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ARTICLE

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ST2472: a new potential antipsychotic with very low liability to induce side-effects

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Abstract

ST2472 was shown to bind to multiple receptors, thus resembling the affinity spectrum of atypical antipsychotics. The present study investigates its in-vivo potential antipsychotic effects. ST2472 is effective in the conditioned avoidance response (CAR) test in rats (ED_{50} = 1.5 mg/kg p.o.), a model sensitive to antipsychotics. It antagonizes amphetamine-induced hypermotility at dosages (minimal effective dose = 0.7 mg/kg p.o.) that are lower than those necessary to antagonize amphetamine-induced stereotypy (minimal effective dose = 30 mg/kg p.o.), in rats. This finding, together with the fact that ST2472 does not induce catalepsy in rodents at up to 100 mg/kg p.o., indicates that ST2472 has very low liability to induce extrapyramidal side-effects. ST2472 does not increase prolactinaemia after chronic treatment. In mice, ST2472 does not appear to alter blood pressure and heart rate in a significant fashion. In conclusion, ST2472 seems to be an antipsychotic with lower liability to produce side-effects than other antipsychotics, such as haloperidol, risperidone, olanzapine and clozapine, which were evaluated as reference drugs.

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Key words: Atypical antipsychotic, conditioned avoidance response, catalepsy, extrapyramidal side-effects, haloperidol, mice, olanzapine, prolactinaemia, rats, risperidone, ST2472.

Introduction

Despite the fact that several lines of research have been undertaken to explore the role of genetic, viral, environmental and neurodevelopmental factors in the aetiopathology of schizophrenia (Weinberger, 1995), no clear and simple mechanistic hypothesis has so far been proposed. Up to now, no drug that is not a dopaminergic D_2 receptor antagonist has proved to be an effective antipsychotic. Serotonergic 5-HT_{2A} antagonism is considered important in reducing extrapyramidal side-effects (EPS), while maintaining of therapeutical benefits (Meltzer et al., 1989). However, 5-HT_{2A} antagonism is also linked to possible QT wave prolongation (Frishman and Grewall, 2000). Moreover, other receptor bindings are also believed to play an important role in reducing schizophrenia symptoms, but compounds with specific action on these receptors failed in clinical trials, namely SKF38393 (D_1 agonist; Davidson et al., 1990), SCH39166/ecopipam (D_1 antagonist; Karlsson et al., 1995), fananserin (D_4 and 5-HT_{2A} antagonist; Truffinet et al., 1999), SR141716 (cannabinoid CB_1 antagonist; Meltzer et al., 2004), SR48692 (NTS_1 receptor antagonist; Meltzer et al., 2004), prazosin (α_1 antagonist; Hommer et al., 1984), and buspirone (5-HT_{1A} agonist; Friedman, 1991). At the present, the serotonin 5-HT_{2A} receptor inverse agonist, ACP-103 (Vanover et al., 2006) as an add-on therapy with an antipsychotic (www.ClinicalTrials.gov), SR142801 and SB223412/talnetant, neurokinin NK_3 receptor antagonists, and SR46349/eplivanserin, a 5-$HT_{2A/2C}$ antagonist, are under clinical evaluation (Evangelista, 2005; Meltzer et al., 2004). Therefore, the medical need in schizophrenia is to search not only for compounds that treat both positive and negative symptoms as well as impaired cognition, but also for those with low liability to induce side-effects (Gerlach, 2002). These may vary from EPS to hyperprolactinaemia, QT

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interval prolongation, cognitive detrimental effects and iatrogenic diabetes (Kongsamut et al., 2002; Newcomer, 2005).

In consideration of the foregoing, we searched for compounds that could at least have less liability to induce side-effects. Based on a pyrrolo[1,2]benzothiazepine moiety, this research first discovered the 7-chloro derivative of 9-(methyl-1-piperazinyl) pyrrolo[2,1-b][benzothiazepine (ST1460), and then 9-(methyl-1-piperazinyl)pyrrolo[2,1-b]benzothiazepine (ST1899) (Campiani et al., 2002, 2004, 2005). Subsequently, 9-piperazin-1-ylpyrrolo[2,1-b][1,3]benzothiazepine (ST2472) was identified as a compound with the pharmacological requirements we were searching for. In fact, while all three compounds possess similar potential therapeutical effects, the liability of prolonging cardiac QT prolongation, a relevant side-effect of current antipsychotics, decreased ten times. Indeed, the concentration that inhibited the HERG-mediated current by 50% in vitro, taken as an index of QT prolongation, increased from 0.167 μM for ST1460, to 0.22 μM for ST1899, and to 1.4 μM for ST2472 (Stasi et al., 2006), for comparison it was about 10 nM for E-4013, a drug that prolongs ventricular repolarization (Nolan et al., 2006). Another study on rabbit Purkinje fibres did not reveal any QT prolongation by ST2472 (Stasi et al., 2006).

In this paper, we describe the activity of ST2472 in an animal model sensitive to antipsychotics, such as the conditioned avoidance response (CAR) (Arnt, 1982; Millan et al., 2000) and activity against amphetamine-induced hypermotility (Costall et al., 1978; Millan et al., 2000), and in tests used to assess potential EPS, such as activity against amphetamine-induced stereotypy (Costall et al., 1972b; Millan et al., 2000), and catalepsy (Costall et al., 1972a,b). The effect of ST2472 on prolactinaemia is also reported. Since ST2472 has been reported to display great affinity ($K_i < 1$ nM) for NA-α_{1A} and NA-α_{1B} (Stasi et al., 2006, see discussion), whose interference may influence the cardiovascular function, its effect on the in-vivo cardiovascular system was also evaluated.

Materials and methods

Animals

Male rats (Charles River, Calco, Italy), 3 months old, were kept for at least 1 wk at 24 $\pm$ 2 °C, with 55 $\pm$ 15% relative humidity, 15–20 air volume/h changes, and a 12-h light/dark cycle (lights on 07:00 hours). Fisher-344 rats were used for the CAR test, measurement of spontaneous motor activity, and prolactin plasma levels. Wistar rats were used for amphetamine-induced hypermotility or stereotypy, and for catalepsy. Male CD-1 mice (Calco, Como, Italy), weighing 20–22 g, were used for blood pressure and heart rate. The experiments were conducted according to the European Directive no. 86/609 and Italian D.L. no. 116 of 27 Jan. 1992, and approved by the Company veterinarian and by the Italian Ministry of Health.

Conditioned avoidance response

A computer-assisted active avoidance (shuttle box) apparatus equipped with a tilting grid floor with microswitch detection and connected to a high-resistance power supply (Basile, Comerio, Italy) was used. The box is divided into two compartments of equal size by a partition with one opening, both compartments being lit by a 10 W lamp, above the plastic cover. The rat had 1 min to familiarize itself with the cage. Upon presentation of the light conditioned stimulus, the animal had 3 s to move from one compartment of the shuttle box to the other (avoidance response). If the rat remained in the same compartment for more than 3 s, the unconditioned stimulus was presented as an electrical shock (0.3 mA) in the grid floor until an escape was performed (escape response). If the animal did not respond within the subsequent 4 s, the trial was terminated (failure response). Experimental sessions were run for 15 min, resulting in 20 trials (ST2472) or 15 trials (olanzapine and clozapine) in any session. The inter-trial interval was 45 s (ST2472) or 60 s (olanzapine and clozapine). The number of crossings from one compartment to the other was recorded during the inter-trial interval. The number of avoidances, escapes, failures and inter-trial crossings for each trial in each session was averaged. The animals were trained daily until they reached an avoidance response in two consecutive sessions of approximately 75%. Oral ST27472, at doses of 0.25, 0.5, 1 and 2 mg/kg, or vehicle was administered to animals 1 h before testing. The dose response was not performed in a single randomized experiment, but each dose was tested separately, with the vehicle being injected into the same animal the day before compound administration. In the same experimental conditions, oral olanzapine was administered at 0.75, 1.5, 3 and 6 mg/kg and oral clozapine at 1.5, 3, 6 and 9 mg/kg.

Motor activity

This activity was measured in two cages 40 cm $\times$ 40 cm $\times$ 30.5 cm high (Harvard Apparatus Inc., Holliston, MA, USA). Two lines of horizontal infrared

photocells were embedded in the wall 2.5 cm apart. The lower line (2 cm from the bottom) served to measure motor activity that is expressed as distance walked in centimetres. The upper line (8 cm from the bottom) served to measure vertical activity expressed as number of photocell interruptions. Oral ST27472, at doses of 0.125, 0.25, 0.5, 1, 2 or 4 mg/kg, or vehicle was administered to animals which were unhabituated to the motor activity cage. One hour after oral administration, animals were placed in the motor activity cages and motor activity was recorded for 30 min. The dose response was not performed in a single randomized experiment, but each dose was tested separately with the vehicle. In the same experimental conditions, oral olanzapine was administered at 0.77, 2.3 and 4.6 mg/kg and oral clozapine at 2, 4 and 8 mg/kg.

Amphetamine-induced hypermotility and stereotypy

Each animal was acclimatized to a cage for 60 min before subcutaneous amphetamine administration. Immediately after administration, motor activity was measured every 10 min in cages as described above. Stereotypy was observed in a normal cage for 5 s every 15 min and scored according to Costall and Naylor (1974): score of 0 for non-stereotyped behaviour; score of 1 for periodic sniffing and/or rearing behaviour; score of 2 for continuous sniffing and/or rearing behaviour; score of 3 for periodic biting and/or licking; score of 4 for continuous biting and/or licking behaviour. A dose response of d-amphetamine was performed (data not shown) to select the best dose to induce hypermotility (1 mg/kg) or stereotypy (10 mg/kg) for the subsequent interaction studies. Amphetamine-induced hypermotility and stereotypy lasted 2 and 5 h, respectively. Time-course (15, 30, 60, 90 or 120 min before amphetamine) experiments with oral ST2472, olanzapine, clozapine, haloperidol and risperidone were carried out to select the best pre-treatment time for each compound before d-amphetamine (data not shown). The selected pre-treatment times for dose-response studies were 15 min for ST2472, 30 min for clozapine, 60 min for olanzapine and risperidone, and 90 min for haloperidol.

Catalepsy

Animals were gently placed by their forelegs on a small metal bar 5 mm in diameter, about 35 cm long at a height of 10 cm from the supporting surface. The time that the animal employed to change position and return to the work surface was recorded by an observer unaware of the treatment. This time was taken as an index of catalepsy. The animal was observed up to 60 s. After oral drug or vehicle administration, catalepsy was recorded at 15, 30, 60, 120, 180, 240 and 300 min. Data were expressed as mean of values from 15 min to 300 min.

Prolactin

Oral ST2472 (1.4 mg/kg), haloperidol (0.5 mg/kg), olanzapine (1.5 mg/kg) and clozapine (4.9 mg/kg) were orally administered once daily for 21 consecutive days. Compound doses were selected on the basis that they were equiactive in the experiment on the CAR experiment. One hour after the last administration, animals were sacrificed by decapitation and trunk blood was collected. Serum prolactin levels were measured by the enzyme immunoassay (EIA) system (Biotrack-Amersham Life Sciences, Milan, Italy; code RPN2563). The assay is based on competition between unlabelled rPRL (rat prolactin) and a fixed quantity of biotin-labelled rPRL for a limited amount of rPRL-specific antibody. The sensitivity of the assay is 0.44 ng/ml.

Blood pressure and heart rate

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious restrained mice with a computerized tail-cuff system (BP-2000, Visitech Systems Inc., Apex, NC, USA) that determines SBP using a photoelectric sensor. The mice, brought to the testing room where the lights were dimmed, were allowed to acclimatize for 20 min prior to testing. Measurements of SBP and HR were obtained every 10 min. Each value is the average of measurements obtained during 10 consecutive computer-automated inflation/deflation cycles of the balloon cuff. Two training sessions preceded the experimental session so that the mice could become accustomed to the tail-cuff procedure. The first one consisted of a 40-min recording, while the second was identical to the experimental session except that the mice received vehicle or the compounds. Prazosin (5 mg/kg) and olanzapine were used orally as reference compounds. ST2472 and olanzapine were tested at a dose (5 mg/kg) shown to be fully active in the previous behavioural experiments.

Drugs

ST2472, synthesized in Sigma-Tau's Chemistry Department, was dissolved in 0.5% carboxymethylcellulose sodium salt with 0.1% HCl, except for the dose-response experiment, in the CAR and spontaneous motor activity where it was dissolved in

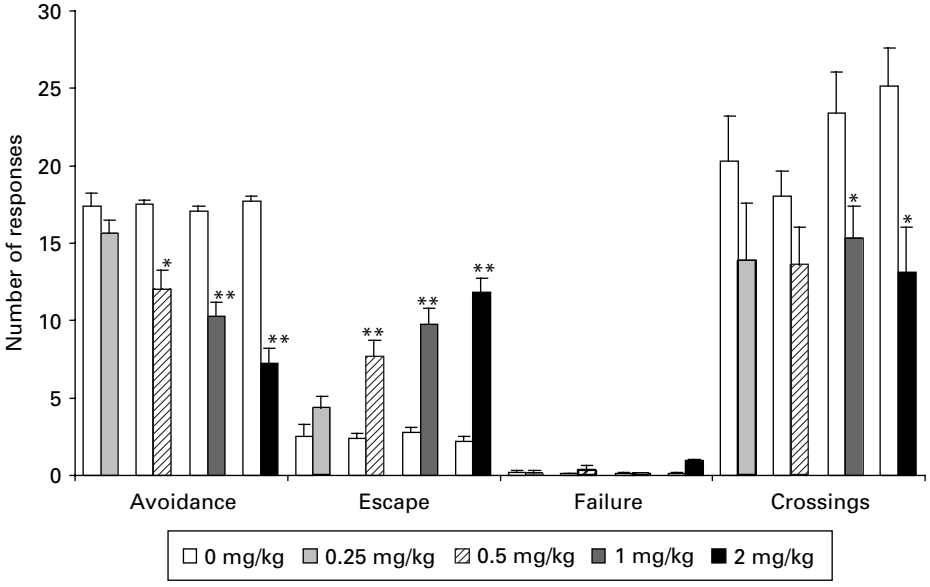

Figure 1. Dose response of ST2472 on the conditioned avoidance response (CAR) test. Columns represent mean ± S.E.M. from 10–16 rats. ST2472 was given orally 60 min before testing. Paired Student's *t* test: * $p < 0.05$, ** $p < 0.01$ vs. dose 0.

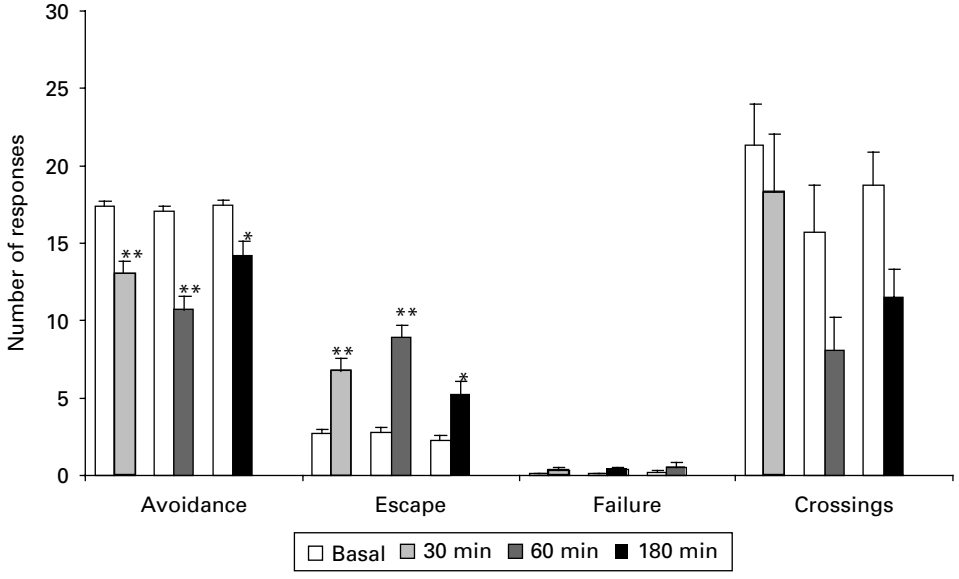

Figure 2. Time-course of ST2472 on the conditioned avoidance response (CAR) test. Columns represent mean ± S.E.M. from 15 rats. ST2472 was given orally, 60 min before testing, at a dose equal to ED_{50} in reducing avoidance responses (1.4 mg/kg).

40% 2-hydroxypropyl-β-cyclodextrine in water and then diluted again in 90% water. Clozapine, haloperidol, risperidone and prazosin were purchased from Sigma-Aldrich (St Louis, MO, USA) and olanzapine in the pharmacy as Zyprexa®. Clozapine, olanzapine and prazosin were dissolved in a few drops of 1 N HCl and then in water, haloperidol in 0.5% carboxymethylcellulose sodium salt plus acetic acid.

Statistics

Conditioned active avoidance and motor activity: paired or unpaired Student's *t* test was used depending on the type of experiment. Mann–Whitney was used to assess statistical probabilities when variances were not homogeneous. The ED_{50} value, with confidence limits, was calculated by nonlinear

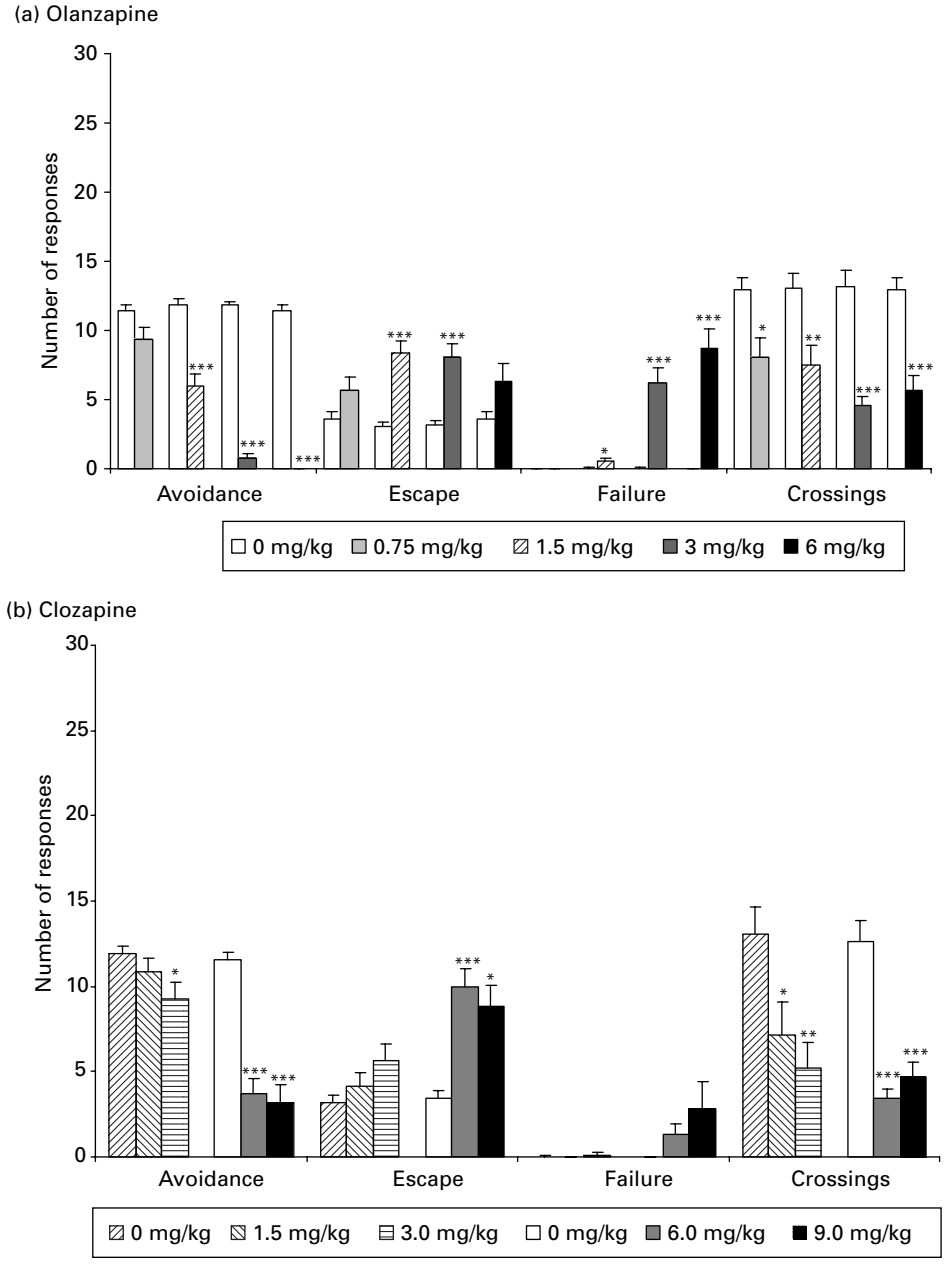

Figure 3. Effects of dose response of (a) olanzapine and (b) clozapine on the conditioned avoidance response (CAR) test. Columns represent mean ± S.E.M. from 10-16 rats. The compounds were given orally 60 min before testing. Paired Student's *t* test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. dose 0.

regression analysis using the GraphPad® StatMate version 1.01i (GraphPad software, San Diego, CA, USA). For amphetamine-induced hypermotility and stereotypy, AUCs were calculated (120 min for hypermotility and 90 min for stereotypy) and one-way ANOVA was performed, followed by Dunnett's test. For catalepsy, two-way ANOVA for repeated measures was used.

Results

Conditioned avoidance response

Sixty minutes after administration, ST2472 dose-dependently reduced avoidance responses and increased escape responses, 0.5 mg/kg being the first statistically significant dose (Figure 1). Failure responses did not increase at any dose. Reduction in

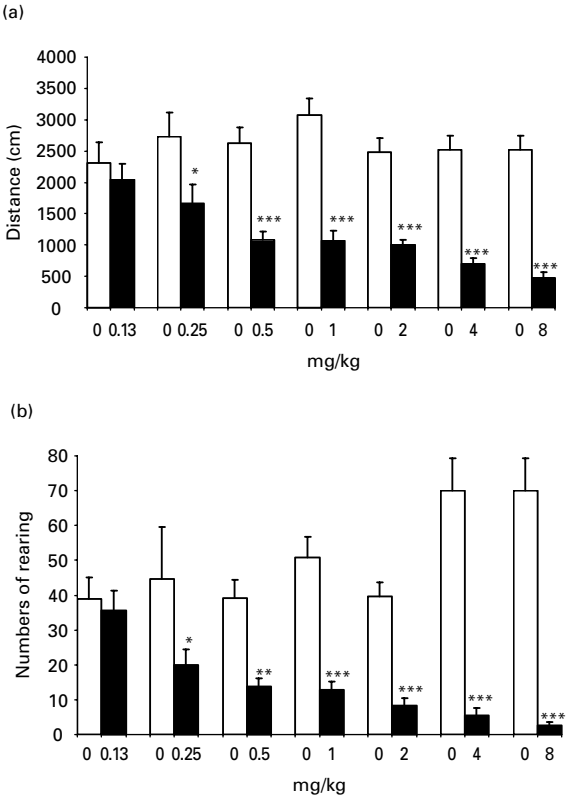

Figure 4. Effects of ST2472 on spontaneous motor activity. Columns represent mean ± S.E.M. from 7–18 rats. ST2472 was given orally 60 min before unhabituated rats were placed in the motor activity cage. Motor activity was measured for 30 min. (a) Horizontal motor activity, (b) vertical motor activity. Student's *t* test for independent groups: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. dose 0.

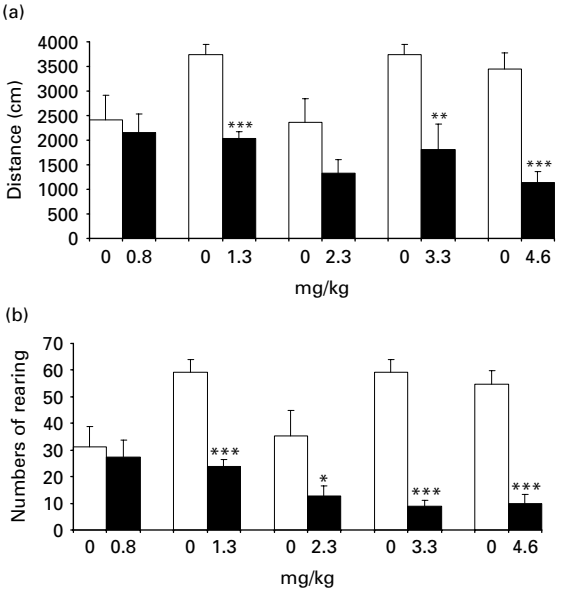

Figure 5. Effect of olanzapine on spontaneous motor activity. Columns represent mean ± S.E.M. from 7–13 rats. The compounds were given orally 60 min before unhabituated rats were placed in the motor activity cage. Motor activity was measured for 30 min. (a) Horizontal motor activity, (b) vertical motor activity. Student's *t* test for independent groups: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. dose 0.

inter-trial crossings was observed at 1 and 2 mg/kg. The time-course revealed that the ST2472 peak effect is between 30 and 180 min after its administration (Figure 2). Olanzapine dose-dependently reduced avoidance responses and increased escape responses, 1.5 mg/kg being the first statistically significant dose (Figure 3). Failure responses were increased at 1.5, 3 and 6 mg/kg. Reduction in inter-trial crossings was observed at all doses.

Clozapine dose-dependently reduced avoidance responses and increased escape responses, the first statistically significant doses being 3 and 6 mg/kg, respectively (Figure 3). Failure responses did not increase at any dose. Reduction in inter-trial crossings was observed at all doses.

Spontaneous motor activity

ST2472 dose-dependently reduced both horizontal (Figure 4a) and vertical (Figure 4b) motor activity, 0.25 mg/kg being the first significant dose. Olanzapine reduced horizontal and vertical (Figure 5a, b) motor activity, 1.3 mg/kg being the first significant dose. Likewise, clozapine reduced first the vertical activity at a dose of 0.25 mg/kg, then horizontal activity at a dose of 6 mg/kg (Figure 6a, b).

Amphetamine-induced hypermotility and stereotypy

ST2472 reduced hypermotility at a dose as low as 0.7 mg/kg (Figure 7) and stereotypy at a dose of 30 mg/kg (Figure 8). Clozapine already reduced hypermotility at 15 mg/kg (Figure 7) and did not affect stereotypy up to 80 mg/kg (Figure 8). Olanzapine reduced hypermotility a lower dose that was necessary to reduce stereotypy (Figure 7). Haloperidol and risperidone reduced stereotypy at doses lower than those necessary to reduce hypermotility (Figures 7 and 8).

Catalepsy

ST2472 did not induce catalepsy at up to 100 mg/kg (Figure 9), whereas haloperidol, risperidone, clozapine and olanzapine induced catalepsy at the highest doses (Figure 9).

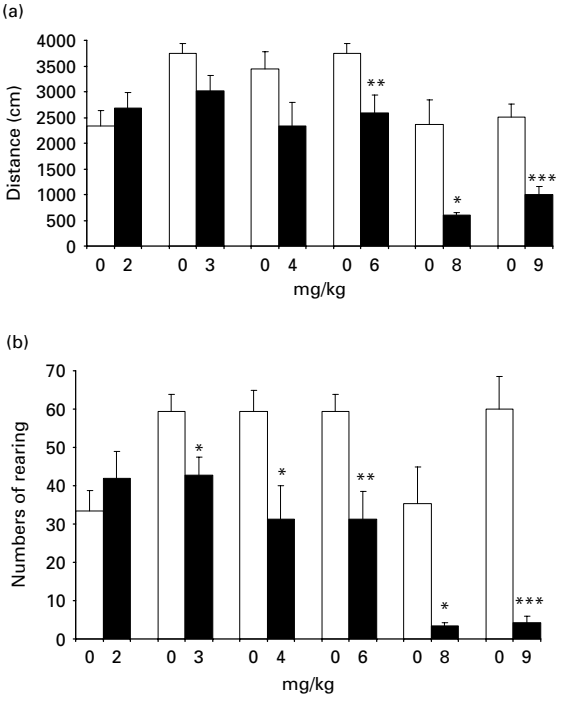

Figure 6. Effect of clozapine on spontaneous motor activity. Columns represent mean ± S.E.M. from 7–13 rats. The compounds were given orally 60 min before unhabituated rats were placed in the motor activity cage. Motor activity was measured for 30 min. (a) Horizontal motor activity, (b) vertical motor activity. Student's *t* test for independent groups: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. dose 0.

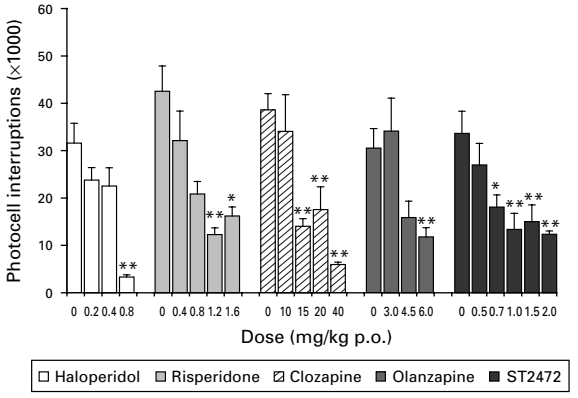

Figure 7. Effect of various compounds on amphetamine-induced hypermotility. Columns represent mean ± S.E.M. from 4–8 rats, values represent the total motor activity in 120 min. Haloperidol, risperidone, clozapine, olanzapine and ST2472 were administered orally 90, 60, 30, 60 and 15 min, respectively before d-amphetamine. d-amphetamine was given subcutaneously at 1 mg/kg to induce hypermotility. ANOVA on hypermotility: haloperidol [F(df 3, 15) = 10.85, $p<0.001$]; risperidone [Kruskal–Wallis: H(df 4) = 24.52, $p<0.001$]; clozapine [Kruskal–Wallis: H(df 4) = 22.77, $p<0.001$]; olanzapine [F(df 3, 22) = 6.02, $p<0.01$]; ST2472 [F(df 5, 34) = 5.55, $p<0.001$]. Dunnett's test: * $p<0.05$, ** $p<0.01$ vs. control.

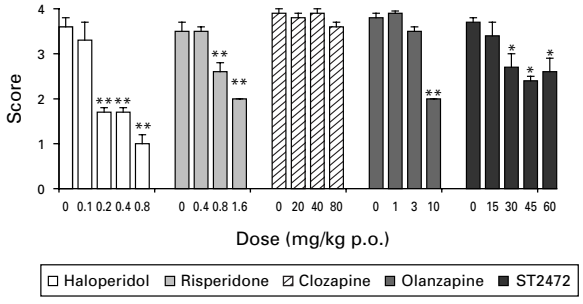

Figure 8. Effect of various compounds on amphetamine-induced stereotypy. Columns represent mean ± S.E.M. from 4–8 rats, values represent the average stereotypy in 90 min. Haloperidol, risperidone, clozapine, olanzapine and ST2472 were administered orally 90, 60, 30, 60 and 15 min, respectively before d-amphetamine. d-amphetamine was given subcutaneously at 10 mg/kg for stereotypy. ANOVA on stereotypy: haloperidol [Kruskal–Wallis: H(df 4) = 28.54, $p<0.001$]; risperidone [Kruskal–Wallis: H(df 3) = 16.40, $p<0.001$]; clozapine [Kruskal–Wallis: H(df 3) = 2.56, $p=0.46$]; olanzapine [Kruskal–Wallis: H(df 3) = 17.84, $p<0.001$]; ST2472 [F(df 4, 25) = 5.109, $p<0.001$]. Dunnett's test: * $p<0.05$, ** $p<0.01$ vs. control.

Prolactin

Body weight of animals was similar throughout the experiment for all treatments (data not shown). Haloperidol and olanzapine increased prolactinaemia. In contrast, clozapine and ST2472 did not statistically change serum prolactin levels (Table 1).

Blood pressure and heart rate

Blood pressure

Two-way ANOVA revealed that there was an effect on BP for the factor 'drug' ($F=8.929$, df = 3, 242, $p<0.0001$), but not for the factor 'time' ($F=0.6648$, df = 7, 242, n.s.) nor for the interaction between the two factors ($F=0.4173$, df = 21, 242, n.s.). The significant difference of the factor 'drug' was ascribable to prazosin, even if post-hoc analysis did not reveal any single point that was statistically different between vehicle- and prazosin-treated mice (Figure 10).

Heart rate

Two-way ANOVA revealed that there was an effect on HR for the factor 'drug' ($F=2.763$, df = 3, 243, $p<0.05$)

Table 1. Effect of oral ST2472, clozapine, haloperidol and olanzapine on serum prolactin

Treatment	Dose (mg/kg)	Prolactinaemia (ng/ml)
Vehicle	–	67.1±7.6
ST2472	1.4	85.0±5.7
Clozapine	4.9	75.3±5.5
Haloperidol	0.5	169.8±14.6**
Olanzapine	1.5	116.5±42.1*

Values are mean±S.E.M. from 6–9 rats. Compounds were administered orally once daily for 21 consecutive days at equiactive doses in the conditioned avoidance response (CAR) test. Prolactinaemia was assessed 1 h after the last administration.
Dunnett's test, after one-way ANOVA: * $p<0.05$; ** $p<0.01$ vs. vehicle.

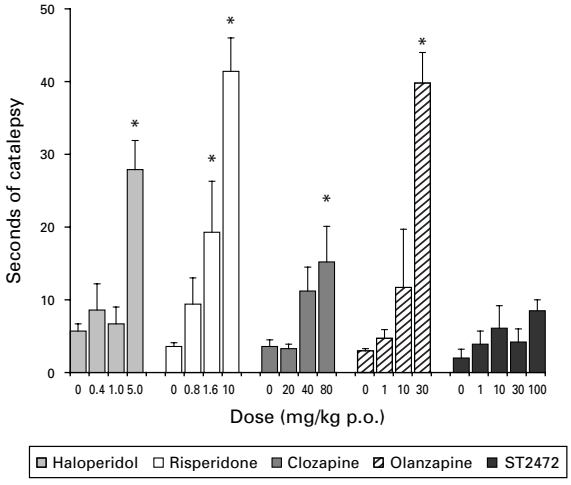

Figure 9. Effect of various compounds on catalepsy in rats. Columns represent mean±S.E.M. from 4–6 rats and are mean of values from 15 to 300 min. ANOVA: haloperidol [F(df 3, 19)=12.58, $p<0.001$]; risperidone [F(df 3, 19)=13.92; $p<0.001$]; clozapine [F(df 3, 20)=3.83, $p<0.05$]; olanzapine [Kruskal–Wallis: H(df 3)=11.31, $p<0.01$]; ST2472 [Kruskal–Wallis: H(df 4)=8, 265, n.s.]. Dunnett's test: * $p<0.05$ vs. control.

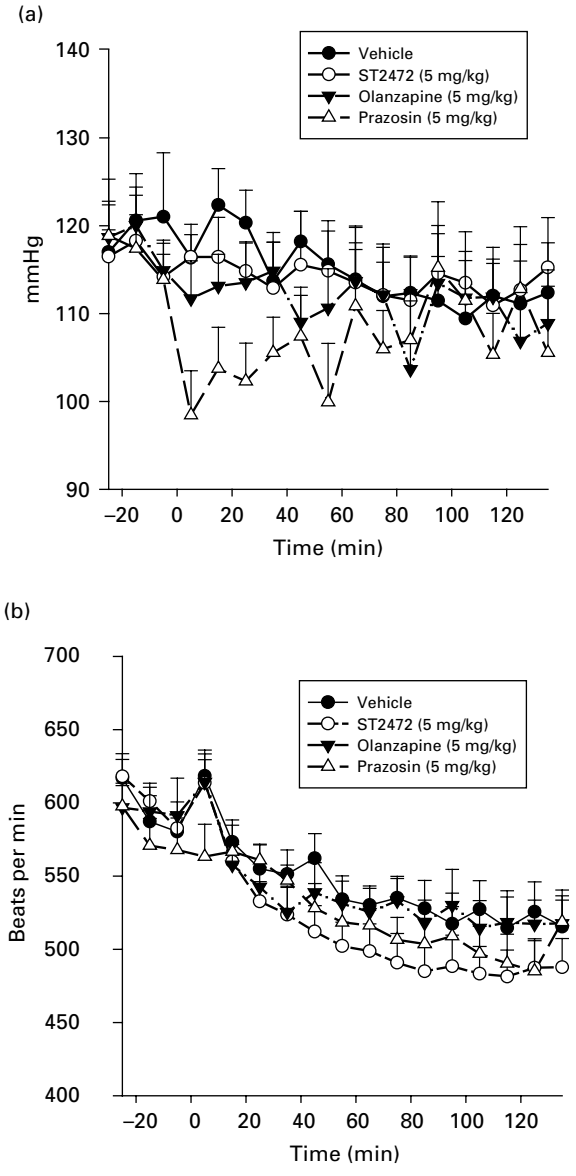

Figure 10. Effects of various compounds on (a) blood pressure (BP) and (b) heart rate (HR) in restrained mice. Points represent mean±S.E.M. from 8–9 mice. Two-way ANOVA revealed a significant drug effect on both BP and HR, but post-hoc analysis (Bonferroni test) did not reveal any statistical difference with control group.

and the factor 'time' ($F=12.93$, df=7, 243, $p<0.0001$), but not for the interaction between the two factors ($F=0.642$, df=21, 243, n.s.). Over the time, all the groups reduced their HR. Post-hoc analysis did not reveal any single point that was statistically different from vehicle; however, it seems that prazosin reduced HR in the first 5 min and PST2472 at about 40–60 min.

Discussion

Like proven antipsychotics, ST2472 antagonized conditioned avoidance responses. Even if ST2472 reduced motor activity, such hypomotility did not produce failures in the CAR test. The cortex seems the brain structure primarily involved in mediating such behaviour (Takano et al., 2004; Wadenberg et al., 2006). In contrast, ST2472 appears to poorly affect behaviours that depend on striatal structures, which is considered predictive of EPS (Costall et al., 1972a,b). In fact, ST2472 does not induce catalepsy at up to 100 mg/kg

Table 2. Oral ED_{50} or minimal effective dose (MED) values (mg/kg) of ST2472, clozapine, haloperidol and olanzapine in some behavioural parameters

			Antagonism of amphetamine-induced		
	CAR (ED_{50})	Motor activity (ED_{50})	Hypermotility (MED)	Stereotypy (MED)	Catalepsy (MED)
ST2472	1.4 (0.8–2.6)	0.7 (0.3–1.5)	0.7	30	>100
Olanzapine	1.5 (1.2–1.8)	2.6 (1.3–5.0)	6.0	10	30
Clozapine	4.9 (3.3–7.3)	4.9 (4.7–10.2)	15	>80	80
Haloperidol	0.5 (0.3–0.8)	1.3 (1.0–1.6)	0.8	0.2	5
Risperidone			1.2	1.2	1.6

CAR, Conditioned avoidance response.
Compounds were given 60 min before the 15-min CAR or the 30-min spontaneous motor activity recording. ST2472, olanzapine, clozapine and ST2472 were given 15, 60, 30 and 90 min before the 120-min amphetamine-induced hypermotility or stereotypy. Catalepsy was measured for 6 h.

(the maximal dose tested), more than 70 times the active dose used in the CAR test. In contrast, haloperidol, risperidone and olanzapine induced catalepsy at doses between 10–20 times those active in the CAR test. The poor activity of ST2472 on the basal ganglia was also confirmed when a more direct behavioural comparison between the involvement of limbic vs. striatal structures was carried out by means of amphetamine-induced hypermotility and stereotypy (Costall and Naylor, 1974), respectively. In fact, a dose 40 times higher than that active in reducing amphetamine-induced hypermotility was needed to attenuate striatal stereotypies induced by amphetamine. For comparison, haloperidol and risperidone showed reverse selectivity, in that they antagonized stereotypy at lower doses than those that reduced amphetamine-induced hypermotility. The olanzapine dose that reduced amphetamine-induced stereotypy was just a bit higher than the one that antagonized amphetamine-induced hypermotility. We could not administer clozapine at doses higher than 80 mg/kg as animals were deeply prostrated at this dosage.

ST2472 has greater affinity (K_i < 1 nM) for NA-α_{1A}, NA-α_{1B} and 5-HT_{2C} receptors, than for 5-HT_{2A}, $D_{4.4}$, H_1, 5-HT_6 and 5-HT_7 receptors (>1 nM and <10 nM), and D_1, D_2, D_3 and D_5 and M4 receptors (1>10 nM and <50 nM) (Stasi et al., 2006). The selectivity of ST2472 for behaviours that depend on the mesocorticolimbic system cannot be derived from its binding profile. The relative role of various receptors in mediating the effect of compounds in the various tests for antipsychotics has already been evaluated (Hertel, 2006; Millan et al., 2000; Volontè et al., 1997). Such brain regional selectivity is also confirmed by the apparent lack of activity of ST2472 on other brain systems. In fact, in contrast to haloperidol and olanzapine, ST2472 did not increase serum prolactin at doses active in the CAR test. Thus, it appears that whatever the combination of effects on the various receptors, ST2472 is very selective in modulating mesocorticolimbic activities. Further experiments (electrophysiology, microdialysis) are needed to understand whether this activity is exerted at post- and/or presynaptic levels. The relative role played by each pharmacological component also needs further investigation, as, for example, in the presence of low dopamine D_2 receptor occupancy, additional α_1-adrenoceptor blockade might improve antipsychotic efficacy (Wadenberg et al., 2000) and may reduce EPS (Dickinson et al., 1988).

The blockade of 5-HT_{2A} receptors has been indicated as a possible risk for QT prolongation (Frishman and Grewall, 2000). However, ST2472 seems to be safe on the cardiovascular system, as it blocks HERG currents only with an IC_{50} of 1.4 μM and does not prolong QT waves in rabbit Purkinje fibres at up to 10 μM (Stasi et al., 2006). Additionally, despite its affinity for α-noradrenergic receptors, ST2472, at doses active in the CAR test, does not appear to modify BP, even if a non-statistically significant bradycardia was observed. ST2472 possesses affinity for the noradrenaline transporter (K_i = 21 nM; Stasi et al., 2006) and changes in noradrenaline extracellular concentrations may influence ST2472 direct activity, at least on noradrenergic receptors.

Thus, in terms of a safety profile, ST2472 seems better than haloperidol, risperidone and olanzapine (see Table 2). A quantitative comparison with

clozapine was not possible due to extreme behavioural prostration induced by high doses of this drug. ST2472 also seems better than recently proposed potential antipsychotics, in terms of a safety profile in the same tests (Millan et al., 2000).

In conclusion, ST2472 is a potential antipsychotic with a liability to induce side-effects lower than that of current antipsychotics.

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Statement of Interest

None.

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