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Pharmacology, Biochemistry and Behavior 71 (2002) 635–643

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Effects of the 5-HT$_6$ receptor antagonist, SB-271046, in animal models for schizophrenia

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Received 19 July 2001; received in revised form 22 October 2001; accepted 31 October 2001

Abstract

The 5-HT$_6$ receptor is targeted by several new antipsychotics such as clozapine, olanzapine, and sertindole. We studied the effect of SB-271046 [5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide], a specific 5-HT$_6$ receptor antagonist, in three models for the positive symptoms of schizophrenia—D-amphetamine-induced hyperactivity, and D-amphetamine- or phencyclidine (PCP)-disrupted prepulse inhibition (PPI). We also tested this compound in a model for the negative symptoms of schizophrenia, 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-271046 was compared to clozapine in all models tested. This study showed that SB-271046 had no beneficial effect in PCP-disrupted SIT. However, SB-271046 dose-dependently normalised D-amphetamine-disrupted PPI, but did not reverse PCP-disrupted PPI. In addition, SB-271046 did not antagonise D-amphetamine-induced hyperactivity. Thus, this specific 5-HT$_6$ receptor antagonist was associated with a clear positive outcome in only one model for the positive symptoms of schizophrenia, and had no beneficial effect in the model for negative symptoms. Consequently, it is clear that SB-271046 is not expected to have an antipsychotic efficacy, at least when given as monotherapy.

Keywords: SB-271046; 5-HT$_6$; Prepulse inhibition; Schizophrenia; Activity; Social interaction; Catalepsy

1. Introduction

The 5-HT$_6$ receptor is targeted by several second-generation antipsychotics such as clozapine, olanzapine, and sertindole (Roth et al., 1994; Arnt and Skarsfeldt, 1998), but also by some classical antipsychotics (Roth et al., 1994). In in vitro assays, clozapine has been shown to down-regulate the expression of the 5-HT$_6$ receptor (Frederick and Meador-Woodruff, 1999) and 5-HT$_6$ receptor density (Zhukovskaya and Neumaier, 2000). This receptor is of additional interest because of its specific expression in the CNS (Monsma et al., 1993). Immunohistological studies have demonstrated that the 5-HT$_6$ receptor is localised at the level of the cortex (frontal and entorhinal), the nucleus accumbens, the striatum, and the hippocampus of rats (Gérard et al., 1997; Hamon et al., 1999). This localisation suggests a possible beneficial role for the treatment of schizophrenia. However, the role per se of this receptor in the clinical action of antipsychotics is still unknown (see, for review, Branchek and Blackburn, 2000). Pharmacological studies with compounds having a moderate affinity (pK_i = 7.3) for the 5-HT$_6$ receptor (Ro 04-6790) (Sleight et al., 1998) but poor brain penetrability (< 1%) (or studies with oligonucleotide antisense for the receptor) have suggested that this receptor could be of interest for the treatment of cognitive dysfunctions via its regulation of the activity of the cholinergic system (Bourson et al., 1995, 1998). Studies with antisense oligonucleotide technologies have also suggested that this receptor could mediate anxiety responses (Otano et al., 1999; Yoshioka et al., 1998), and could be involved in the regulation of body weight via modulation of feeding (Bentley et al., 1997). However, no preclinical studies have demonstrated the effect or lack of effects of a 5-HT$_6$ receptor antagonist in models related to schizophrenia.

This lack of knowledge is largely due to a lack of potent and selective 5-HT$_6$ receptor antagonists. Recently, however, SmithKline Beecham have generated series of 5-HT$_6$ receptor antagonists, SB-271046 [5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide], showing high affinity for the 5-HT$_6$ receptor

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0091-3057/02/$ – see front matter
PII: S0091-3057(01)00743-2

($pK_i = 8.9$) and a more than 200-fold selectivity over a panel of related receptors (Bromidge et al., 1999; Routledge et al., 2000). This compound has been proposed to act as a cognitive enhancer (Rogers et al., 1999). This is supported by microdialysis studies showing that this compound increased glutamate and aspartate levels in the frontal cortex without affecting noradrenaline, dopamine, or 5-HT levels in the frontal cortex and striatum (Dawson et al., 2000). This compound has also been shown to dose-dependently increase the threshold for generalized seizures in rats, and has consequently also been considered to be of interest as an anticonvulsant (Routledge et al., 2000).

In order to clarify the potential role of a 5-HT_6 receptor antagonist for the treatment of schizophrenia, we studied the effect of SB-271046 in three models related to positive symptoms of schizophrenia, and which are considered predictive of antipsychotic action. D-amphetamine-induced hyperactivity (Arnt, 1995), and D-amphetamine- (Feifel and Reza, 1999; Paabøl Andersen and Pouzet, 2001) or phencyclidine (PCP)- (Mansbach and Geyer, 1989) antagonised prepulse inhibition (PPI) in rats. We also tested this compound in a putative model for negative symptoms, PCP-disrupted social interaction (SIT) in rats (Corbett et al., 1995; Sams-Dodd, 1995, 1996, 1998). Side-effects potential was evaluated by testing its ability to inhibit spontaneous motility, and to induce catalepsy in rats (Arnt, 1982).

The effect of SB-271046 was compared to clozapine in all models tested, since clozapine (as with olanzapine) has one of the highest affinities for the 5-HT_6 receptor of all marketed antipsychotics (Roth et al., 1994). The potential role of a 5-HT_6 receptor antagonist for the 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 Møllegor (Denmark). Rats used in the PPI studies 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 paradigms, but were generally 200–250 g when used in the catalepsy, motility, and D-amphetamine hyperactivity paradigms. Animals were housed four per cage for the motility, D-amphetamine hyperactivity, and catalepsy paradigms, but two per cage for the SIT and PPI paradigms. They were housed in climate-controlled animal facilities (temperature at 21 ± 2 °C and relative humidity at $60 \pm 10\%$). Animals were housed and studied 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 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. Material 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 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. PPI

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. SIT

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, NL) in order to evaluate activity, active, and passive SIT of rats. SIT is defined as the duration during which animals have their centres of gravity less than 20 cm from each other. During each single trial, SIT was subsequently divided into an active and passive component for each rat based upon whether the rat moved around in the arena 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-271046 and PCP (2 mg/kg) for three consecutive days, 24 and 48 h before the last injection. Subjects were tested on the last day of injection, 45 min after the last two consecutive injections of SB-271046 and PCP. 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 cataleptic. Observation for catalepsy occurred once each hour during the first 6 h, and once 24 h after dosing.

2.3. Drugs

Phencyclidine 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 dissolved in 0.9% NaCl. SB-271046 hydrochloride was synthesised at H. Lundbeck and dissolved in water. Clozapine was supplied courtesy of Novartis (Basel) and dissolved in 0.1 M HCl diluted in 0.9% NaCl. Vehicle controls were always 0.9% NaCl. All compounds were injected at volumes of 5 ml/kg. All doses are expressed in milligrams per kilogram, according to the amount of free base, except for PCP and D-amphetamine, which are expressed as their respective salts. All compounds were administrated via subcutaneous route.

2.4. Data analysis

2.4.1. D-Amphetamine-induced hyperactivity

Hyperactivity was analysed by two-way repeated measures ANOVA consisting of a between-subjects factor of drug (six levels for SB-271046: VEH–VEH; VEH–AMPH; 0.60, 4.5, 9.0, and 18 mg/kg AMPH; seven levels for clozapine: VEH–VEH; VEH–AMPH; 0.31, 0.63, 1.3, 2.5, and 10 mg/kg AMPH), and a within-subjects factor of time (8 bins of 15 min each). A post hoc analysis via Fisher's PLSD was performed to complete the analysis of activity between groups, and of within time intervals. Several replications were performed with proper controls for this model and the motility in order to define a full dose–response effect. Consequently, the population involved in the control groups is larger than in the drug groups.

2.4.2. Motility

Motility was analysed by one-way ANOVA consisting of a between-subjects factor of drug (five levels for SB-271046: VEH; 1.2, 4.5, 9.0, and 18 mg/kg; five levels for clozapine: VEH; 0.16, 0.63, 1.3, and 2.5 mg/kg). A post hoc analysis via Fisher's PLSD was performed to complete the analysis of motility between groups.

2.4.3. D-Amphetamine- or PCP-disrupted PPI

The startle amplitude was analysed by 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, and 9.0 mg/kg for SB-271046, and VEH; 2.5, 5.0, or 10 mg/kg for clozapine). 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 via Fisher's PLSD was performed to complete

the analysis of the startle amplitude and mean percent PPI between groups.

2.4.4. PCP-disrupted SIT

Hyperactivity, active, and passive SIT were analysed by 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; 4.5 and 14 mg/kg for SB-271046). A post hoc analysis via Fisher's LSD was performed to complete the analysis of hyperactivity and SIT between groups. Clozapine was not tested in this model, since its effects have already been extensively described via data generated in our own laboratories (Sams-Dodd, 1996, 1998).

2.4.5. Catalepsy

Catalepsy was analysed by two-way repeated measures ANOVA consisting of a between-subjects factor of drug (three levels for SB-271046: 4.5, 9.0, and 18 mg/kg; four levels for clozapine: 2.5, 5.0, 10, and 20 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 raw data and were calculated using the SigmaStat (2.03) software system [except PPI data, which were analysed using the StatView (5.0) software system].

3. Results

3.1. D-Amphetamine-induced hyperactivity

3.1.1. SB-271046

D-Amphetamine induced hyperactivity (Fig. 1) at all time intervals (P's $< .05$). The 6×8 (Drug $\times$ Time) repeated-measures ANOVA [$F(35,714) = 6.47$, $P < .001$] completed by a post hoc analysis showed that SB-271046 (18 mg/kg) reduced the activity of rats during the 15 min exploratory period when directly compared to VEH–VEH-treated animals. SB-271046 weakly antagonised the effect of D-amphetamine only during the first 45 min of the observation.

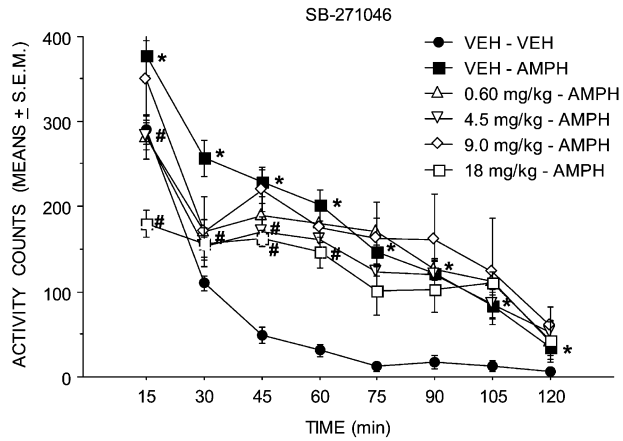

Fig. 1. Effects of acute treatment with SB-271046 on D-amphetamine-induced (0.5 mg/kg) hyperactivity in rats. $n = 8$ per Drug $\times$ Treatment group, except $n = 24$ in the 4.5-mg/kg AMPH and $n = 32$ in VEH–VEH and VEH–AMPH groups. $^{*}P < .05$ versus VEH–VEH; $^{\#}P < .05$ versus VEH–AMPH.

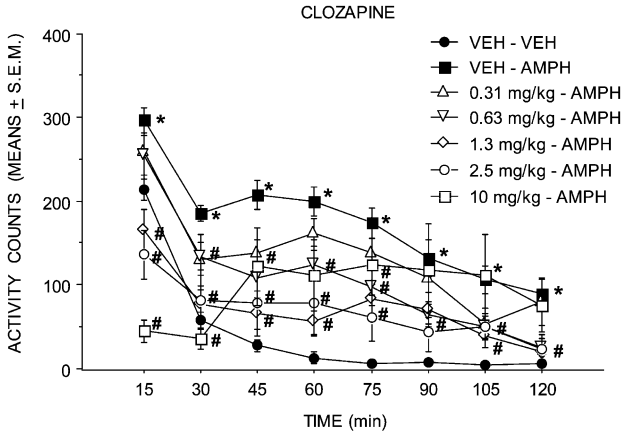

Fig. 2. Effects of acute treatment with clozapine on D-amphetamine-induced (0.5 mg/kg) hyperactivity in rats. $n = 7$ per Drug $\times$ Treatment group, except $n = 8$ in the 2.5-mg/kg AMPH, $n = 23$ in the VEH–AMPH group, and $n = 15$ in VEH–VEH and 0.63-mg/kg AMPH groups. $^{*}P < .05$ versus VEH–VEH; $^{\#}P < .05$ versus VEH–AMPH.

3.1.2. Clozapine

D-Amphetamine induced hyperactivity (Fig. 2) at all time intervals (P's $< .001$). The 7×8 (Drug $\times$ Time) repeated-measures ANOVA [$F(42,525) = 5.48$, $P < .001$] completed by a post hoc analysis showed that, with the exception of the lowest dose (0.31 mg/kg) and, surprisingly, the highest dose (10 mg/kg), all other doses tested antagonised D-amphetamine-induced hyperactivity at all time intervals (P's $< .05$). During the exploratory period, the two highest doses of clozapine (2.5 and 10 mg/kg), given in combination with

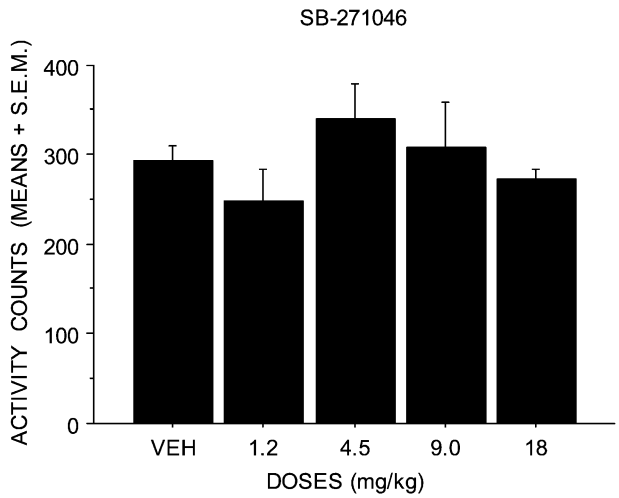

Fig. 3. Effects of acute treatment with SB-271046 on spontaneous motility in rats. $n = 4$ per drug group, except $n = 12$ in VEH and $n = 8$ in 4.5 mg/kg.

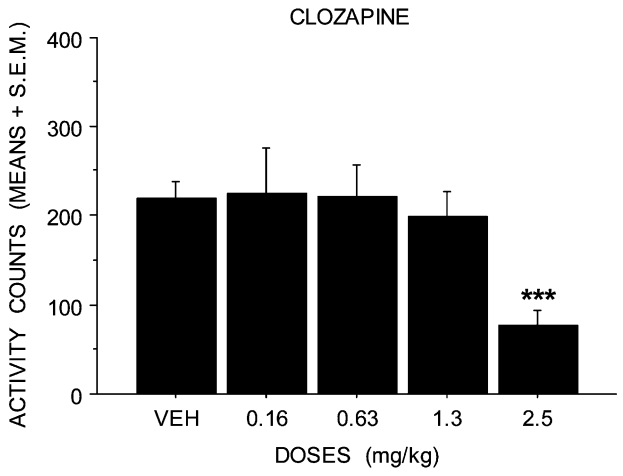

Fig. 4. Effects of acute treatment with clozapine on spontaneous motility in rats. $n=4$ per drug group, except $n=16$ in VEH and $n=8$ in 2.5 mg/kg. ***$P<.001$ versus VEH.

D-amphetamine, reduced the activity of rats when directly compared to VEH–VEH-treated animals (P's $<.02$).

3.2. Motility

3.2.1. SB-271046

SB-271046 had no effect on the motility of rats at any of the doses tested [$F(4,27)=1.06$, $P>.35$] (Fig. 3).

3.2.2. Clozapine

A significant main effect of drug [$F(4,31)=6.62$, $P<.001$] completed by a post hoc analysis demonstrated that clozapine reduced the motility of rats (P's $<.001$), but only at the 2.5 mg/kg dose (Fig. 4).

3.3. D-Amphetamine-disrupted PPI

3.3.1. SB-271046

As shown in Fig. 5A, there was a significant PPI effect, as reflected in the main effect of prepulse [$F(2,84)=101.09$, $P<.001$]. A significant main effect of drug [$F(2,42)=5.89$, $P<.01$] demonstrated that PPI had a higher amplitude in the group treated with SB-271046 9.0 mg/kg ($P<.005$). D-Amphetamine disrupted PPI

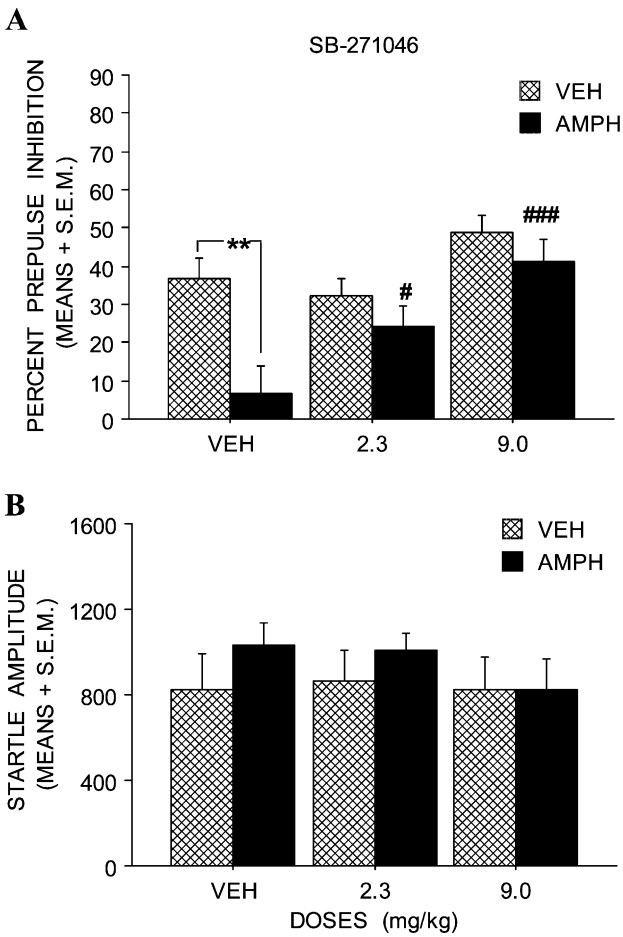

Fig. 5. (A) Effects of acute treatment with SB-271046 on D-amphetamine-disrupted (2.0 mg/kg) PPI in rats. (B) Effects of SB-271046 on startle amplitude in rats. $n=8$ per Drug × Treatment group. **$P<.01$; $^{\#}P<.05$ versus VEH–AMPH; $^{\#\#\#}P<.001$ versus VEH–AMPH.

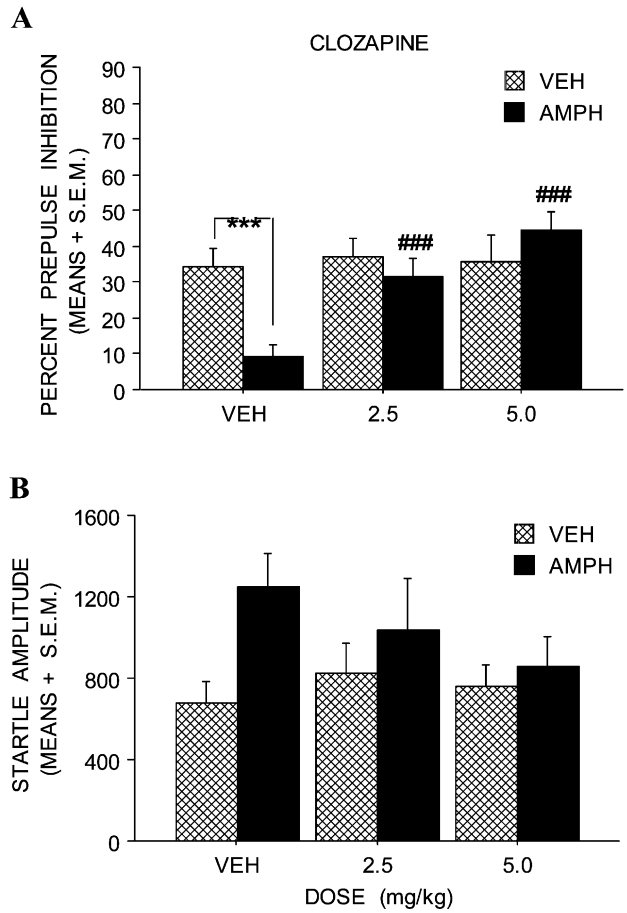

Fig. 6. (A) Effects of acute treatment with clozapine on D-amphetamine-disrupted (2.0 mg/kg) PPI in rats. (B) Effects of clozapine on startle amplitude in rats. $n=8$ per Drug × Treatment group. ***$P<.001$; $^{\#\#\#}P<.001$ versus VEH–AMPH.

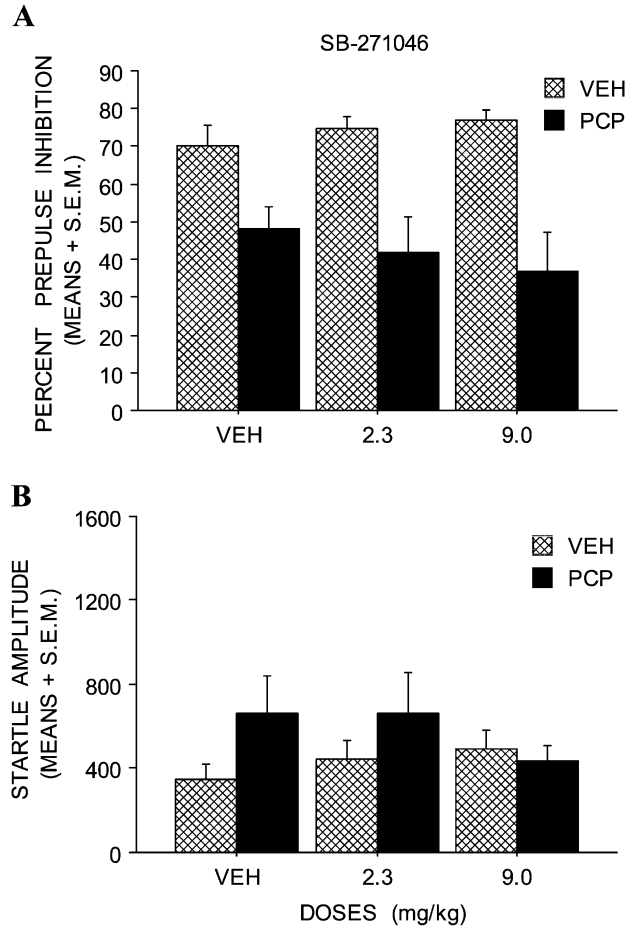

Fig. 7. (A) Effects of acute treatment with SB-271046 on PCP- (2 mg/kg) disrupted PPI in rats. (B) Effects of SB-271046 on startle amplitude in rats. $n=8$ per Drug × Treatment group.

[$F(1,42)=6.91$, $P<.02$]. A post hoc analysis indicated a significant difference only between vehicle and D-amphetamine groups in those animals receiving no treatment with SB-271046 ($P<.005$). Further, SB-271046 (2.3; 9.0 mg/kg) reversed the disruptive effect of D-amphetamine ($P<.05$ and $P<.001$, respectively). As shown on Fig. 5B, startle amplitude was not affected by any of the factors analysed (all P's >.30).

3.3.2. Clozapine

As shown in Fig. 6A, there was a significant PPI effect, as reflected in the main effect of prepulse [$F(2,84)=45.51$, $P<.001$]. Clozapine normalised D-amphetamine-disrupted PPI, as reflected by the near-significant Treatment × Drug interaction [$F(2,42)=2.88$, $P=.067$]. Post hoc analysis indicated a significant difference only between vehicle and D-amphetamine groups in those animals receiving no treatment with clozapine ($P<.001$). Both doses of clozapine reversed the disruptive effect of D-amphetamine (P's <.001). A main effect of treatment [$F(2,42)=4.95$, $P<.04$] reflected that D-amphetamine increased the startle amplitude of rats.

3.4. PCP-disrupted PPI

3.4.1. SB-271046

There was a significant PPI effect, as reflected in the main effect of prepulse [$F(2,84)=125.23$, $P<.001$]. As shown in Fig. 7A, SB-271046 did not antagonise (P's >.40) PCP-disrupted PPI [$F(1,42)=31.92$, $P<.001$]. As shown in Fig. 7B, startle amplitude was not affected by any of the factors analysed (P's >.10).

3.4.2. Clozapine

As shown in Fig. 8A, there was a significant PPI effect, as reflected in the main effect of prepulse [$F(2,76)=38.37$, $P<.001$]. Clozapine did not antagonise (P's >.55) the disruptive effect of PCP [$F(1,38)=14.40$, $P<.001$]. As shown in Fig. 8B, startle amplitude was not affected by any of the factors analysed (all P's >.20).

3.5. PCP-disrupted SIT

3.5.1. SB-271046

As shown in Fig. 9A, PCP-induced hyperactivity in rats [$F(1,66)=70.79$, $P<.001$] was not antagonised by

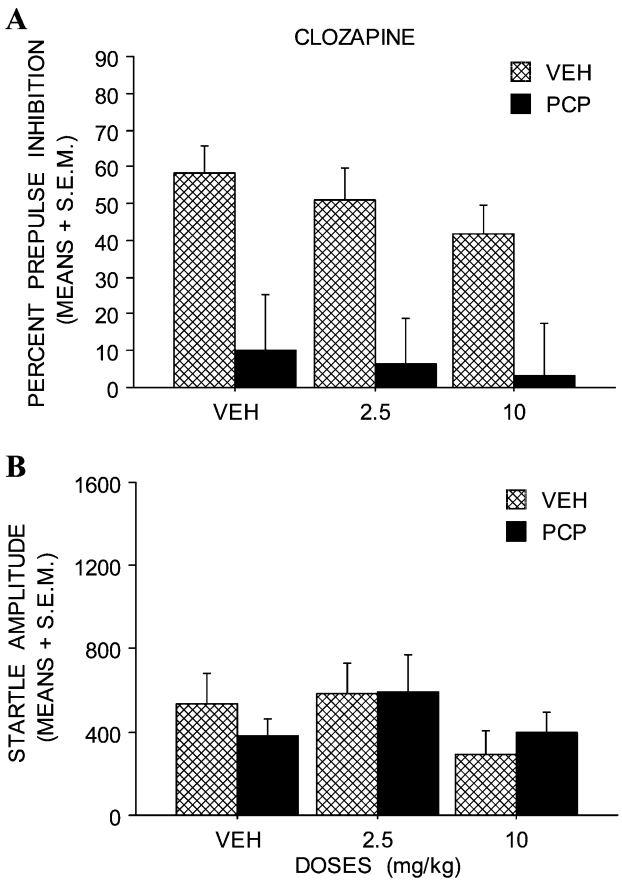

Fig. 8. (A) Effects of acute treatment with clozapine on PCP- (2.0 mg/kg) disrupted PPI in rats. (B) Effects of clozapine on startle amplitude in rats. $n=8$ in the VEH–VEH and VEH–AMPH groups, but $n=7$ in all other groups.

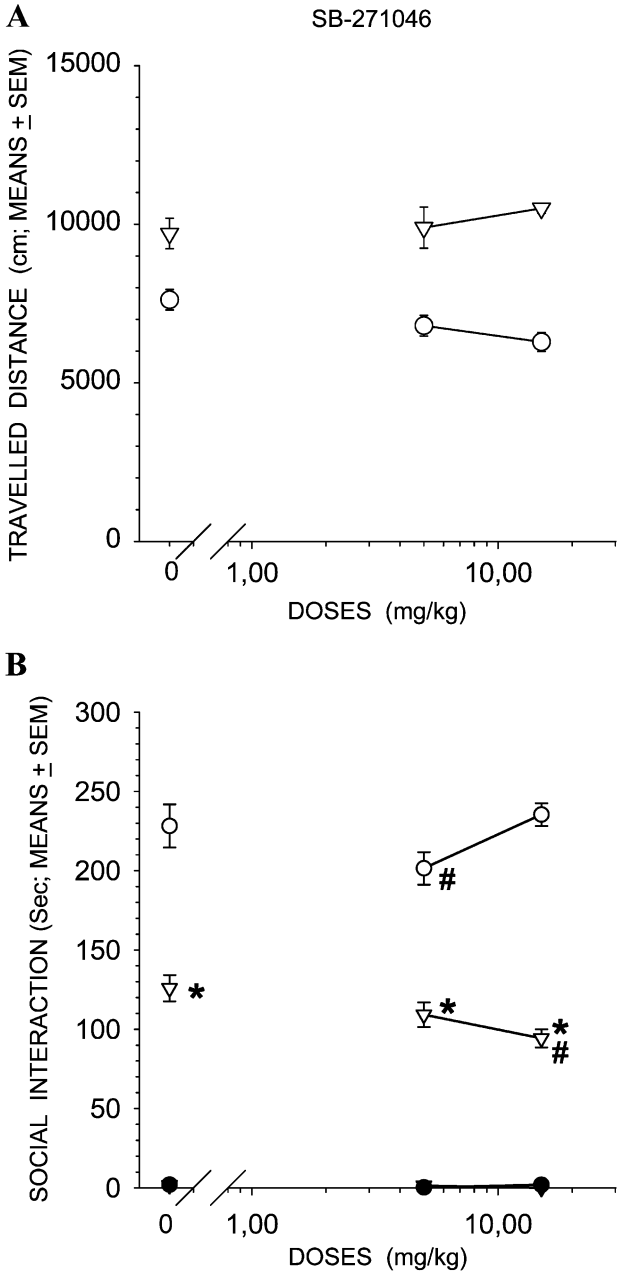

Fig. 9. (A) Effects of subchronic (3 days) treatment with SB-271046 on PCP- (2.0 mg/kg) induced hyperactivity in rats. (B) Effects of subchronic (3 days) treatment with SB-271046 on PCP-disrupted SIT in rats. $n=6$ pairs of rats per Drug × Treatment group. Symbols: (○) SB-271046 in combination with vehicle; (▽) SB-271046 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.

SB-271046 ($P>.07$) at the two doses tested. SB-271046 did not decrease spontaneous activity either ($P>.75$).

As shown in Fig. 9B, PCP disrupted the active SIT of rats [$F(1,66)=224.08$, $P<.001$] and a significant Drug × Treatment interaction [$F(2,66)=3.97$, $P<.03$], completed by a post hoc analysis, demonstrated that at 14 mg/kg, SB-271046 enhanced this disruptive effect of PCP. SB-271046 (4.5 mg/kg) reduced the level of spontaneous active SIT in rats.

As shown in Fig. 9B, SB-271046 did not induce passive SIT ($P>.25$).

3.6. Catalepsy

3.6.1. SB-271046

SB-271046 did not induce any catalepsy at any of the doses up to 18 mg/kg, and for up to 24 h after administration (P's = 1) (data not shown).

3.6.2. Clozapine

Clozapine did not induce catalepsy at any of the doses up to 20 mg/kg, and for up to 24 h after administration (P's = 1) (data not shown).

4. Discussion

This is the first attempt to study the potential antipsychotic activity of a selective 5-HT_6 receptor antagonist. SB-271046, which appears to be highly selective across a broad range of receptor targets (Bromidge et al., 1999; Routledge et al., 2000), has in this study a significant effect in only one model known to be predictive of antipsychotic action: D-amphetamine-disrupted PPI in rats (Feifel and Reza, 1999; Feifel et al., 1999b; Paabøl Andersen and Pouzet, 2001). The effect obtained on this model is similar to the one obtained with clozapine in this study. Disruption of PPI by a dopamine enhancer is reversed by both classical and second-generation antipsychotics (Swerdlow and Geyer, 1993; Swerdlow et al., 1994; Yamada et al., 1999). This model can consequently be considered as predictive of a general antipsychotic effect in the clinic (Swerdlow and Geyer, 1993). However, in contrast to clozapine, SB-271046 did not reverse D-amphetamine-induced hyperactivity in rats — another model considered to be predictive for a general antipsychotic effect (Arnt, 1995, 2000). Consequently, the present results indicate some discrepancy between these two results.

Other compounds, such as oxytocin or caerulein, have been shown to antagonise D-amphetamine-disrupted PPI in rats (see, for review, Geyer et al., 2001). These compounds are without confirmed antipsychotic effect in the clinic, but show positive effects in other models that have been investigated in this study such as NMDA antagonist-disrupted PPI (Feifel and Reza, 1999) or D-amphetamine-induced hyperactivity (Feifel et al., 1999a). This is not the case with SB-271046, and it consequently suggests doubtful antipsychotic-like effect of this compound. The dose-dependent response of SB-271046 in the D-amphetamine–PPI model is also somewhat contradictory to microdialysis (Dawson et al., 2000) and pharmacological (Bourson et al., 1998) studies showing that the 5-HT_6 receptor is not involved in dopaminergic transmission. However, other

microdialysis studies suggest that the 5-HT_6 receptor may interact with dopaminergic mechanisms in the rat medial prefrontal cortex as antisense oligonucleotides partially antagonised the fluoxetine-induced cortical dopamine release (Matsumoto et al., 1999). Thus, we cannot exclude the possibility that in CNS structures involved in the PPI phenomenon, SB-271046 may directly or indirectly regulate dopaminergic function.

SB-271046 did not antagonise PCP-disrupted PPI. PPI disruption produced by noncompetitive NMDA antagonists (Mansbach and Geyer, 1989) is of interest since this is suggested to be specifically reversed by second-generation antipsychotics (Bakshi and Geyer, 1995; Bakshi et al., 1994; Yamada et al., 1999). On the other hand, controversial effects in this model have also been claimed for clozapine or risperidone (Johansson et al., 1994; Swerdlow et al., 1996). In support of the studies conducted by Johansson et al. (1994), we could not observe any effect of clozapine in this model. Our results suggest that the effect of clozapine in this rat model is weak (Johansson et al., 1994) and variable between laboratories (Geyer et al., 2001). According to the observed efficacy of risperidone in this model (Pouzet et al., 2002), we believe that we can exclude the possibility that a problem occurred with the set-up of our equipment. The reversal effect of SB-271046 on D-amphetamine-disrupted PPI suggested that this compound could have an antipsychotic-like effect, but this compound showed a lack of effect in PCP-disrupted PPI. Moreover, this lack of effect versus PCP is consistent with the lack of effect obtained with SB-271046 in the D-amphetamine-induced hyperactivity model. Thus, according to results obtained in these three models, the probability that SB-271046 can, on its own, treat positive symptoms in schizophrenic patients seems, as previously suggested, weak.

With respect to the proposed model of negative symptoms of schizophrenia, i.e., PCP-disrupted SIT in rats (Sams-Dodd, 1995, 1997, 1998), SB-271046 did not show any beneficial effect. Moreover, when given at the highest dose, it enhanced the disruptive effect of PCP on active SIT. Given at the lower dose, SB-271046 reduced spontaneous SIT of rats, a finding apparently similar to that reported for oligonucleotide antisense studies of SIT in rats (Hamon et al., 1999; Otano et al., 1999).

With regard to side effects, similarly to clozapine, SB-271046 tested at 18 mg/kg (sc) did not induce catalepsy. As this model is a predictor of extrapyramidal side effects (Arnt, 1982), it is unlikely that antagonism of the 5-HT_6 receptor would induce EPS. SB-271046 did not reduce the motility of rats either. This result is at variance with that obtained using an alternative 5-HT_6 receptor antagonist, Ro 04-6790. The latter compound has been shown to reduce the motility of rats when given at high doses (Bentley et al., 1999). However, our results are consistent with those observed following administration of antisense oligonucleotides in rats (Bourson et al., 1995; Hamon et al., 1999; Otano et al., 1999). These apparently conflicting results could be explained on the basis of different experimental conditions. Bentley et al. (1999) tested animals under normal light conditions, but we tested them in a dark room. Further, we failed to observe the 'stretching' effects in response to SB-271046 that have been reported for both Ro 04-6790 (Bentley et al., 1999; Sleight et al., 1998) and antisense nucleotide treatment (Bourson et al., 1995).

The current study has demonstrated that an antipsychotic action of SB-271046 is unlikely. This is consistent with a previous study showing that SB-271046, in contrast to clozapine, or haloperidol, did not enhance expression of c-fos in dopamine nerve terminal regions (Branchek and Blackburn, 2000; Ireland et al., 1999). According to the known specificity of this compound (Bromidge et al., 1999; Routledge et al., 2000), it does not seem probable that 5-HT_6 receptor antagonists would have antipsychotic action on their own. Moreover, testing SB-271046 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), we did not observe any enhancement of the beneficial effect of these two compounds (unpublished data).

Acknowledgments

The authors thank Mrs. L. Jeel and her animal facility team for their invaluable help with animal care, as well as the technicians of the Psychopharmacology, Psychosis Department Lundbeck for their assistance in conducting these experiments. Finally, the authors thank Dr. R. Blackburn-Munro for her assistance in the preparation of this publication, and Dr. R. Dancer for synthesis of SB-271046.

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