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Effect of blonanserin on methamphetamine-induced disruption of latent inhibition and c-Fos expression in rats

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HIGHLIGHTS

- We examined the pharmacological profile of blonanserin, a novel antipsychotic.
- Blonanserin ameliorates methamphetamine-induced disruption of latent inhibition.
- Blonanserin increases c-Fos expression in the shell area of the nucleus accumbens.

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ABSTRACT

To clarify the psychopharmacological profile of blonanserin, a novel antipsychotic, we examined its effect on the methamphetamine-induced disruption of latent inhibition (LI) and the neural activation related to this effect in rats. To evaluate the LI, we used a conditioned emotional response in which a tone (conditioned stimulus) was paired with a mild foot shock (unconditioned stimulus). This paradigm was presented to rats licking water. Methamphetamine-induced (1.0 mg/kg, i.p.) disruption of LI was significantly improved by the administration of a higher dose (3.0 mg/kg, i.p.) of blonanserin and tended to be improved by 1.0-mg/kg blonanserin and 0.2-mg/kg haloperidol but not by a lower dose (0.3 mg/kg) of blonanserin. Immunohistochemical examination showed blonanserin (3.0 mg/kg, i.p.) increased c-Fos expression in the shell area but not in the core area of the nucleus accumbens while methamphetamine (3.0 mg/kg, i.p.) produced the opposite expression pattern. Blonanserin also increased the number of c-Fos expressions in the central amygdala nucleus but not in the basolateral amygdala nucleus or the prefrontal cortex. Blonanserin ameliorates the methamphetamine-induced disruption of LI, as other antipsychotics do, and a neuronal activation and/or modulation of neurotransmission in the nucleus accumbens is related to the disruption of LI by methamphetamine and to its amelioration by blonanserin.

1. Introduction

The latent inhibition (LI) effect is the suppression of performance on a classical conditioning task when a conditioned stimulus (CS) is pre-exposed before conditioning [8]. That is, if a CS is presented several times without an unconditioned stimulus (US), the person or animal learns to ignore, or not to pay attention to, the stimulus, and, consequently, the strength of the subsequent conditioning is inhibited [23]. The disruption of LI has been observed in patients with schizophrenia and in healthy humans and rats under dopamine releaser treatment [22] and is considered to be related to the functional decline in attentional filtering, a cognitive deficit observed in schizophrenia [23]. Typical and atypical antipsychotics, which mainly block dopamine receptors, ameliorate the disrupted LI and even enhance LI [3,5,9,16,17,25,26]. Therefore, we can estimate the potential antipsychotic profiles, especially for cognitive deficits, of novel compounds by examining their effects on LI.

Blonanserin, 2-(4-ethyl-1-piperazinyl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta [b] pyridine, is a novel atypical antipsychotic that has a binding profile different from that of other atypical antipsychotics: it has high affinity for dopamine D_2 and serotonin 5-HT_{2A} receptors and weak affinity for D_1, 5-HT_{1A}, 5-HT_6, 5-HT_7, histamine H_1, and muscarinic M_1 receptors and

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for α_1, α_{2c}, and β adrenoceptors [11,14]. Blonanserin has the highest affinity for D_2 receptors, and its affinity is the highest among all antipsychotics [11]. Clinically, blonanserin exhibits atypical antipsychotic properties with efficacy against positive and negative symptoms of schizophrenia [4,7]; however, the effects of blonanserin on cognitive deficits in schizophrenia have not been fully examined. Basic research with rodents showed that amphetamine-induced disruption of pre-pulse inhibition (PPI) [20] and phencyclidine-induced impairment in a novel object recognition test [6] were relieved by blonanserin. That is, there are only two reports on the potential ameliorating effect and mechanism of blonanserin of the cognitive deficits in schizophrenic model animals. To examine the remedial effects of blonanserin on disrupted LI and its underling neurobiological mechanisms, we conducted behavioral and morphological studies focusing on the brain areas potentially related to LI.

2. Materials and methods

2.1. Animals

We used 157 adult male Sprague–Dawley rats (Charles River Laboratories, Japan), weighing 250–330 g. They were housed in a temperature-controlled colony room in separate cages at 23 ± 1 °C with 50 ± 10% relative humidity on a 12:12-h light: dark cycle (lights on from 08:00 to 20:00 h). Water access was restricted, as described below, and food access was unrestricted. The experimental procedures were performed in strict adherence with the guidelines of the University of Miyazaki for the care and use of experimental animals and with the approval of the ethical committee for animal experimentation at the University of Miyazaki.

2.2. LI evaluation

We used 93 rats in a behavioral experiment designed to evaluate LI. LI was assessed using an off-baseline conditioned emotional response procedure in rats licking water. The test chamber was 24.0 cm long, 29.0 cm wide, and 25.0 cm high (Ohara Ika Sangyo, Tokyo, Japan) with a retractable water bottle and a speaker. The chamber was placed in a soundproof box. Licks were detected with a drinkometer (Ohara Ika), and the data were captured using a program written in LabVIEW (National Instruments Co., Tokyo, Japan).

In an initial training procedure (days 1–9), the rats (after being handled for 5 min) were placed one by one in the test chamber for 5 min on four consecutive days. For the next five consecutive days, they were water restricted overnight and then placed in the chamber and allowed to take water freely for 15 min by licking the tip of the water bottle. The rats were given free access to water for 1 h after the training procedure, at which time the water was again removed.

The LI indexing procedure was conducted on days 10–13 and consisted of four sessions, with a 24-h break between them. (1) In a pre-exposure session, one group of rats (the pre-exposed (PE) group) were placed one at a time in the chamber and received 20 tone presentations (2.8 kHz, 90 dB, 10 s) with an inter-stimulus interval of 50 s without the water bottle. The remaining rats (the non-pre-exposed (NPE) group) were likewise placed in the chamber for the same period of time without being exposed to the tone. (2) In a conditioning session, all the rats were placed one at a time in the chamber without the water bottle, and a CS (tone, 2.8 kHz, 90 dB, 10 s) was presented, immediately followed by an US (0.5 mA, 1.0 s, foot shock via floor grid). The first stimulus pairing was administered 5 min after the start of the session, and the pairings were given 5 times with an inter-pairing interval of 5 min. (3) In a retraining session, all the rats were placed one at a time in the chamber and took water freely for 15 min by licking the tip of the water bottle, as in the initial training. (4) In a test session, all the rats were placed one at a time in the chamber and took water freely from the tip of the water bottle. When the number of licks reached 300, the CS was continuously presented until 600 licks had been taken. The times taken to take licks 150–250 (period *A*) and 302–402 (period *B*) were measured. The suppression ratio was defined as $B/(A+B)$.

2.3. Drug treatment in LI evaluation

All drug treatments were conducted before the conditioning session, and the effects of 0.2-mg/kg haloperidol (Hal 0.2) and 0.3-, 1.0-, and 3.0-mg/kg blonanserin (BNS 0.3, BNS 1.0, and BNS 3.0) on methamphetamine-induced (1.0 mg/kg) disruption of LI were examined. The drug dosages were determined by reference to previous studies that examined the effects of intraperitoneal injection of antipsychotics on LI [3,9,25,26]. Sixty-nine rats were randomly assigned to ten experimental groups in a 2×5 factorial design with main factors of pre-exposure (NPE, PE) and drug (saline (SAL), Hal 0.2, BNS 0.3, BNS 1.0, and BNS 3.0). Blonanserin (Dainippon Sumitomo Pharma. Co., Ltd., Japan) was suspended in and diluted with an appropriate amount of 0.5% tragacanth (Sigma Aldrich, Co., Ltd., USA) to make 0.3-, 1.0-, and 3.0-mg/mL blonanserin solutions. Haloperidol and methamphetamine (Dainippon Sumitomo Pharma. Co., Ltd., Japan) were diluted with appropriate amounts of saline to make 0.2- and 1.0-mg/mL solutions, respectively. The rats were injected intraperitoneally with a 1.0-mL/kg antipsychotic (or saline for the control group), and methamphetamine was injected intraperitoneally 75 min after the injection of the antipsychotic and 15 min before the conditioning session. The timings of the methamphetamine and antipsychotics treatments were determined by referring to a previous report [26] and to one stating that dopamine release reaches a maximum level 90–120 min after the treatment of blonanserin [13]. To obtain a standard suppression ratio, we prepared PE and NPE group rats (naïve) without any drug treatment ($n = 12$ for each).

2.4. c-Fos evaluation

An immunohistochemical experiment was used to examine the patterns of c-Fos immunopositive cells in the prefrontal cortex (PFC), accumbens core area (AcbC), accumbens shell area (AcbSh), central amygdala nucleus (Ce), and basolateral amygdala area (BLA) after the injection of an antipsychotic and/or methamphetamine. Sixty-four rats were randomly assigned to nine experimental groups in a 3×3 factorial design with main factors of antipsychotics (vehicle (Veh), 0.2-mg/kg haloperidol (Hal 0.2), or 3.0-mg/kg blonanserin (BNS 3.0)) and methamphetamine (SAL, 1.0-mg/kg methamphetamine (MAP 1.0), or 3.0-mg/kg methamphetamine (MAP 3.0)). All drugs were diluted in the same manner as in the behavioral experiment. The Veh group was treated with 0.5% tragacanth.

Following handling of the rats for 5 min over 4 days, an antipsychotic (or Veh) was intraperitoneally administrated. Methamphetamine (or SAL) was injected intraperitoneally 75 min later and 120 min before perfusion. Our timing of the methamphetamine treatment is based on a finding that c-Fos expressions are detected 15–120 min after methamphetamine treatment [19]. The rats were then deeply anesthetized with sodium pentobarbital and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1-M phosphate buffer (PB; pH 7.4). Their brains were removed immediately and postfixed at 4 °C for 1 h in the above fixative. After fixation, the samples were immersed