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Pharmacology, Biochemistry and Behavior 71 (2002) 655–665

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Effects of the 5-HT$_7$ receptor antagonist SB-258741 in animal models for schizophrenia

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Received 10 July 2001; received in revised form 22 October 2001; accepted 31 October 2001

Abstract

The 5-HT$_7$ receptor is targeted by several new antipsychotics such as clozapine and risperidone. We studied the effect of *R*-(+)-1-(toluene-3-sulfonyl)-2-[2-(4-methylpiperidin-1-yl)ethyl]-pyrrolidine (SB-258741), a specific 5-HT$_7$ receptor antagonist, in three models for positive symptoms, D-amphetamine-induced hyperactivity and D-amphetamine- and phencyclidine (PCP)-disrupted prepulse inhibition (PPI) in rats, with the aim of investigating the role of this receptor in the clinical effect of antipsychotics. We also tested this compound in a model for negative symptoms, PCP-disrupted social interaction (SIT) in rats. Induction of side effects by this compound was evaluated by testing its potency to reduce spontaneous motility and to induce catalepsy in rats. The effect of SB-258741 was compared to risperidone in all models. This study showed that SB-258741 had no beneficial effect on PCP-disrupted SIT. SB-258741 did not reverse D-amphetamine-disrupted PPI; however, it dose-dependently normalised PCP-disrupted PPI. SB-258741 antagonised D-amphetamine-induced hyperactivity but reduced motility of rats at similar doses. Thus, this specific 5-HT$_7$ receptor antagonist brought a clear positive outcome in only one model for positive symptoms of schizophrenia and had no beneficial effect in the model for negative symptoms. Consequently, it is clear that SB-258741 affects the PPI phenomenon but is not expected to have an antipsychotic effect on its own in clinic.

Keywords: SB-258741; 5-HT$_7$; Prepulse inhibition; Schizophrenia; Activity; Social interaction; Catalepsy

1. Introduction

The 5-HT$_7$ receptor is targeted by several antipsychotics of second generation such as clozapine, risperidone, and zotepine, and with lower affinity, by olanzapine and sertindole (Arnt and Skarsfeldt, 1998; Eglen et al., 1997; Roth et al., 1994; Terrón and Falcón-Neri, 1999). In in vitro assays, clozapine also up-regulates the expression of the 5-HT$_7$ receptor (Zhukovskaya and Neumaier, 2000). The localisation of the 5-HT$_7$ receptor at the level of limbic structures (Branchek et al., 1995; Doyland et al., 1995; Hagan et al., 2000; Le Corre et al., 1997; Thomas et al., 1999; To et al., 1995) also suggests a possible role for treatment of schizophrenia. However, the role per se of this receptor in the clinical action of antipsychotics is still unknown. Various in vivo studies have been conducted in order to define the role of this receptor, and sometimes with nonspecific compounds (Meneses and Terrón, 2001). This receptor has been suggested to be of interest for treatment of cognitive dysfunction (Meneses and Terrón, 2001), depression (Yau et al., 1997; Schwartz, 1993; Sleight et al., 1995), and sleep disorders (Schwartz, 1993; Tsou et al., 1994) by regulation of circadian rhythms (Lena Mullins et al., 1999; Lovenberg et al., 1993). However, no preclinical studies described its role in animal models related to schizophrenia.

This lack of knowledge is basically due to a lack of potent and specific 5-HT$_7$ ligands. Recently, 5-HT$_7$ receptor antagonists have been developed (Forbes et al., 1998; Hagan et al., 2000; Lovell et al., 2000), *R*-(+)-1-(toluene-3-sulfonyl)-2-[2-(4-methylpiperidin-1-yl)ethyl]-pyrrolidine (SB-258741) being one of the most specific (see compound "13" in Lovell et al., 2000).

In order to clarify the potential role of a 5-HT$_7$ receptor antagonist for the treatment of schizophrenia, we studied the effect of SB-258741 in three models related to positive symptoms of schizophrenia, D-amphetamine-induced hyperactivity and D-amphetamine- and phencyclidine (PCP)-disrupted prepulse inhibition (PPI) in rats. We also tested this compound in a putative model for negative symptoms, PCP-disrupted social interaction (SIT) in rats. Induction of motor

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0091-3057/02/$ – see front matter
PII: S0091-3057(01)00744-4

side effects by this compound was evaluated by testing its potency to reduce spontaneous motility and to induce catalepsy in rats.

The D-amphetamine-induced hyperactivity in rats has the advantage of being a predictive and reproducible model for the selection of compounds with antipsychotic-like action (Arnt, 1995, 2000). Consequently, it should indicate whether SB-258741 has a general antipsychotic potential.

PPI (Braff and Geyer, 1990; Swerdlow et al., 1994) is disrupted in patients with schizophrenia (Swerdlow et al., 1994; Braff and Grillon, 1992), and this is modeled in the rat by systemic administration of the indirect dopamine (DA) agonist D-amphetamine (Druhan et al., 1998; Swerdlow et al., 1986; Mansbach et al., 1988; Zhang et al., 2000; Johansson et al., 1995; Paabøl Andersen and Pouzet, 2001; but see Davis, 1988), or the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist PCP (Mansbach and Geyer, 1989; Bakshi et al., 1994). PPI disruption in rodents is antagonised by antipsychotic drugs, and this effect is used as an animal model of antipsychotic action. Interestingly, while disruption of PPI by a dopamine enhancer is reversed by both classical and the second generation of antipsychotics (Swerdlow and Geyer, 1993; Swerdlow et al., 1994; Yamada et al., 1999; Paabøl Andersen and Pouzet, 2001), PPI disruption produced by noncompetitive NMDA antagonists is apparently specifically reversed by the second generation of antipsychotics (Bakshi and Geyer, 1995; Bakshi et al., 1994; Yamada et al., 1999). On the other hand, a lack of effect in this model has also been claimed for clozapine or risperidone (Johansson et al., 1994; Swerdlow et al., 1996). Thus, we investigated the effect of SB-258741 in D-amphetamine-disrupted PPI in order to determine the possible general antipsychotic-like effect of this compound, and we considered of interest to test the effect of SB-258741 in PCP-disrupted PPI in order to define similarities with the new generation of antipsychotics.

A specific advantage of the second generation of antipsychotics is that they have a lower potential to induce extrapyramidal side effects (EPS) than classical antipsychotics (Goldstein, 2000; Glazer, 2000), and they also partially reduce negative symptoms such as social withdrawal in schizophrenic patients (Blind, 1999). In order to illustrate the capacity of compounds to reduce social withdrawal, we studied their effect in PCP-disrupted SIT in rats (Corbett et al., 1995; Sams-Dodd, 1995). Few antipsychotics (clozapine, risperidone, and sertindole) show capacity to antagonise PCP-disrupted SIT (Sams-Dodd, 1997; 1998). As these three antipsychotics (Arnt and Skarsfeldt, 1998; Roth et al., 1994) have affinity for the 5-HT_7 receptor, it was consequently of interest to test whether SB-258741 would have a positive effect in this model as well.

The effect of SB-258741 was compared to risperidone in all models tested, as this antipsychotic is the one on the market with the highest affinity for the 5-HT_7 receptor (Roth et al., 1994). The potential interest of a 5-HT_7 receptor antagonist for treatment of schizophrenia will be discussed.

2. Materials and methods

2.1. Subjects

Rats used in all experimental paradigms except PPI were male Wistar rats supplied by Møllegård (Denmark). Rats used in the PPI were supplied by Charles River (Germany). Animals were approximately 2 months old and weighed approximately 250–300 g when used in the PPI and SIT paradigm, but 200–250 g when used in the catalepsy, motility, and D-amphetamine hyperactivity paradigms. New animals were used in each paradigm. Animals were housed four per cage (macrolon type III) for the motility, D-amphetamine hyperactivity, and catalepsy paradigms, but two per cage for the SIT and PPI paradigms. They were kept in climate-controlled animal facilities (temperature at 21 ± 2 °C and relative humidity at $60 \pm 10\%$). Animals were housed under a normal light–dark cycle (lights on 06:00–18:00 h) with free access to food and water, except for the PCP-disrupted SIT model, in which animals were housed under a reversed light–dark cycle (lights on 18:00–06:00 h). Animals were randomly divided into treatment and drug groups. All procedures were in strict accordance with the Danish Committee on Care and Use of Laboratory Animals.

2.2. Materials and experimental design

2.2.1. D-Amphetamine-induced hyperactivity and motility

The test cages were macrolon type III, high model ($42.5 \times 26.5 \times 18.5$ cm) equipped with four infrared light sources and photocells 4 cm above the bottom of the cages. The D-amphetamine-induced hyperactivity experiments were run in normal light conditions in an undisturbed room. The test substances were injected subcutaneously (sc) 30 min before injection (sc) of D-amphetamine sulphate (0.5 mg/kg). Immediately after injection of D-amphetamine, the rats were placed individually in test cages, and locomotor activity was measured in 15 min intervals for 2 h. The motility experiments were performed in identical cages, but were located in a dark room in order to obtain a better spontaneous activity and, consequently, optimal conditions to highlight possible motor inhibition after administration of test substances. The test substances were injected subcutaneously 30 min before measuring motility for 15 min, which means during the exploratory phase of rats.

2.2.2. Prepulse inhibition

The apparatus consisted of four startle chambers (MOPS 2b; Metod och Produkt, Göteborg, Sweden). Each rat was placed in a wire-mesh cage ($18.5 \times 7 \times 6.5$ cm) that was suspended at one point to a piston within a stabilimeter in such a way that this cage could move freely under the piston. Each cage was enclosed within an individual sound-insulated box ($52 \times 42 \times 38$ cm). Each movement of the piston was converted to an analogue signal by an accelerometer. Signals were transferred to a computer using an

analog-to-digital converter from National Instruments. Custom-designed StarWin software package from Ellegaard Systems (Denmark) ran the startle-stimuli schedules and the real-time data analysis. Acoustic noise bursts were presented via two speakers mounted 15 cm behind the cages. A background noise (62 or 70 dB) was maintained throughout the session. Startle amplitude was defined as the maximal of one hundred 1-ms stabilimeter readings collected from stimulus onset. The four cages were calibrated for equal sensitivity before starting experimental sessions. Stimulus consisted of a burst of white noise superimposed to the background noise with a rise time of less than 1 ms.

The PCP-disrupted PPI procedure was designed as follows. After a 10 min acclimatization with the background sound on, eight startle pulses of 105 dB broad band burst for 30 ms were presented to test for basal startle responsiveness. Then eight blocks of six different trial types were presented to measure PPI. Trials were presented pseudorandomly throughout the session, i.e., pulse alone (105 dB), prepulse alone (77 dB), prepulse followed by pulse (three trial types: 67 + 105 dB; 72 + 105 dB; 77 + 105 dB) or no pulse. The three different prepulses had an intensity of either 5, 10, or 15 dB above the background sound (62 dB) and a duration of 20 ms. The time interval between the prepulse offset and the pulse onset was 60 ms. The intertrial period was constant and lasted 15 s. The percentage PPI induced by each prepulse intensity was calculated as: [100 − (100 × startle amplitude on prepulse trial)/(startle amplitude on pulse alone trial)]. PCP (2 mg/kg) or vehicle was injected subcutaneously 10 min before starting acclimatization. Compounds to be tested were injected 15 min before PCP administration.

The D-amphetamine-disrupted PPI design differed for some parameters. After a 5 min acclimatization period with the background sound on, eight startle pulses of 120-dB broad band burst for 40 ms were presented to test for basal startle responsiveness. The three different prepulses had an intensity of either 4, 8, or 12 dB above the background sound (70 dB) and a duration of 30 ms. The time interval between the prepulse offset and the pulse onset was 100 ms. The intertrial period was constant and lasted 15 s. D-Amphetamine (2 mg/kg) or vehicle was injected subcutaneously 25 min before starting acclimatization. Compounds to be tested were injected 15 min before D-amphetamine administration.

2.2.3. Social interaction

The test was performed in an open arena (150 × 100 × 40 cm). The behaviour of rats was recorded by a video camera placed above the arena and connected to an S-VHS video recorder. Lighting in the room consisted of dark-red diffused light. Videos were analysed off-line by the Ethovision programme (Noldus) in order to evaluate activity, active SIT, and passive SIT of rats. SIT is defined as the duration during which animals were less than 20 cm from each other. SIT was subsequently divided into an active and passive component for each rat based upon whether the rat moved or was inactive (Sams-Dodd, 1996). The method applied for this experiment is the same as the 3-day treatment protocol described in detail elsewhere (Sams-Dodd, 1997). To summarise shortly, 2 weeks after arrival in our facilities, half of the rats within a drug group were dyed with black hair colour except on the head. Rats received a daily injection of SB-258741 and PCP (2 mg/kg) for 3 consecutive days. Subjects were tested on the last day of injection. SIT of rats was measured for 10 min after placing two unfamiliar rats (one white and one dyed black) in the open arena, on the same wall side but at opposite corners. Both rats received the same drug treatment.

2.2.4. Catalepsy

Catalepsy was assessed on a vertical wire mesh frame (50 × 50 cm). Mesh opening was 1 × 1 cm and mesh diameter was 2 mm. Animals were considered cataleptic when they remained immobile during a period of 15 s. Rats showing muscle relaxation were not considered cataleptic. Rats that did not move their paws but showed active body or head movements were also not considered as cataleptic. Observation for catalepsy occurred once each hour during the first 6 h and once 24 h after dosing.

2.3. Drugs

PCP hydrochloride was synthesized at H. Lundbeck and dissolved in 0.1 M methanesulfonic acid diluted in 0.9% NaCl. D-Amphetamine sulfate was supplied by Nomeco (Copenhagen) and was dissolved in 0.9% NaCl. SB-258741 was synthesised at H. Lundbeck and was dissolved in water. Risperidone was a courtesy of Janssen Pharmaceutical (Beerse) and was dissolved in 0.1 M HCl diluted in 0.9% NaCl. Solution used as vehicle control was always 0.9% NaCl. All compounds were injected at volumes of 5 ml/kg. Except for PCP and D-amphetamine, all doses are expressed in milligrams per kilogram, according to the amount of free base.

2.4. Data analysis

2.4.1. D-Amphetamine-induced hyperactivity

Hyperactivity was analysed by a two-way repeated measurement ANOVA consisting of a between-subjects factor of treatment (six levels for SB-258741: VEH–VEH; VEH–AMPH; 0.56 mg/kg AMPH; 2.3 mg/kg AMPH; 4.6 mg/kg AMPH; 9.1 mg/kg AMPH; five levels for risperidone: VEH–VEH; VEH–AMPH; 0.080 mg/kg AMPH; 0.31 mg/kg AMPH; 1.3 mg/kg AMPH) and a within-subjects factor of Time (eight bins of 15 min each). A post hoc analysis Fisher's PLSD was performed to compare between groups and time intervals.

2.4.2. Motility

Motility was analysed by a one-way ANOVA consisting of a between-subjects factor of drug (five levels for SB-

258741: VEH; 1.1 mg/kg; 2.3 mg/kg; 4.6 mg/kg; 9.1 mg/kg; four levels for risperidone: VEH; 0.31 mg/kg; 1.3 mg/kg; 4.9 mg/kg). A post hoc analysis Fisher's PLSD was performed to compare between groups.

2.4.3. D-Amphetamine- or PCP-disrupted PPI

The startle amplitude was analysed by a two-way ANOVA consisting of a between-subjects factor of treatment (two levels: VEH; and AMPH or PCP) and a between-subjects factor of drug (three levels: VEH; 2.3 mg/kg; 9.1 mg/kg, for SB-258741; and VEH; 0.080 or 0.16 mg/kg; and 0.16 or 0.31 mg/kg for risperidone). Mean percentage PPI was analysed by three-way repeated-measures ANOVA consisting of a between-subjects factor of treatment, a between-subjects factor of drug, and a within-subjects factor of three prepulse intensities. A post hoc analysis Fisher's PLSD was performed to compare the startle amplitude and the mean percent PPI between groups.

2.4.4. PCP-disrupted SIT

Activity, active SIT, and passive SIT were analysed by a two-way ANOVA consisting of a between-subjects factor of treatment (two levels: VEH and PCP) and a between-subjects factor of drug (three levels: VEH; 2.3 mg/kg; 9.1 mg/kg, for SB-258741; and VEH; 0.16 mg/kg; 0.31 mg/kg, for risperidone). A post hoc analysis Fisher's PLSD was performed to compare activity and SIT between groups.

2.4.5. Catalepsy

The total catalepsy score was expressed as percent of the maximum achievable score for the individual rat and the result presented as mean values per treatment group. Catalepsy was analysed by a two-way repeated-measures ANOVA consisting of a between-subjects factor of drug (two levels for SB-258741: 9.1 and 18 mg/kg; three levels for risperidone: 2.5 mg/kg; 5.0 mg/kg; 10 mg/kg), and a within-subjects factor of Time (seven levels: 1, 2, 3, 4, 5, 6, and 24 h).

All analyses were based on the raw data and were calculated with the SigmaStat (2.03) software system, except for the PPI data, which were analysed with the StatView (5.0) software system. Preliminary results of this study were presented as a poster at the SFN meeting 2000.

3. Results

3.1. D-Amphetamine-induced hyperactivity

3.1.1. SB-258741

D-Amphetamine-induced hyperactivity (Fig. 1) as shown by the significant difference of activity between the VEH–VEH- and VEH–AMPH-treated groups on the time intervals 30–105 min postinjection (P's < .01). The post hoc analysis conducted on the significant Treatment × Time interaction, $F(35,406) = 7.40$, $P < .001$, showed that SB-258741 administered at 0.56 mg/kg did not reverse the effect of D-amphetamine. SB-258741 administered at 2.3 mg/kg reversed hyperactivity induced by D-amphetamine up to 45 min after starting measurement (P's < .04). SB-258741 given at the two highest doses (4.6 and 9.1 mg/kg) reversed hyperactivity induced by D-amphetamine up to 60 min after starting measurement (P's < .03 and P's < .002, respectively). At 15 min after starting the observation, the two highest doses of SB-258741 given in addition to D-amphetamine reduced motor activity below that of VEH–VEH-treated animals (P's < .001). On the time interval 120 min, the groups treated with the three highest doses were more active than the VEH–AMPH group (P's < .03) and the VEH–VEH group (P's < .003).

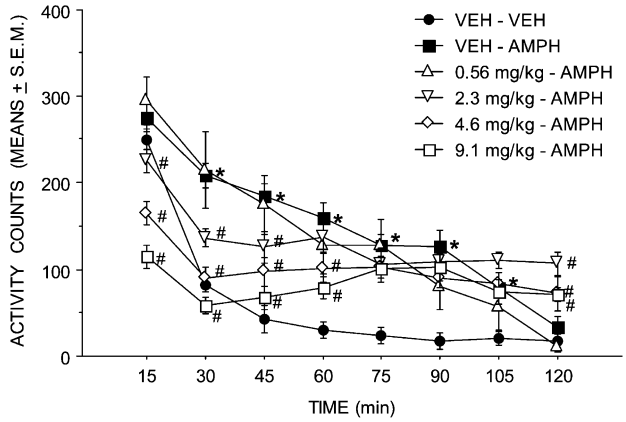

Fig. 1. Effects of acute treatment with SB-258741 on D-amphetamine-induced (0.5 mg/kg) hyperactivity in rats. $n = 8$ per Drug × Treatment group, but $n = 16$ in VEH–VEH and VEH–AMPH groups. * $P < .05$ versus VEH–VEH; # $P < .05$ versus VEH–AMPH.

3.1.2. Risperidone

D-Amphetamine-induced hyperactivity (Fig. 2) as shown by the significant difference of activity between the VEH–VEH- and VEH–AMPH-treated groups on the time intervals 30–105 min postinjection (P's < .03). The post hoc analysis conducted on the significant Treatment × Time interaction, $F(28,245) = 6.84$, $P < .001$, showed that only risperidone given at the two highest doses (0.31 and 1.3 mg/kg) reversed hyperactivity induced by D-amphetamine up to 45 and 60 min after starting measurement (P's < .001). During the first 30 min after starting the observation, the highest dose of risperidone (1.3 mg/kg) given in addition to D-amphetamine reduced activity of rats below the level of VEH–VEH-treated animals (P's < .005).

3.2. Motility

3.2.1. SB-258741

A significant main effect of drug, $F(4,27) = 7.59$, $P < .001$, demonstrated that SB-258741 tested at 4.6 and 9.1 mg/kg reduced motility of rats (P's < .03) (Fig. 3).

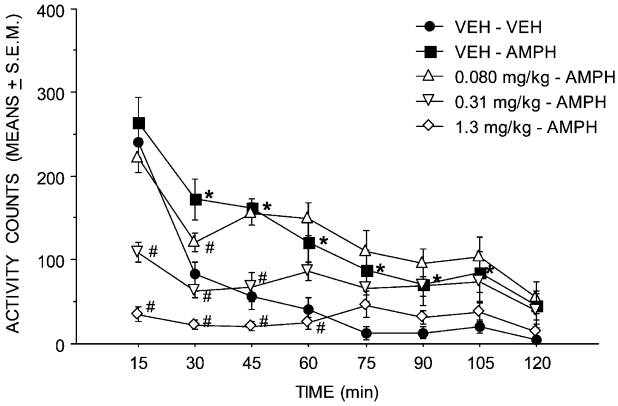

Fig. 2. Effects of acute treatment with risperidone on D-amphetamine-induced (0.5 mg/kg) hyperactivity in rats. $n=8$ per Drug × Treatment group. * $P<.05$ versus VEH–VEH; # $P<.05$ versus VEH–AMPH.

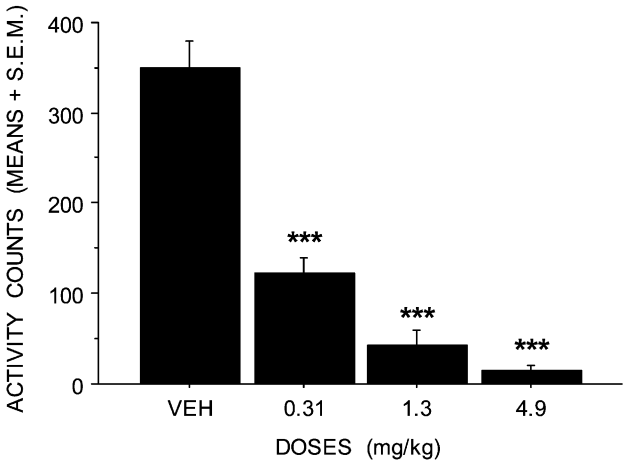

Fig. 4. Effects of acute treatment with risperidone on spontaneous motility in rats. $n=4$ per drug group, but $n=8$ in VEH. *** $P<.001$ versus VEH.

3.2.2. Risperidone

A significant main effect of drug, $F(3,16)=40.84$, $P<.001$, demonstrated that risperidone reduced motility of rats (P's $<.001$) on all doses tested (Fig. 4).

3.3. D-Amphetamine-disrupted PPI

3.3.1. SB-258741

As shown in Fig. 5A, there was a significant PPI effect, as reflected in the main effect of prepulse, $F(2,66)=43.19$, $P<.001$. The disruptive effect of D-amphetamine, $F(1,33)=6.15$, $P<.02$, was not reversed by SB-258741 as neither the main effect of drug, $F(2,33)=0.08$, $P>.92$, nor any of the interactions reached the significant level (P's $>.18$). As shown in Fig. 5B, startle amplitude was not affected by the factors analysed (all P's $>.31$).

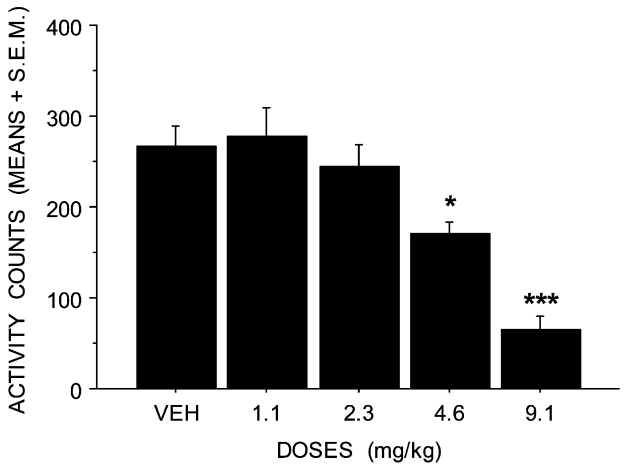

Fig. 3. Effects of acute treatment with SB-258741 on spontaneous motility in rats. $n=4$ per drug group, but $n=16$ in VEH. * $P<.05$ versus VEH; *** $P<.001$ versus VEH.

3.3.2. Risperidone

As shown in Fig. 6A, there was a significant PPI effect, as reflected in the main effect of prepulse, $F(2,84)=102.63$, $P<.001$. Risperidone normalised D-amphetamine-disrupted

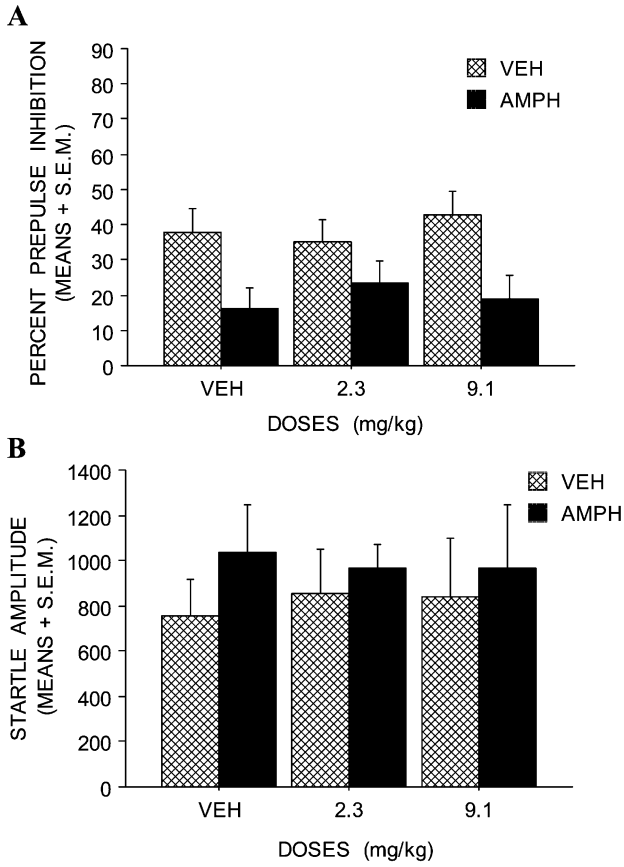

Fig. 5. (A) Effects of acute treatment with SB-258741 on D-amphetamine-disrupted (2 mg/kg) PPI in rats. (B) Effects of SB-258741 on startle amplitude. $n=6$ in the VEH–AMPH, 2.3 mg/kg VEH, and 9.1 mg/kg groups, and $n=7$ in all the other groups.

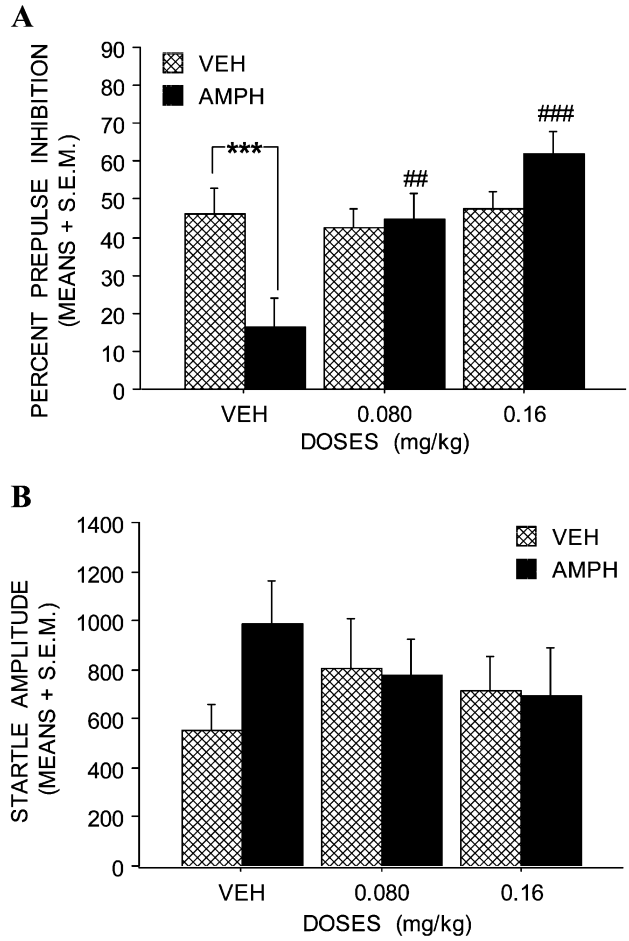

Fig. 6. (A) Effects of acute treatment with risperidone on D-amphetamine-disrupted (2 mg/kg) PPI in rats. (B) Effects of risperidone on startle amplitude. $n=8$ per Drug × Treatment group. *** $P<.001$. ## $P<.01$ versus VEH–AMPH; ### $P<.001$ versus VEH–AMPH.

PPI, as reflected by the significant Treatment × Drug interaction, $F(2,42)=6.98$, $P<.003$. Post hoc analysis indicated a significant difference only between vehicle and D-amphetamine groups in those animals receiving no treatment with risperidone ($P<.001$). Moreover, groups treated with risperidone at both doses in addition to D-amphetamine were significantly different from the AMPH–VEH-treated group (P's $<.01$). As shown in Fig. 6B, startle amplitude was not affected by the factors analysed (P's $>.25$).

3.4. PCP-disrupted PPI

3.4.1. SB-258741

As shown in Fig. 7A, there was a significant PPI effect, as reflected in the main effect of prepulse, $F(2,84)=98.61$, $P<.001$. There was a significant disruptive effect of PCP reflected by the main effect of treatment, $F(1,42)=17.52$, $P<.001$. SB-258741 enhanced PPI, as reflected by a significant effect of drug, $F(2,42)=6.49$, $P<.005$. According to the double significant effect of treatment and drug, a Fisher PLSD post hoc test showed that there was a significant difference between vehicle and PCP groups in the animal groups not treated with SB-258741 ($P<.02$) or treated with 2.3 mg/kg SB-258741 ($P<.001$). The animal group treated with SB-258741 at 9.1 mg/kg in addition to PCP was significantly different from the VEH–PCP-treated group ($P<.003$). As shown in Fig. 7B, a significant Treatment × Drug interaction, $F(2,42)=3.93$, $P<.03$, completed with a Fisher PLSD post hoc test confirmed that both doses of SB-258741 ($P<.03$) antagonised PCP-reduced startle amplitude ($P=.053$) in this experiment. Consequently, we cannot exclude the possibility that the effect of SB-258741 on the PCP-disrupted PPI model is simply a consequence of SB-258741 reversing PCP-reduced startle amplitude of rats.

3.4.2. Risperidone

As shown in Fig. 8A, there was a significant PPI effect, as reflected in the main effect of prepulse, $F(2,84)=66.96$, $P<.001$. There was a significant disruptive effect of PCP, $F(1,42)=51.29$, $P<.001$, which was dose-dependently reversed by risperidone as reflected by the significant Treatment × Drug interaction, $F(2,42)=3.80$, $P<.04$. Post

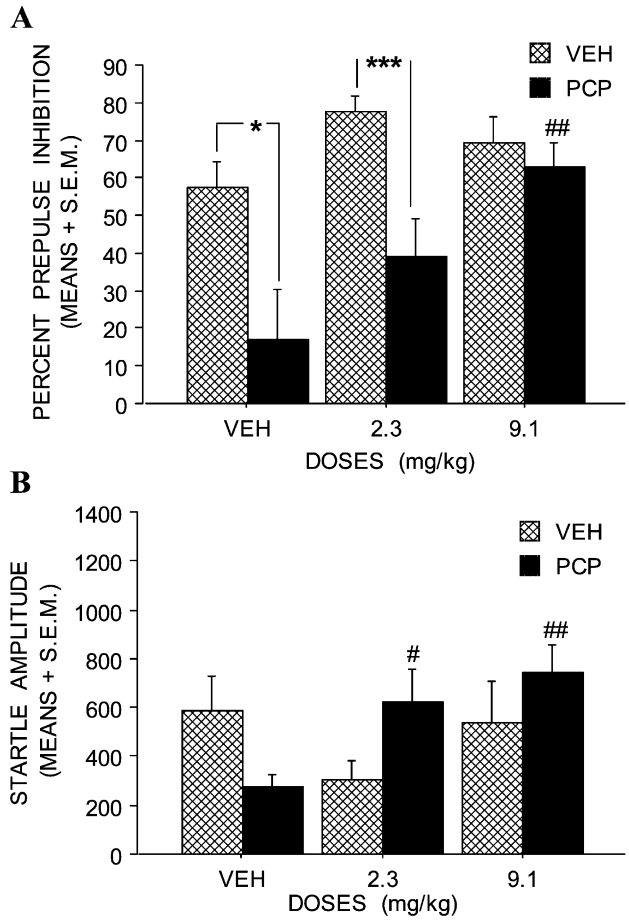

Fig. 7. (A) Effects of acute treatment with SB-258741 on PCP (2 mg/kg)-disrupted PPI in rats. (B) Effects of SB-258741 on startle amplitude. $n=8$ per Drug × Treatment group. * $P<.05$; *** $P<.001$. # $P<.05$ versus VEH–PCP; ## $P<.01$ versus VEH–PCP.

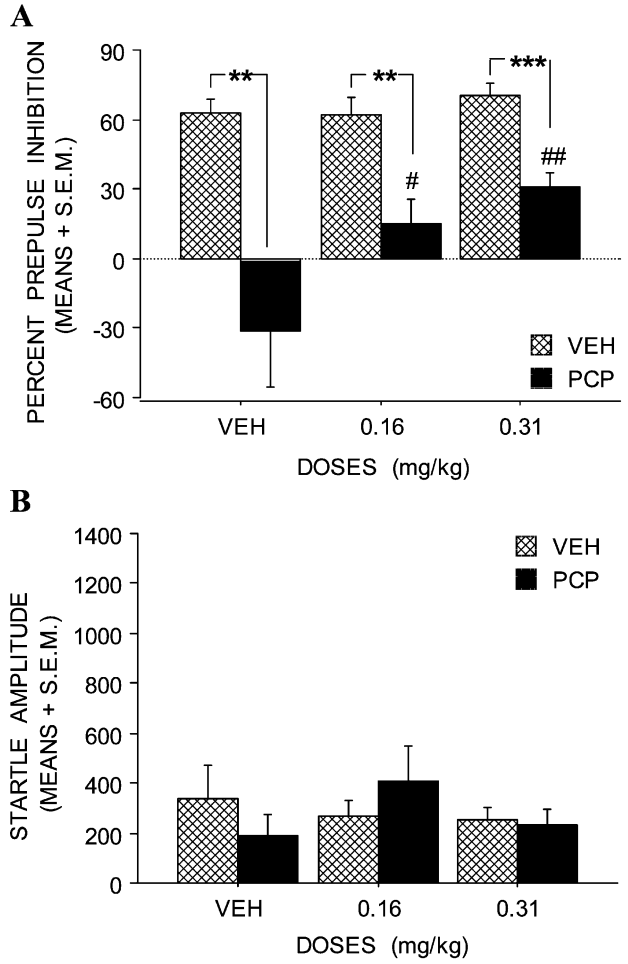

Fig. 8. (A) Effects of acute treatment with risperidone on PCP (2 mg/kg)-disrupted PPI in rats. (B) Effects of risperidone on startle amplitude. $n=8$ per Drug × Treatment group. ** $P<.01$; *** $P<.001$. # $P<.05$ versus VEH–PCP; ## $P<.01$ versus VEH–PCP.

hoc analysis indicated a significant difference between vehicle and PCP groups in the three treatment groups (P's $<.003$). However, this analysis showed that those groups treated with risperidone at both doses in addition to PCP were significantly different from the PCP–VEH-treated group (P's $<.04$). As shown in Fig. 8B, startle amplitude was not affected by factors analysed (all P's $>.30$).

3.5. PCP-disrupted SIT

3.5.1. SB-258741

3.5.1.1. Hyperactivity. A significant Drug × Treatment interaction, $F(2,66)=8.66$, $P<.001$, showed that the various doses of SB-258741 interfered differently with PCP on activity (Fig. 9A). At the lowest dose (2.3 mg/kg), SB-258741 decreased activity similarly in the vehicle- and PCP-treated groups. At the highest dose (9.1 mg/kg), reduction of spontaneous activity induced by SB-258741 in addition to vehicle was much more pronounced than in the PCP-treated group.

3.5.1.2. Active SIT. As shown in Fig. 9B, PCP disrupted active SIT of rats, $F(1,66)=88.14$, $P<.001$, and SB-258741 reduced the level of active SIT of both vehicle-

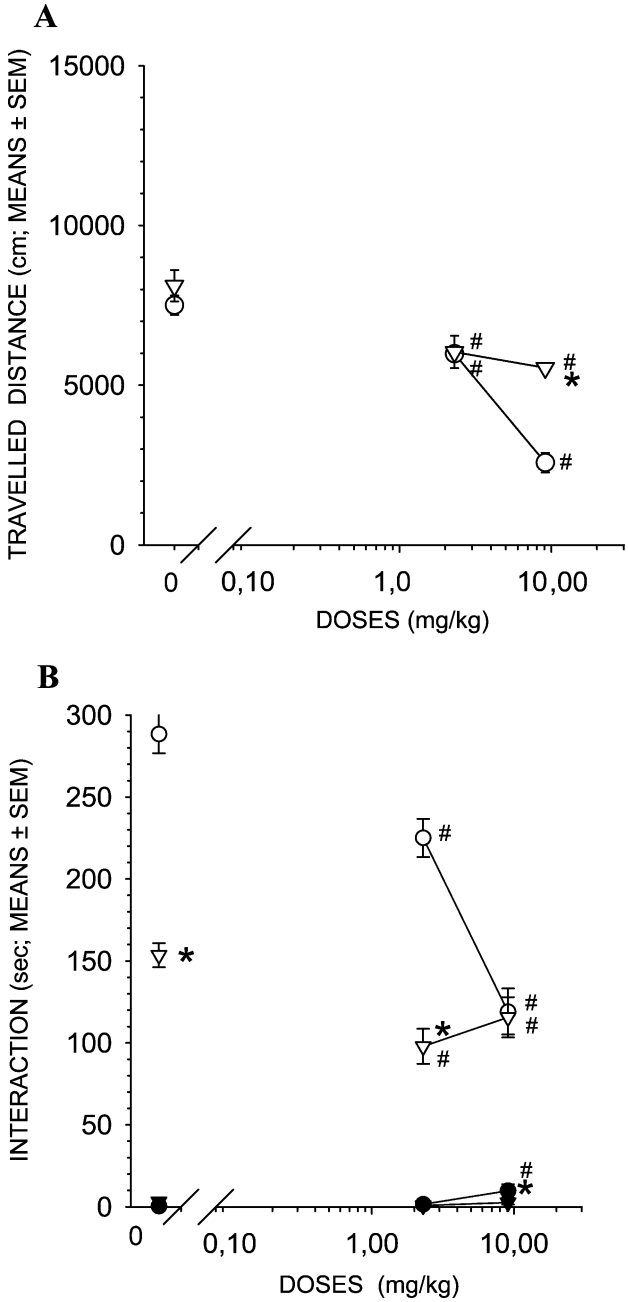

Fig. 9. (A) Effects of subchronic (3 days) treatment with SB-258741 on PCP (2 mg/kg)-induced hyperactivity in rats. (B) Effects of subchronic (3 days) treatment with SB-258741 on PCP-disrupted SIT in rats. $n=6$ pairs of rats per Drug × Treatment group. Symbols: (○) SB-258741 in combination with vehicle; (▽) SB-258741 in combination with PCP. Open symbols indicate active SIT, and dark symbols indicate passive SIT * $P<.05$ versus the respective vehicle-treated group. # $P<.05$ versus VEH–VEH or VEH–PCP.

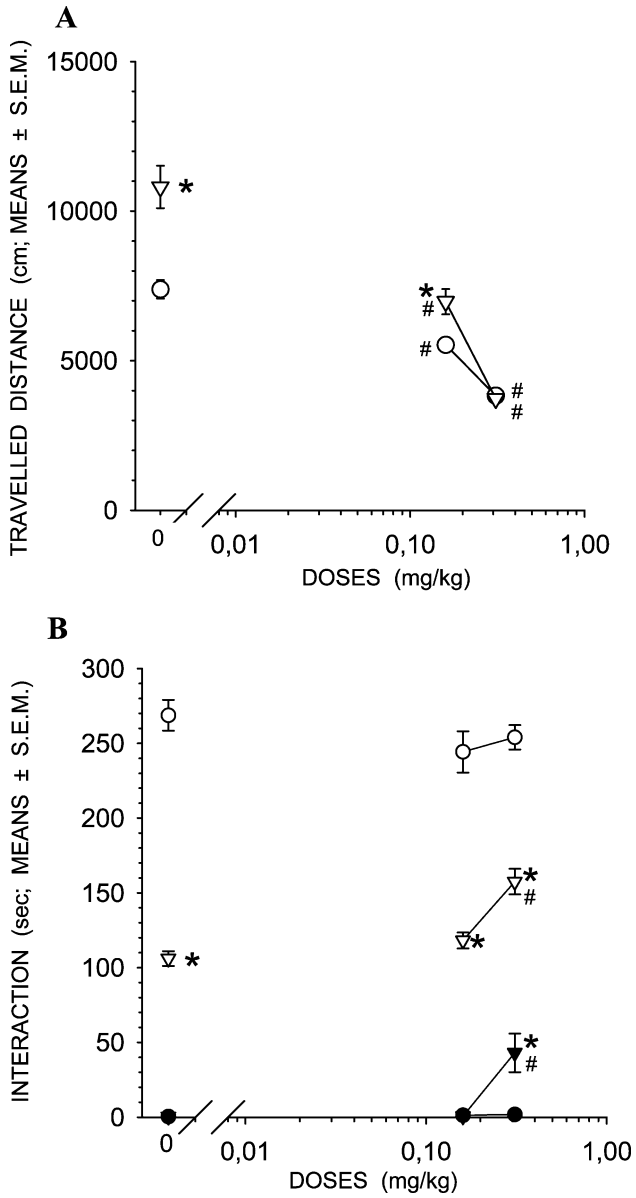

Fig. 10. (A) Effects of subchronic (3 days) treatment with risperidone on PCP (2 mg/kg)-induced hyperactivity in rats. (B) Effects of subchronic (3 days) treatment with risperidone on PCP-disrupted SIT in rats. $n=6$ pairs of rats per Drug × Treatment group. Symbols: (○) risperidone in combination with vehicle; (▽) risperidone in combination with PCP. Open symbols indicate active SIT, and dark symbols indicate passive SIT $*P<.05$ versus the respective vehicle-treated group. $\#P<.05$ versus VEH–VEH or VEH–PCP.

and PCP-treated rats, $F(2,66)=40.68$, $P<.001$. A significant Drug × Treatment interaction, $F(2,66)=20.46$, $P<.001$, as shown by a post hoc analysis demonstrated that 2.3 mg/kg of SB-258741 reduced the level of active SIT to the same extent in the vehicle- and PCP-treated groups. At 9.1 mg/kg, SB-258741 strongly reduced the level of active SIT in the vehicle-treated group in comparison to the PCP-treated group. This reduction of active SIT appears directly correlated to the reduction of activity described previously.

3.5.1.3. Passive SIT. As shown in Fig. 9B, SB-258741 given at 9.1 mg/kg induced marginal passive SIT, $F(2,66)=3.80$, $P<.05$, which is also related to the reduction of spontaneous activity previously observed at this dose.

3.5.2. Risperidone

3.5.2.1. Hyperactivity. As shown in Fig. 10A, PCP induced hyperactivity in rats, $F(1,66)=22.48$, $P<.001$, and risperidone reduced the activity of these rats, $F(2,66)=80.04$, $P<.001$. A significant Drug × Treatment interaction, $F(2,66)=8.66$, $P<.001$, completed by a post hoc analysis showed that the lowest dose (0.16 mg/kg) of risperidone normalised hyperactivity in the PCP-treated groups and slightly reduced the spontaneous activity of rats. However, there was still a significant difference between the activity level of the VEH- and PCP-treated groups at this dose of risperidone. At the highest dose (0.31 mg/kg), the risperidone-induced decrease in activity was much more pronounced in the PCP-treated group than in the vehicle-treated group, with the consequence that both groups reached the same low level of activity.

3.5.2.2. Active SIT. As shown in Fig. 10B, PCP disrupted active SIT of rats, $F(1,66)=303.33$, $P<.001$, and risperidone increased the level of active SIT of rats as demonstrated by a main effect of drug, $F(2,66)=3.99$, $P<.05$. A significant Drug × Treatment interaction, $F(2,66)=6.73$, $P<.005$, completed by a post hoc analysis demonstrated that at 0.16 mg/kg, risperidone did not reverse PCP-disrupted SIT. At 0.31 mg/kg, risperidone partially reversed this disruptive effect of PCP, without significant modification of the spontaneous SIT of rats.

3.5.2.3. Passive SIT. As shown in Fig. 10B, 0.31 mg/kg induced passive SIT, $F(2,66)=11.29$, $P<.001$, which is correlated to the increase in active SIT previously described at this dose, and probably to the reduction of activity as well.

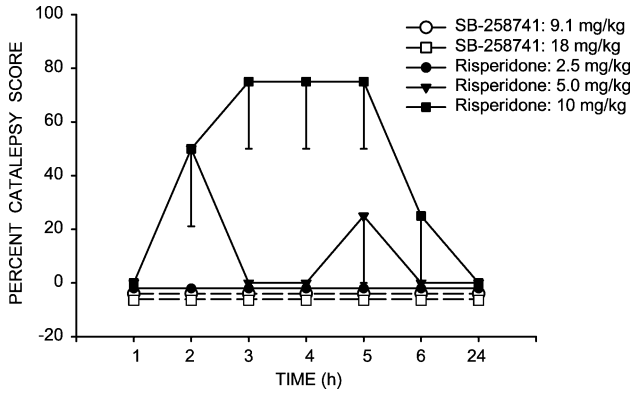

Fig. 11. Cataleptogenic effect of SB-258741 (dash line) and risperidone (full line). $n=4$ per drug group.

3.6. Catalepsy

3.6.1. SB-258741

As shown in Fig. 11, SB-258741 did not induce any catalepsy for doses up to 18 mg/kg and up to 24 h after administration (P's = 1).

3.6.2. Risperidone

As shown in Fig. 11, risperidone induced dose-dependent catalepsy, $F(2,9) = 6.84$, $P < .02$, for the two highest doses.

4. Discussion

This is the first attempt to study the potential antipsychotic activity of a selective 5-HT_7 receptor antagonist. SB-258741 is 200-fold selective over other 5-HT or dopaminergic receptors (see compound "13" in Lovell et al., 2000) and has significant effects in two models known to be predictive of antipsychotic action: D-amphetamine-induced hyperactivity in rats (Arnt, 1995, 2000) and PCP-disrupted PPI (Mansbach and Geyer, 1989; Bakshi and Geyer, 1995; Bakshi et al., 1994; Yamada et al., 1999). However, in both cases, this effect might be a consequence of a modification of motricity induced by SB-258741. SB-258741 dose-dependently antagonised D-amphetamine-induced hyperactivity but also reduced spontaneous activity of rats at the same doses (4.6 and 9.1 mg/kg). It is only at 2.3 mg/kg that SB-258741 antagonised specifically D-amphetamine-induced hyperactivity. It is also noted that rats treated with SB-258741 at this mid-dose were more active than VEH–AMPH rats when the effect of D-amphetamine elapsed. Thus, SB-258741 seems to have a dual effect on activity of rats. At high doses, it reduced activity of rats during the exploratory part of the test, but at lower doses, it keeps rats more active than VEH–AMPH-treated rats. This observation is difficult to explain, as we cannot conclude whether this effect is specific to SB-258741 or to a possible metabolite, due to a real pharmacodynamic effect of SB-258741 or to an effect on the pharmacokinetics of D-amphetamine. In comparison, risperidone dose-dependently reversed D-amphetamine-induced hyperactivity without inducing such hyperactivity at the end of the session. At the two highest doses risperidone also reduced activity of rats during the exploratory period, which questions the validity of the results obtained versus D-amphetamine in this model. In a similar fashion to SB-258741, risperidone significantly reduced the spontaneous activity of rats in the motility test when tested with doses that were effective in the D-amphetamine-induced hyperactivity model. Consequently, the effect of SB-258741 in this model is inconclusive as this compound antagonised D-amphetamine at doses that were similar to those inhibiting spontaneous activity of rats. However, risperidone, an antipsychotic with demonstrated clinical effect, suppresses locomotor activity at the same doses that were effective in the D-amphetamine-induced hyperactivity model.

SB-258741 also dose-dependently normalised the effect of PCP in PCP-disrupted PPI in rats. However, on both doses tested, SB-258741 also normalised the reduction of startle amplitude induced by PCP. Thus, the beneficial effect of SB-258741 in PCP-disrupted PPI is certainly not independent of its effect on the startle amplitude of rats in the PCP-treated groups. This means that the positive effect of SB-258741 in this model is probably not related to a sensory gating process (Swerdlow et al., 2000). Consequently, the beneficial effect of SB-258741 in the PCP-disrupted PPI model should be considered carefully, and it seems difficult to predict an antipsychotic action for SB-258741 in this model as well. Contrary to SB-258741, risperidone antagonised PCP-disrupted PPI without effect on startle amplitude and under condition at which PCP did not affect startle amplitude as well. This result with risperidone confirms another previous report (Yamada et al., 1999) but is contrary to another publication showing that risperidone was not effective in this model (Swerdlow et al., 1996). It shows that like clozapine (Bakshi et al., 1994; Yamada et al., 1999; Johansson et al., 1994), the antipsychotic-like effect of risperidone in this model is variable between laboratories.

On D-amphetamine-disrupted PPI, a model predictive of antipsychotic action (Swerdlow and Geyer, 1993; Paabøl Andersen and Pouzet, 2001), SB-258741 did not reverse the effect of D-amphetamine. This effect is opposite to the one obtained with risperidone but is consistent with the lack of clear effect obtained with SB-258741 on both the D-amphetamine-induced hyperactivity and PCP-disrupted PPI models. Thus, according to results obtained in these three models, the probability that SB-258741 can, on its own, treat positive symptoms in schizophrenic patients seems weak.

With respect to the model indicative of effect on negative symptoms of schizophrenia, i.e., PCP-disrupted SIT in rats (Sams-Dodd, 1997, 1998), SB-258741 did not show any beneficial effect, as it enhanced the disruptive effect of PCP on active SIT instead of reducing it. Contrary to the motility test, all doses tested for both SB-258741 and risperidone reduced spontaneous activity. This difference can certainly be explained by different experimental conditions (acute versus 3 days of treatment; macrolon type III cages versus large arena as boxes for testing). However, only risperidone antagonised PCP-disrupted SIT, although passive SIT was increased to the same extent. Consequently, specific antagonism of the 5-HT_7 receptor does not seem to explain the beneficial effect of risperidone observed in this study and previously reported for this model (Sams-Dodd, 1997). However, the possibility that a combination of the effect of 5-HT_7 receptor antagonism plus the dopaminergic D_2 and/or 5-HT_{2A} receptor antagonism of risperidone has a positive effect in this model cannot be excluded.

As regards side effects, SB-258741 tested at 18 mg/kg did not induce catalepsy. As this model is a predictor of

EPS (Arnt, 1982), we can exclude the possibility that antagonism on the 5-HT_7 receptor would induce EPS. This lack of cataleptogenic effect of SB-258741 is in contrast to the induction of catalepsy obtained with risperidone in this study. As previously suggested, we cannot exclude the possibility that a combination of antagonism on the 5-HT_7 receptor plus the dopaminergic D_2 and/or 5-HT_{2A} receptor would change the pattern of results obtained both in models predictive of antipsychotic action and EPS. However, a preliminary study conducted in our laboratory, testing SB-258741 in combination with the dopaminergic D_2 receptor antagonist remoxipride, or the 5-HT_{2A} receptor antagonist MDL-100151 in another model predictive of antipsychotic action, PCP-induced hyperactivity in mice (Gleason and Shannon, 1997), did not show any enhancement of the beneficial effect of these two compounds (unpublished data).

This study demonstrated that we should not expect to obtain an antipsychotic action with SB-258741. According to the specificity of this compound (see compound "13" in Lovell et al., 2000), it seems doubtful that other specific 5-HT_7 receptor antagonists would have any antipsychotic action on their own as well. On the other hand, a lack of information about the pharmacokinetics of this compound does not permit exclusion of the possibility that its central effect is weak due to a poor brain penetrability. However, two arguments go against this hypothesis. First, we described in this study that SB-258741 antagonised the effect of PCP on startle amplitude of rats but did not affect the spontaneous startle amplitude on its own. This suggests an effect of this compound on central structures involved in regulation of startle. Second, SB-258741 and SB-269970 have very similar chemical structures (Lovell et al., 2000), with similar biological activities, and SB-269970 is known to have a good brain penetrability (Hagan et al., 2000).

Acknowledgments

The authors thank Mrs. Lisbeth Jeel and her animal facility team for their invaluable help with animal care, as well as technicians of the Psychopharmacology–Psychosis Department at Lundbeck for their assistance in conducting these experiments. The authors would also like to thank Dr. Robert Dancer (H. Lundbeck) for his comments and assistance in the preparation of this publication.

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