Behavioural Brain Research 237 (2013) 86–95

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

New insights into pharmacological profile of LASSBio-579, a multi-target *N*-phenylpiperazine derivative active on animal models of schizophrenia

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HIGHLIGHTS

- The *N*-phenylpiperazine LASSBio-579 is effective in a model of sensoriomotor gating.
- LASSBio-579 is a multi-target ligand with potential antipsychotic activity.
- Binding to D2, D4 and 5-HT_{1A} receptors might account for LASSBio-579 mode of action.

ARTICLE INFO

Article history:
Received 24 August 2012
Accepted 7 September 2012
Available online 19 September 2012

Keywords:
Antipsychotic
Multi-target compound
Apomorphine-induced climbing
Pre-pulse inhibition
Catalepsy

ABSTRACT

Previous behavioral and receptor binding studies on *N*-phenylpiperazine derivatives by our group indicated that LASSBio-579, LASSBio-580 and LASSBio-581 could be potential antipsychotic lead compounds. The present study identified LASSBio-579 as the most promising among the three compounds, since it was the only one that inhibited apomorphine-induced climbing (5 mg/kg p.o.) and apomorphine-induced hypothermia (15 mg/kg p.o.). Furthermore, LASSBio-579 (0.5 mg/kg p.o.) was effective in the ketamine-induced hyperlocomotion test and prevented the prepulse inhibition deficits induced by apomorphine, DOI and ketamine with different potencies (1 mg/kg, 0.5 mg/kg and 5 mg/kg p.o., respectively). LASSBio-579 also induced a motor impairment, catalepsy and a mild sedative effect but only at doses 3–120 times higher than those with antipsychotic-like effects. In addition, LASSBio-579 (0.5 and 1 mg/kg p.o.) reversed the catalepsy induced by WAY 100,635, corroborating its action on both dopaminergic and serotonergic neurotransmission and pointing to the contribution of 5-HT_{1A} receptor activation to its pharmacological profile. Moreover, co-administration of sub-effective doses of LASSBio-579 with sub-effective doses of clozapine or haloperidol prevented the apomorphine-induced climbing without induction of catalepsy. In summary, our results characterize LASSBio-579 as a multi-target ligand active in pharmacological animal models of schizophrenia, confirming that this compound could be included in development programs aiming at a new drug for treating schizophrenia.

1. Introduction

Schizophrenia is a challenging disorder for the development of new drugs due to its complexity. It is characterized by several neurochemical and metabolic changes in the central nervous system that lead to altered connectivity and impaired processing of information. As a result, patients with schizophrenia develop three main kinds of symptoms: positive (hyperactivity, delusions, hallucinations, disorganized speech), negative (avolition, anhedonia, social isolation) and cognitive (attentional impairment, memory deficits) [1]. The annual incidence of schizophrenia averages 15 per 100,000 and there is a 0.7% risk of developing the illness over one's lifetime [2].

Pharmacological treatment of schizophrenia includes two classes of drugs: the typical or first generation antipsychotics (FGAs) (mainly haloperidol and chlorpromazine) and the atypical or second generation antipsychotics (SGAs) (for example clozapine, amisulpride, risperidone, olanzapine, quetiapine, aripiprazole). The pharmacological property shared by all currently available antipsychotic agents is their ability to block dopamine D_2 receptor at concentrations well correlated to their clinical antipsychotic

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http://dx.doi.org/10.1016/j.bbr.2012.09.016

potencies [3,4]. However, while FGAs act mainly on D_2-like receptors, most SGAs present a multiple receptor binding profile, that includes serotonin receptors (mainly 5-HT_{1A} and 5-HT_{2A}) which play an important role in their antipsychotic effectiveness and side effects profile [5–7].

Considered more effective and safer than FGAs, SGAs have progressively displaced the older agents in the treatment of schizophrenia. Besides literature data are still controversial about SGAs superior effectiveness regarding negative and cognitive symptoms [8,9], they present a lower risk of causing extrapyramidal side effects, which is an unequivocal advantage over FGAs [6,10]. This relative lack of extrapyramidal effects presented by SGAs has been attributed to partial activation of 5-HT_{1A} receptors and blocking of 5-HT_{2A} receptors [5,7,11]. On the other hand, increasing concern has emerged with SGAs potential to cause severe metabolic side effects (weight gain, dyslipidemia and diabetes mellitus) [12,13]. Concomitant with this, the relative failure to treat all the symptoms of schizophrenia and the refractoriness of approximately 15% of patients to both FGAs and SGAs [9,14,15] support the need for developing more effective and safer antipsychotics.

In previous studies our group described the design and synthesis of a series of *N*-phenylpiperazine derivatives aiming to achieve a new antipsychotic prototype. Indirect electrophysiological studies carried out in cultured hippocampal neurons suggested that LASSBio-579 (1-[1-(4-chlorophenyl)-1*H*-4-pyrazolylmethyl]-4-phenylhexahydropyperazine) and LASSBio-581 (1-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-ylmethyl]-4-phenylhexahydropyperazine)] could act as agonists at pre-synaptic dopamine D_2-like receptors while LASSBio-580 could act as antagonist at these same receptors [16]. In accordance with this observation, LASSBio-579 inhibited the amphetamine-induced stereotypy in rats and apomorphine induced-climbing in mice [17,18] and both LASSBio-579 and LASSBio-581 induced a mild catalepsy when administered intraperitoneally to mice [17]. The multi-receptor profile of this molecular scaffold was thereafter confirmed in vitro and in vivo. LASSBio-579, LASSBio-580 and LASSBio-581 bind to 5-HT_{1A} receptors with affinities similar to those for D_2-like receptors. On the other hand, the affinities estimated for 5-HT_{2A} was 7 to 25 fold lower than for 5-HT_{1A} and D_2-like receptors [18,19]. In vivo studies demonstrated that LASSBio-579 and LASSBio-581 cause a significant reduction in mice core temperature that was blocked by WAY 100,635 (a 5-HT_{1A} receptor antagonist) and inhibited head-twitches and ear scratches induced by (±)–DOI, two responses related with 5-$HT_{2A/C}$ receptor activation in rodents [20]. LASSBio-580 has not been evaluated in vivo until now. Taking together, these preliminary results indicate that heterocyclic *N*-arylpiperazine compounds have a pharmacological profile that may be useful for the development of new antipsychotics.

In this study we carried on a further pharmacological evaluation of LASSBio-579, LASSBio-580 and LASSBio-581 in order to select the most promising compound with potential antipsychotic activity.

2. Materials and methods

2.1. Animals

Adult male CF1 mice (25–35 g) from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS-RS) breeding colony were used. Animals were housed in plastic cages (17 cm × 28 cm × 13 cm) with free access to food (Nuvital®) and water. Mice were kept at constant room temperature (22 ± 2 °C) and humidity (60%), under a 12 h light-dark cycle (lights off at 7:00 pm) and were adapted to local conditions for at least 72 h before the experiments. All experimental protocols were approved by CONEP-Brazil (National Commission of Research Ethics–Protocol 2006541) and performed according to guidelines of The National Research Ethical Committee (published by National Heath Council–MS, 1998) and Brazilian law [21], which are in compliance with the International Guiding Principles for Biomedical Research Involving Animals [22].

2.2. Drugs and treatments

LASSBio-579, LASSBio-580 and LASSBio-581 were synthesized, purified and structurally characterized as described elsewhere [16]. Apomorphine hydrochloride hemihydrate (Sigma, São Paulo, Brazil), clozapine (Novartis, São Paulo, Brazil), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride ((±)-DOI, Sigma, São Paulo, Brazil), haloperidol (Galena, São Paulo, SP, Brazil), ketamine hydrochloride (Cristália, São Paulo, Brazil), sodium pentobarbital (Cristália, São Paulo, Brazil) and WAY 100,635 maleate (Sigma, São Paulo, Brazil) were used.

LASSBio-579, LASSBio-580, LASSBio-581 and haloperidol were suspended in saline with addition of 1% (v/v) polissorbate 80. (±)-DOI, ketamine, sodium pentobarbital and WAY 100,635 were directly dissolved in saline. Apomorphine was dissolved in saline with addition of 0.1% ascorbic acid and clozapine in saline with addition of 0.1% acetic acid 0.1 M. Vehicle groups received 1% (v/v) polissorbate 80 in saline. The drugs were administered by intraperitoneal and oral routes (10 ml/kg body weight) or subcutaneously (5 ml/kg body weight). All animals were fasted for 6 h before oral drug administration. All doses are expressed as free base.

2.3. Apomorphine-induced climbing

This test was conducted as described by Neves and coworkers [18]. Briefly, mice were treated with one of the test compounds or vehicle (first treatment) and immediately put in cages (29 cm × 23 cm × 19 cm) with the floor, walls and top consisting of parallel metal bars (2 mm diameter). Animals were allowed to freely explore the cages for 30 min. After that they were treated with apomorphine 4 mg/kg or vehicle s.c. (second treatment). The climbing behavior score was evaluated as: normal behavior (0 point), increased activity and sniffing (1 point), occasional clinging to sides of cage with forepaws (2 points), intermittent clinging to sides or top of cage with all four paws (3 points) and uninterrupted climbing with all four paws (4 points). Climbing behavior was scored at 5, 10, 15, 20, 25 and 30 min after second treatment administration. The period of observation in each interval was 1 min. The climbing index was calculated as the sum of all scores obtained by the same animal at each time interval.

In order to evaluate the combined effect of LASSBio-579 with antipsychotics, the animals received a combination of sub-effective dose of these drugs, i.e., LASSBio-579 plus haloperidol, LASSBio-579 plus clozapine or haloperidol plus clozapine. The drug mixtures were prepared immediately before the administration and constituted the first treatment. The test was performed in the same conditions described above.

2.4. Apomorphine-induced hypothermia

Core temperature was recorded with a digital thermomether (ProCheck®) with reading precision of 0.1 °C. Temperature measures were carried out between 9:00 and 11:00 a.m. at controlled room temperature (24 ± 1 °C). Mice were gently immobilized and the apparatus lubricated with vaseline was inserted 1.5 cm into animal's rectum. Mice were pretreated with one of the test drugs or vehicle p.o. Thirty minutes later, all animals received a second treatment with apomorphine 1 mg/kg i.p. The body temperature of each animal was taken immediately prior (basal temperature), 45 and 60 min after the pre-treatment (i.e. 15 and 30 min after apomorphine). Temperature decrease (°C) was calculated by the difference between basal and the core temperature after apomorphine administration at each time of measure.

2.5. Locomotor activity

Locomotor activity was monitored in an area made of acrylic (transparent walls and black floor, 30 cm × 30 cm × 45 cm) with the floor divided into 24 squares of equal area. Mice were treated with the test substance and immediately positioned at the apparatus center. Mice were allowed to freely explore during thirty minutes and then observed during 20 min. The total number of squares crossings during the last 20 min was recorded by a trained observer unaware of the treatments. All procedures were done in a room dimly lit.

2.6. Ketamine-induced hyperlocomotion

The same apparatus and room conditions used to measure spontaneous locomotor activity were maintained. Mice were treated with the test substances, positioned at the apparatus center and allowed to free exploration. Thirty minutes later they were treated with ketamine 10 mg/kg s.c. and observed during 20 min. The total number of squares crossings was recorded by a trained observer unaware of the treatments.

2.7. Prepulse inhibition of startle reflex (PPI)

The PPI test occurred in a startle chamber (Insight®, São Paulo, Brazil) in which a loudspeaker produced a continuous background noise of 65 dB of sound as well as the acoustic startle pulses. A white noise pulse was used as the startle stimulus, which had an intensity of 115 dB and duration of 50 ms; three different noise intensities (80, 85 and 90 dB, duration 20 ms) were used as prepulses. An acclimatization time of 5 min, during which the mice received no stimulus except the background