Behavioural Brain Research 224 (2011) 159–165

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

p-Hydroxyamphetamine causes prepulse inhibition disruption in mice: Contribution of serotonin neurotransmission

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ARTICLE INFO

Article history:
Received 15 October 2010
Received in revised form 28 May 2011
Accepted 2 June 2011

Key words:
p-Hydroxyamphetamine
Prepulse inhibition
Serotonin

ABSTRACT

p-Hydroxyamphetamine (*p*-OHA) has been shown to have a number of pharmacological actions, including causing abnormal behaviors such as increased locomotor activity and head-twitch response in rodents. We have recently reported that intracerebroventricular (i.c.v.) administration of *p*-OHA dose-dependently induces prepulse inhibition (PPI) disruption in mice, which is attenuated by pretreatment with haloperidol, clozapine or several dopaminergic agents. Haloperidol and clozapine have affinities for serotonergic (especially 5-HT_{2A}) receptors. To investigate the involvement of the central serotonergic systems in *p*-OHA-induced PPI disruption, herein we tested several serotonergic agents to determine their effects on *p*-OHA-induced PPI disruption. *p*-OHA-induced PPI disruption was attenuated by pretreatment with 5,7-dihydroxytryptamine (5,7-DHT, a neurotoxin which targets serotonin-containing neurons) and *p*-chlorophenylalanine (PCPA, a serotonin synthesis inhibitor). *p*-OHA-induced PPI disruption was also attenuated by pretreatment with ketanserin (a 5-$HT_{2A/2C}$ receptor antagonist) and MDL100,907 (a selective 5-HT_{2A} receptor antagonist). These data suggest that *p*-OHA-induced PPI disruption may involve increased serotonin release into the synaptic cleft, which then interacts with the post-synaptic 5-HT_{2A} receptor.

1. Introduction

p-Hydroxyamphetamine (*p*-OHA), an amphetamine (AMPH) and methamphetamine (METH) metabolite, has been shown to have a number of pharmacological actions, including causing psychostimulant-induced behaviors such as those induced by drugs of abuse [3,8,17,36]. For example, we have recently reported that intracerebroventricular (i.c.v.) administration of *p*-OHA induces hyperlocomotion and prepulse inhibition (PPI) disruption in mice, the hyperlocomotion is inhibited by pretreatment with a dopamine (DA)-reuptake inhibitor [30], and the PPI disruption is attenuated by pretreatment with haloperidol, clozapine, L-741,626 (a selective D_2 receptor antagonist), L-745,870 (a selective D_4 receptor antagonist) or 6-hydroxydopamine (a neurotoxin which targets DA-containing neurons) (Table 1, [31]). PPI of the acoustic startle response (ASR) is a form of sensorimotor gating, defined as an inhibition of the startle reflex that occurs when the startling stimulus is preceded by a weak prestimulus [12]. Schizophrenia patients exhibit impairment in PPI of the ASR, which is commonly interpreted as a sensorimotor gating deficit [40]. PPI is also diminished in rodents treated with psychotomimetic drugs such as AMPH, METH and phencyclidine [2,22,23]. PPI has been suggested to be a relevant model for studying the pathophysiology of schizophrenia [12]. Our reports indicate that the central dopaminergic system may be involved in abnormal behaviors induced by *p*-OHA [30,31]. However, *p*-OHA has been assumed to induce the release of neurotransmitters from presynaptic monoaminergic neurons. Indeed, previous reports have shown that the release of DA and serotonin (5-HT) can be induced by *p*-OHA [17,27]. We have previously reported that i.c.v. administration of *p*-OHA induces a head-twitch response (HTR) in mice, a possible model of hallucination [41]. HTR is markedly reduced by pretreatment with a

Abbreviations: AMPH, amphetamine; ANOVA, analysis of variance; ASR, acoustic startle response; DA, dopamine; 5,7-DHT, 5,7-dihydroxytryptamine; DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine; HTR, head-twitch response; i.c.v., intracerebroventricular; METH, metamphetamine; MRN, median raphe nucleus; NA, noradrenaline; PCPA*p*-, chlorophenylalanine; *p*-OHA, *p*-hydroxyamphetamine; PPI, prepulse inhibition; SEM, standard errors of the mean; SERT, serotonin transporter.

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doi:10.1016/j.bbr.2011.06.002

Table 1
Effects of antipsychotics and dopaminergic agents on *p*-hydroxyamphetamine (*p*-OHA)-induced prepulse inhibition (PPI) disruption [31].

Drug	Classification	Attenuate/ineffective
Haloperidol	A typical antipsychotic	Attenuate
Clozapine	An atypical antipsychotic	Attenuate
SCH 23390	A selective D_1 antagonist	Ineffective
L-741,626	A selective D_2 antagonist	Attenuate
Eticlopride	A D_2/D_3 antagonist	Ineffective
L-745,870	A selective D_4 antagonist	Attenuate
6-Hydroxydopamine	A neurotoxin for DA neurons	Attenuate
GBR 12909	A DA-reuptake inhibitor	Ineffective

Pretreatment with haloperidol, clozapine, SCH 23390, L-741,626, eticlopride, L-745,870, GBR 12909 or their solvents as vehicle was performed by intraperitoneal (i.p.) or subcutaneous (s.c.) administration 30 min before intracerebroventricular (i.c.v.) administration of *p*-OHA (80 μg/mouse) or Ringer's solution. Pretreatment with 6-hydroxydopamine (40 μg/mouse) or vehicle was performed by i.c.v. administration 7 days before i.c.v. administration of *p*-OHA or Ringer's solution; i.c.v. administration of *p*-OHA or Ringer's solution was performed 25 min before the start of PPI testing.

5-HT synthesis inhibitor such as *p*-chlorophenylalanine (PCPA) or pretreatment with 5-HT receptor antagonists such as cyproheptadine and dimethothiazine, indicating the involvement of the central serotonergic system [41].

Thus, to investigate the involvement of the central serotonergic system in *p*-OHA-induced PPI disruption, we investigated the effects of several serotonergic agents on PPI disruption. First, we used presynaptic serotonergic agents i.e., 5,7-dihydroxytriptamine (5,7-DHT, a neurotoxin which targets 5-HT-containing neurons) or PCPA (a 5-HT synthesis inhibitor) on *p*-OHA-induced PPI disruption. Both these agents were shown to reduce 5-HT levels [15,26,38]. If increased 5-HT levels from presynaptic 5-HT nerve terminals are involved in *p*-OHA-induced PPI disruption, then 5,7-DHT or PCPA treatment is expected to attenuate PPI disruption. Next, we tested the effects of ketanserin or MDL100,907, for the purpose of inhibiting the 5-HT_{2A} receptor, which, along with the D_2 receptor, is one of the main targets of antipsychotics.

2. Materials and methods

All experiments were performed with the approval of the Ethics Committee of Animal Experiments in Tohoku Pharmaceutical University and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize suffering and to reduce the number of animals used.

2.1. Animals

Male ddY strain mice (weighing 28–34 g, Japan SLC, Japan) were used for all experiments. Approximately 5–7 mice were housed in cages with free access to food and water under conditions of constant temperature (22 ± 2 °C) and humidity (55 ± 5%), on a 12 h light–dark cycle (lights on: 07:00 to 19:00).

2.2. Drugs and treatments

The following drugs were used: the racemic form of *p*-OHA hydrobromide (Smith-Kline and French, USA), 5,7-DHT, PCPA, ketanserin tartrate salt (Sigma–Aldrich, USA) and MDL100,907 (ABX Advanced Biochemical Compounds, Germany). *p*-OHA was dissolved in Ringer's solution. 5,7-DHT was dissolved in Ringer's solution containing 0.1% ascorbic acid. PCPA and MDL100,907 were dissolved in 0.5% Tween 80 and ketanserin was dissolved in 0.9% saline. For all solutions, pH was adjusted to 7.4 at 37 °C. Doses of all drugs were based on previous studies [4,9,26,31,35,38].

The technique employed herein for i.c.v. injection into mice was the same as that originally described by Brittain and Handley [7]. Briefly, i.c.v. administration of *p*-OHA, 5,7-DHT or Ringer's solution was performed with a disposable 27-G needle attached to a 50 μL Hamilton microsyringe and inserted into the left lateral ventricle of unanesthetized mice. *p*-OHA, 5,7-DHT or Ringer's solution was administered in a total volume of 5 μL at a constant rate of 1 μL/2 s. Administration (i.c.v.) of *p*-OHA or Ringer's solution was performed 25 min before the start of PPI testing. Pretreatment with ketanserin, MDL100,907 or their solvents as vehicle was performed by intraperitoneal (i.p.) administration 30 min before i.c.v. administration of *p*-OHA or Ringer's solution. Pretreatment with PCPA or 0.5% Tween 80 as vehicle was performed by i.p. administration 12 h before i.c.v. administration of *p*-OHA or Ringer's solution (Fig. 1). After the experimental period, brains were removed, snap-frozen, and cut into 20 μm coronal sections using a Cryostat HM560 M (Carl Zeiss, Germany). Accuracy of all i.c.v. injection sites were visually confirmed by examining cresyl violet-stained coronal sections.

2.3. 5,7-DHT lesion

The 5,7-DHT lesioning technique is based on a previous report [38] with minor modifications. Briefly, 20 min prior to 5,7-DHT injection, mice were administered desipramine (25 mg/kg, i.p.) to protect noradrenaline (NA)-containing neurons. 5,7-DHT (50 μg/mouse) or vehicle was injected into the left lateral ventricle of unanesthetized mice. Seven days after i.c.v. administration of 5,7-DHT or vehicle, *p*-OHA or Ringer's solution was administered i.c.v. 25 min before the start of PPI testing (Fig. 1).

2.4. Quantitative autoradiography

To confirm the 5,7-DHT lesion effect, two cohorts of mice were used for autoradiographic labeling of the serotonin transporter (SERT), which is located on serotonergic terminals. After the behavioral experiments, the vehicle (i.c.v.) + Ringer's (i.c.v.) group and 5,7-DHT (i.c.v.) + Ringer's (i.c.v.) group were sacrificed by decapitation. Brains were quickly removed, frozen in isopentane at −40 °C and then stored at −80 °C. Using a Cryostat, 20 μm coronal sections including both the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) were obtained on gelatin-coated slides and stored at −80 °C until use. SERT binding sites were visualized using [^{3}H]-citalopram (79.0 Ci/mmol) (PerkinElmer Life and Analytical Sciences, USA). The protocol was adapted from previous studies [20] with minor modifications. Briefly, the slides were warmed to room temperature for an hour and preincubated in 50 mM Tris–HCl buffer (NaCl 120 mM, $CaCl_2$ 4 mM, pH 7.4) at room temperature for 15 min. The slides were then incubated in an assay buffer containing 2 nM [^{3}H]-citalopram in the same buffer for 60 min at room temperature to determine total binding. Non-specific binding was determined by incubation of 10 μM fluoxetine (TOCRIS, USA) in the assay buffer, which already contained 2 nM [^{3}H]-citalopram. At the end of incubation, sections were rinsed four times, 2 min each in ice-cold buffer as described above, then dipped in deionized water to remove salts and rapidly dried. All slides were exposed to a tritium sensitive imaging plate (Fuji Film, Japan) together with [^{3}H] microscale standards (GE Healthcare UK Ltd., UK) for 10 days to determine [^{3}H]-citalopram binding. Specific labeling was quantified (nGi/mg wet weight) using a computer-assisted Image Reader BAS 5000 ver. 1. 12 (Fuji Film).

2.5. PPI of the acoustic startle response

Tests were conducted using the SR-LAB system (SR-LAB, San Diego Instruments, USA) that comprised two sound-attenuating chambers each equipped with a cylindrical Plexiglas animal enclosure (length: 6.7 cm; inner diameter: 3.8 cm). Ventilation was provided by a small electric fan that also generated a 70 dB background noise. Tone pulses were presented by a speaker positioned 24 cm directly above the animal enclosure. A piezoelectric accelerometer affixed to the animal enclosure frame was used to detect and transduce motion resulting from the animal's response. Tone pulse parameters were controlled by a computer using a commercial software package (SR-LAB) and interface assembly that also digitized, rectified and recorded stabilimeter readings.

Measures of both ASR and PPI were obtained in a single session as described by Nakagawasai et al. [29]. Twenty-five minutes after i.c.v. administration of *p*-OHA or Ringer's solution, animals were placed in the Plexiglas enclosure and allowed to acclimatize to the environment for 5 min before being tested during 42 discrete trials. On the first two trials, the magnitude of the ASR to a 120 dB tone lasting 50 ms was measured. These first two startle tones were presented in order to habituate the animals to the testing procedure. Therefore, the ASR magnitude of these two trials was omitted from the statistical analysis of the mean ASR amplitude. On the subsequent 40 trials, the startle tone was either presented alone or 100 ms after presentation of 30 ms duration prepulse. Prepulse intensity ranged from 3 to 15 dB above background noise and was varied randomly between trials in 3 dB steps. Measures were taken at each of the five prepulse intensities during five trials; animals were randomly presented with the startle tone alone during another ten trials; null trials (background tone alone) during the other five trials. The same stimulus condition was never presented on more than two consecutive trials. The interval between each trial was programmed to a variable time schedule with an average duration of 15 s (range 5–30 s). A measure of startle response amplitude was derived from the mean of 100 digitized data points collected from stimulus onset at a rate of 1 kHz. Prepulse effectiveness in suppressing the startle response was expressed as a percentage based on the mean amplitude of responses to the startle tone alone (10 startle tones) relative to those recorded under the five prepulse conditions (5 startle tones/condition): PPI = 100 − [(mean startle amplitude for prepulse + pulse trials/mean startle amplitude for pulse-alone trials) × 100]%. PPI data also expressed as average across all prepulse intensities (average PPI).