A9 areas, on basal DA efflux in mesolimbic regions, and on the preferential c-Fos protein expression in the n. accumbens and prefrontal cortex *vs* striatum. This limbic *vs*

Table 1 Summary of Effects of SSR181507, in Comparison with Reference Compounds, in Various Behavioral Tests Conducted in our Laboratories

	Amphetamine hyperactivity in rats	Apomorphine stereotypies in rats	Apomorphine climbing (C) and hypothermia (H) in mice	Active avoidance in mice	Catalepsy in rats	Vocalization in pups	Latency to paradoxical sleep in rats	Water maze in rats
SSR181507	MED: 1	ED_{50}: 3.4	C: ED_{50}: 2 H: ED_{50} > 10	MED: 3	MED > 60	MED: 1	3	MED > 3
Clozapine	MED: 6	ED_{50}: > 30	C: ED_{50}: 9.7 H: ED_{50} > 10	MED: 2	MED > 60	MED: 1	5	NT
Haloperidol	MED: 0.1	ED_{50}: 0.2	C: ED_{50}: 0.11 H: ED_{50}: 0.08	MED: 0.3	MED: 0.5	MED > 1	1	MED: 0.3
Risperidone	MED: 0.3	ED_{50}: 0.3	C: ED_{50}: 0.08 H: ED_{50}: > 3	MED: 0.3	MED: 10	NT	NT	MED: 3
Olanzapine	MED: 2	ED_{50}: 4.8	C: ED_{50}: 0.54 H: ED_{50} > 10	MED: 1	MED: 10	NT	5	NT
Amisulpride	MED: 3	ED_{50}: 115	C: ED_{50}: 21 H: ED_{50}: 1.9	MED: 30	MED > 100	MED > 30	30[a]	MED > 30

All numbers are in mg/kg, i.p. ED_{50}: efficacious dose for 50% of maximal effect, MED: minimal efficacious dose, NT: not tested.
[a]Tendency, not confirmed at a higher dose.

striatal selectivity of the expression of blockade of DA D_2 receptors would presumably be responsible for the lack of catalepsy. The implication of 5-HT_{1A} agonist activity in this lack of catalepsy is strongly suggested by the following: (1) cotreatment of SSR181507 with SL88.0338 produced catalepsy, and (2) antagonism of haloperidol-induced catalepsy by SSR181507 was fully reversed by coadministration of SL88.0338. These results are in line with electrophysiological data reported in the companion paper, showing that pretreatment with the 5-HT_{1A} antagonist WAY100635 reversed the limbic *vs* striatal selectivity of SSR181507 (DA cells population experiment). They are also in agreement with reports that activation of the 5-HT_{1A} receptor with full or partial agonists opposes catalepsy produced by various DA D_2 receptor blockers (Broekkamp *et gal*, 1988; Invernizzi *et al*, 1988). Catalepsy is an animal model considered to be predictive of occurrence of EPS, and the absence of cataleptogenic activity of SSR181507 suggests that this compound should be free from motor side effects in patients. Indeed, in the clinic, there are indications that augmentation of antipsychotic therapy with the 5-HT_{1A} receptor partial agonists buspirone (Goff *et al*, 1991; Moss *et al*, 1993) and tandospirone (Yoshida *et al*, 1998) decreases EPS and/or akathisia and/or tardive dyskinesia scores.

Activation of 5-HT_{1A} receptors has been generally associated with occurrence of the serotonergic syndrome (forepaw treading and flat body posture) in rats, seen following treatment with 5-HT_{1A} receptor full agonists such as 8-OH-DPAT (Goodwin *et al*, 1986). SSR181507, unlike 8-OH-DPAT, did not produce this syndrome, and furthermore, antagonized the syndrome produced by 8-OH-DPAT, indicating that it acts as a partial agonist at 5-HT_{1A} receptors mediating this syndrome. (Note: this partial agonist activity is at odds with the full agonist activity in the GTPγ S assay (see companion paper by Claustre *et al*, 2003); this discrepancy might be due to an overexpression of 5-HT_{1A} receptors in the CHO cells used for this assay.) Although not very common, the serotonergic syndrome (restlessness, myoclonus, shivering, etc) has been described in the clinic, and can be potentially fatal (see review by Gillman, 1999). The present data would predict that SSR181507 should not give rise to a serotonergic syndrome in humans.

Schizophrenic patients are notably known to suffer from comorbid psychiatric symptoms: two of which, depression and anxiety, are sufficiently prominent to justify, in a non-negligible proportion of patients, additional prescription of antidepressants and/or anxiolytics (Clark *et al*, 2002). Further, these coexisting depressive/anxiety states have been considered to contribute to poor compliance in certain patients, that may in turn be conducive to a higher rate of relapse of psychotic episodes (Csernansky and Schuchart, 2002), as well as be responsible for the high rate of suicide associated with schizophrenia (Roy, 1982; Caldwell and Gottesman, 1990). The present study provides experimental evidence that SSR181507 possesses activity in animal models of anxiety/depression. (Note: the conservative dissociation between animal models of 'anxiety' and animal models of 'depression' is more and more considered as not being justified, since antidepressant drugs have been repeatedly shown to be clinically active against most, if not all, forms of anxiety: see the recent review by Borsini *et al* (2002). For this reason, the term antidepressant/anxiolytic is being used to translate the activity of SSR181507 in the various tests referred to below.) Guinea-pig pups separated from their mother emit vocalizations, whose duration is decreased by administration of antidepressants such as desipramine and fluvoxamine, of anxiolytics such as diazepam, and of 5-HT_{1A} receptor agonists such as 8-OH-DPAT and flesinoxan (Molewijk *et al*, 1996). SSR181507 dose-dependently reduced the amount of time that pups spent vocalizing during a short-time separation. This experimental suggestion of a potential antidepressant/anxiolytic activity of SSR181507 was further reinforced by its effects in the lithium taste aversion procedure: SSR181507 partially reversed the lithium-induced decrease of saccharin solution intake. This test has been shown to be sensitive to antianxiety agents such as the benzodiazepines lorazepam and diazepam, barbiturates, and to the 5-HT_{1A} receptor agonist 8-OH-DPAT (Ervin and Cooper (1988); Wegener *et al*, 1997). Finally, suggestion of a potential antidepressant/anxiolytic activity was obtained from effects of SSR181507 on the sleep pattern (EcoG activity) in rats. Antidepressants such as fluoxetine and imipramine decrease the time spent in PS by increasing the latency time to enter this stage of sleep (Slater *et al*, 1978; Kleinlogel, 1982). SSR181507, 3 mg/kg i.p., was found to affect the sleep pattern in a way typical of that of antidepressants. An additional finding of interest was that SSR181507 increased SWS to a much lesser extent than drugs such as clozapine and olanzapine, that is, it was less sedative (unpublished data).

The implication of 5-HT_{1A} receptors in the antidepressant/anxiolytic profile of SSR181507 is supported by the finding that SL88.0338 reversed the effects of SSR181507 in these three experimental situations. This implication was further supported by the finding that in the EcoG (see 'Results' section) and the lithium aversion experiments (data not shown), effects of SSR181507 were similarly antagonized by administration of WAY100635, another 5-HT_{1A} receptor antagonist.

The potential antidepressant/anxiolytic activity of SSR181507 was further suggested by the findings that it was efficacious in two additional models sensitive to antidepressant and/or anxiolytic compounds: the ultrasonic vocalization tests in adult rats induced by delivery of electrical shocks, and the 'step-down' passive avoidance test (manuscript in preparation). However, SSR181507 was found to be marginally and not consistently active (data not shown) in classical anxiety tests such as the Vogel conflict test and the elevated plus maze in rats. This might not be surprising, considering that these tests, that have been pharmacologically validated with benzodiazepine-like anxiolytics, are known to be little responsive to buspirone-like agents (ie 5-HT_{1A} receptor agonists).

SSR181507, at doses active in the various antipsychotic and antidepressant/anxiolytic tests above, was devoid of deleterious effects on acquisition (within-session) and retention (between-session) of a Morris water maze task, a rat model of spatial working memory. In contrast, the typical antipsychotic haloperidol and the atypical antipsychotic risperidone, were both found to disrupt acquisition of this task (Table 1), in line with what has been reported

previously for these two compounds, but also for some (clozapine, olanzapine, and ziprasidone), but not other (sertindole, seroquel) antipsychotics (Skarsfeldt, 1996).

Working memory has been shown to be impaired in schizophrenic patients (Goldman-Rakic, 1994). Although there are conflicting data in the literature regarding the possible amelioration of deficits of memory (and in particular, of working memory) with antipsychotics, it has been reported that some atypical antipsychotics have beneficial effects on cognition in schizophrenic patients (Meltzer and McGurk, 1999). Absence of deleterious effects of SSR181507 in the Morris water maze, an animal model of working memory, would appear to constitute a definite advantage in view of the above-exposed arguments. The 5-HT_{1A} agonist activity of SSR181507 might account for this positive aspect. This supposition is substantiated by the recent report that addition of the 5-HT_{1A} receptor partial agonist tandospirone to classical antipsychotics seems to improve some types of memory function deficits associated with schizophrenia (Sumiyoshi *et al*, 2000, 2001).

5-HT_{1A} receptor agonist properties were obtained in a dose range (1–3 mg/kg) similar to that necessary for blockade of DA D_2 receptors. These behavioral results mirror biochemical data, where it was found that SSR181507 increased DA and decreased 5-HT syntheses at roughly equivalent doses (Claustre *et al*, 2003). As such, they indicate that antipsychotic (ie efficacy against positive symptoms) and potential antidepressant/anxiolytic activities of SSR181507 should be obtained at the same dose in schizophrenic patients. Further, the finding that SSR181507 selectively increased extracellular DA levels and c-fos protein expression in the prefrontal cortex of rats (Claustre *et al*, 2003), provides an experimental argument for the hypothesis that SSR181507 might be active against the so-called negative symptoms (and also possibly against some cognitive/attentional and social interaction deficits) commonly encountered in schizophrenic patients. This last assumption is substantiated by the observation that SSR181507 was able to reverse a deficit of social interaction produced by phencyclidine between a pair of rats, as well as to counteract a deficit of selective attention in a social recognition situation between an adult and a pair of juvenile rats, a test sensitive to atypical antipsychotics such as clozapine and amisulpride (manuscripts in preparation).

In conclusion, the present results indicate that SSR181507 has an original behavioral profile that arises from its combined D_2 antagonist/5-HT_{1A} agonist properties, observed at similar dose ranges, suggestive of potential efficacy against several dimensions of schizophrenia, including positive and negative symptoms and cognitive/attentional impairments, ultimately leading to improvement in patients' outcome and rehabilitation. The potential antidepressant/anxiolytic activities linked to the activation of 5-HT_{1A} receptors give SSR181507 an added benefit, as it might allow to dispense with the need for coadministering antidepressant/anxiolytic drugs to patients presenting comorbid anxiety and/or depression states. The interest of the compound is also reinforced by its inability to induce extrapyramidal side effects that markedly affect patients' compliance and relapse. Finally, SSR181507 is devoid of affinity at a variety of receptors thought to mediate adverse effects (Richelson, 1999) such as 5-HT_{2c} receptors (possibly responsible for body weight gain associated with some newer antipsychotics), adrenergic α_1 and histaminergic H_1 receptors (implicated in sedation), α_2 receptors (responsible for autonomic effects), and at muscarinic M_1 receptors (whose blockade exerts deleterious effects on memory). This suggests that SSR181507 should be free from these troublesome side effects that are thought to contribute further to poor compliance (Casey, 2001).

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REFERENCES

Barnes TR, Curson DA, Liddle PF, Patel M (1989). The nature and prevalence of depression in chronic schizophrenic in-patients. *Br J Psychiatry* **154**: 488–491.

Balsara JJ, Jadhav JH, Chandorkar AG (1979). Effect of drugs influencing central serotonergic mechanisms on haloperidol-induced catalepsy. *Psychopharmacology* **62**: 67–69.

Bantick RA, Deakin JF, Grasby PM (2001). The 5-HT_{1A} receptor in schizophrenia: a promising target for novel atypical neuroleptics? *J Psychopharmacology* **15**: 37–46.

Borsini F, Podhorna J, Marazziti D (2002). Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology* **163**: 121–141.

Broekkamp CLE, Oosterloo SK, Berendsen HHG, van Delft AML (1988). Effects of metergoline, fenfluramine, and 8-OHDPAT on catalepsy induced by haloperidol or morphine. *Naunyn-Schmiedeberg's Arch Pharmacol* **338**: 191–195.

Buchanan RW, Kreyenbuhl J, Zito JM, Lehman A (2002). Relationship of the use of adjunctive pharmacological agents to symptoms and level of function in schizophrenia. *Am J Psychiatry* **159**: 1035–1043.

Caldwell CB, Gottesman II (1990). Schizophrenics kill themselves too: a review of risk factors for suicide. *Schizophr Bull* **16**: 571–589.

Casey DE (2001). Barriers to progress—the impact of tolerability problems. *Int Clin Psychopharmacol* **16**(Suppl 1): S15–S19.

Claustre Y, De Peretti D, Brun P, Gueudet C, Allouard N, Alonso R *et al* (2003). SSR181507, a dopamine D_2 receptor antagonist and 5–HT_{1A} receptor agonist. I: Neurochemical and electrophysiological profile. *Neuropsychopharmacology* submitted.

Csernansky JG, Schuchart EK (2002). Relapse and rehospitalisation rates in patients with schizophrenia: effects of second generation antipsychotics. *CNS Drugs* **16**: 473–484.

Costall B, Naylor RJ (1973). On the mode of action of apomorphine. *Eur J Pharmacol* **21**: 350–361.

Christoffersen CL, Meltzer LT (1998). Reversal of haloperidol-induced extrapyramidal side effects in cebus monkeys by 8-hydroxy-2-(di-*n*-propylamino)tetralin and its enantiomers. *Neuropsychopharmacology* **18**: 399–402.

Clark RE, Bartels SJ, Mellman TA, Peacock WJ (2002). Recent trends in antipsychotic combination therapy of schizophrenia and schizoaffective disorder: implications for state mental health policy. *Schizophr Bull* **28**: 75–84.

De Vry J (1995). 5-HT1A receptor agonists: recent developments and controversial issues. *Psychopharmacology* **121**: 1–26.

Ervin GN, Cooper BR (1988). Use of conditioned taste aversion as a conflict model: effects of anxiolytic drugs. *J Pharmacol Exp Ther* **245**: 137–146.

Gillman PK (1999). The serotonin syndrome and its treatment. *J Psychopharmacol* **13**: 100–109.

Goff DC, Midha KK, Brotman AW, McCormick S, Waites M, Amico ET (1991). An open trial of buspirone added to neuroleptics in schizophrenic patients. *J Clin Psychopharmacol* **11**: 193–197.

Goldman-Rakic PS (1994). Working memory dysfunction in schizophrenia. *J Neuropsychiatry Clin Neurosci* **6**: 348–357.

Goodwin GM, De Souza RJ, Wood AJ, Green AR (1986). The enhancement by lithium of the 5-HT1A mediated serotonin syndrome produced by 8-OH-DPAT in the rat: evidence for a post-synaptic mechanism. *Psychopharmacology* **90**: 488–493.

Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY (2001). 5-HT2A and D2 receptor blockade increases cortical DA release via 5-HT1A receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J Neurochem* **76**: 1521–1531.

Invernizzi RW, Cervo L, Samanin R (1988). 8-Hydroxy-2-(di-*n*-propylamino) tetralin, a selective serotonin1A receptor agonist, blocks haloperidol-induced catalepsy by an action on raphe nuclei medianus and dorsalis. *Neuropharmacology* **27**: 515–518.

Kapur S (1996). 5-HT2 antagonism and EPS benefits: is there a causal connection? *Psychopharmacology* **124**: 35–39.

Kapur S, Remington G (2001). Dopamine D_2 receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. *Biol Psychiatry* **50**: 873–883.

Kleinlogel H (1982). The rat paradoxical sleep as a pharmacological test model. In: Herrmann WM (ed) *EEG in Drug Research*. Gustave Fisher: Stuttgart. pp 75–88.

Korsgaard S, Gerlach J, Christensson E (1985). Behavioral aspects of serotonin-dopamine interaction in the monkey. *Eur J Pharmacol* **118**: 245–252.

Liebman JM, Gerhardt SC, Gerber R (1989). Effects of 5-HT1A agonists and 5-HT2 antagonists on haloperidol-induced dyskinesias in squirrel monkeys: no evidence for reciprocal 5-HT-dopamine interaction. *Psychopharmacology* **97**: 456–461.

Meltzer HY (1999). The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* **21**(2 Suppl): 106–115.

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 $(5\text{-HT})_{1A}$ receptors. *J Pharmacol Exp Ther* **295**: 853–861.

Molewijk HE, Hartog K, van der Poel AM, Mos J, Olivier B (1996). Reduction of guinea pig pup isolation calls by anxiolytic and antidepressant drugs. *Psychopharmacology* **128**: 31–38.

Moss LE, Neppe VM, Drevets WC (1993). Buspirone in the treatment of tardive dyskinesia. *J Clin Psychopharmacol* **13**: 204–209.

Prinssen EP, Kleven MS, Koek W (1996). Effects of dopamine antagonists in a two-way active avoidance procedure in rats: interactions with 8-OH-DPAT, ritanserin, and prazosin. *Psychopharmacology* **128**: 191–197.

Richelson E (1999). Receptor pharmacology of neuroleptics: relation to clinical effects. *J Clin Psychiatry* **60**(Suppl 10): 5–14.

Roy A (1982). Risk factors for suicide in psychiatric patients. *Arch Gen Psychiatry* **39**: 1089–1095.

Slater IH, Jones GT, Moore RA (1978). Inhibition of REM sleep by fluoxetine, a specific inhibitor of serotonin uptake. *Neuropharmacology* **17**: 383–389.

Skarsfeldt T (1996). Differential effect of antipsychotics on place navigation of rats in the Morris water maze. A comparative study between novel and reference antipsychotics. *Psychopharmacology* **124**: 126–133.

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.

Tajima O (2001). Mental health care in Japan: recognition and treatment of depression and anxiety disorders. *J Clin Psychiatry* **62**(Suppl 13): 39–44.

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.

Wadenberg ML, Hicks PB (1999). The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? *Neurosci Behav Rev* **23**: 851–862.

Wadenberg ML, Kapur S, Soliman A, Jones C, Vaccarino F (2000). Dopamine D2 receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats. *Psychopharmacology* **150**: 422–429.

Wegener G, Smith DF, Rosenberg R (1997). 5-HT1A receptors in lithium-induced conditioned taste aversion. *Psychopharmacology* **133**: 51–54.

Yoshida K, Sugita T, Higuchi H, Hishikawa Y (1998). Effects of tandospirone on tardive dyskinesia and parkinsonian symptoms. *Eur Psychiatry* **13**: 421–422.