Scientific Research

Effect of Aripiprazole on Methamphetamine-Induced Disruption of Latent Inhibition in Rats

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Received June 23, 2011; revised July 19, 2011; accepted August 2, 2011

Abstract

Objectives: To elucidate the pharmacological profile of aripiprazole, we examined its ameliorating effect on the methamphetamine-induced disruption of latent inhibition (LI) in rats. **Method:** The effect was measured using a conditioned emotional response procedure. The conditioned stimulus was a tone (2.8 kHz, 90 dB), and the unconditioned stimulus was a mild foot shock delivered through a floor grid. The conditioning procedure was repeated five times. **Results:** Methamphetamine-induced (1.0 mg/kg, i.p.) disruption of LI was ameliorated by the administration of haloperidol (0.2 mg/kg, i.p.) and by a moderate dose of aripiprazole (0.3 mg/kg, i.p.) but not by a lower or higher dose (0.1 or 3.0 mg/kg, i.p.) of aripiprazole. However, immunohistochemical examination showed increased levels of c-Fos expression in the shell of the nucleus accumbens after the administration of haloperidol (0.2 mg/kg, i.p.) but not of aripiprazole (0.3 mg/kg, i.p.). **Conclusions:** It is suggested that aripiprazole has an ameliorating effect on methamphetamine-induced disruption of latent inhibition within only a marginal therapeutic window.

Keywords: Aripiprazole, Haloperidol, Latent Inhibition, c-Fos, Immunohistochemistry, Schizophrenia

1. Introduction

The present study aimed to characterize the psychopharmacological profile of aripiprazole, 7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy)-3,4-dihydrocar-bostycil, by examining its ameliorating effect on methamphetamine-induced disruption of latent inhibition (LI) in rats, an animal model of the cognitive impairments seen in schizophrenia [1]. LI is typically measured in classical fear conditioning. That is, if a conditioned stimulus is presented several times without an unconditioned stimulus before conditioning, the animal learns to ignore, or not to pay attention to, the stimulus, and, consequently, the strength of the subsequent conditioning is inhibited [2]. This phenomenon is thought to relate to "attentional filtering mechanism", which enables us to distinguish significant stimuli from irrelevant stimuli, and is known to be disrupted in patients with schizophrenia [1]. LI is also disrupted by the injection of the dopamine releasers, amphetamine and methamphetamine, in humans and rodents, and such disruption is ameliorated by typical and atypical antipsychotic drugs (APDs) [3-6]. Therefore, the

psychopharmacological profiles of novel APDs can be characterized by examining their ameliorating effect on amphetamine- or methamphetamine-induced disruption of LI.

Aripiprazole, is a novel atypical APD that has been reported to have high affinity for dopamine D_2, and it acts as a partial dopamine D_2 receptor agonist [7]. These pharmacological profiles play a central role in the therapeutic effects of aripiprazole on schizophrenia. Although aripiprazole has been reported to enhance some cognitive functions in schizophrenia [8] and some animal model of schizophrenia [9], its effect on disrupted LI has not been examined.

It has been suggested that the remedial effects of APDs on disrupted LI are related to some neural substrates, in which the nucleus accumbens would play a key role [1]. However, no in-depth morphological study has been conducted, and, therefore, we examined the c-Fos expression in brain areas related to LI.

2. Materials and Methods

Seventy-five adult male Sprague-Dawley rats (Charles

River Laboratories, Japan), weighing 260 - 290 g, were used. They were housed in a temperature-controlled colony room (23°C ± 1°C) on a 12:12 h light:dark cycle. Water availability was restricted, as described below, while food was available ad libitum. The experimental procedures were approved by the ethical committee of animal experimentation at the University of Miyazaki.

LI was assessed using an off-baseline conditioned emotional response procedure in rats licking for 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 entire 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).

After being handled for 5 min, a rat was placed in the experimental chamber for 5 min on four consecutive days. For the next five consecutive days, rats were water restricted overnight, then placed in the chamber and allowed to take water freely for 15 min by licking the tip of the water bottle. Rats were given free access to water for 1 h after training at which time 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 the preexposure session, the rats in the preexposed group (PE) received 20 tone presentations (2.8 kHz, 90 dB, 10 s) with an interstimulus interval of 50 s without the water bottle. The rats in the non-preexposed group (NPE) were placed in the chamber for the same period of time without being exposed to the tone. 2) In the conditioning session, without the water bottle, the conditioned stimulus (tone, 2.8 kHz, 90 dB, 10 s) was immediately followed by an unconditioned stimulus (0.5 mA, 1.0 s, foot shock via floor grid). The first pairing was given 5 min after the start of the session, and the pairings were given 5 times with an interpairng interval of 5 min. 3) In the retraining session, the rats took water freely for 15 min by licking the tip of the water bottle as in the initial training. 4) In the test session, which was conducted in the behavioral experiment (experiment 1) but not in the immunohistochemical experiment (experiment 2), the rats took water freely from the tip of the water bottle in the test chamber. When the number of licks reached 300, the conditioned stimulus (2.8 kHz, 90 dB, tone) was continuously presented until the rats completed 1000 licks. The time taken to complete licks 150 - 250 (period A) and 302 - 402 (period B) was measured. The suppression ratio was defined as $B/(A + B)$.

2.1. Drugs

Aripiprazole was supplied by Otsuka Pharmaceutical Co.,

Ltd., Tokyo, Japan as a bulk powder.

Aripiprazole (50 mg) was dissolved in 99.5% N,N-dimethylformamide (Nacalai Tesque, Inc., Kyoto, Japan, 2.0 ml) and 0.1 M citrate buffer (8.0 ml) to make a stock solution with a pH between 4.5 and 5.0. Dilutions were made with saline to make 0.1, 0.3, and 3.0 mg/ml aripiprazole. Haloperidol (Dainippon Sumitomo Pharmaceutical Co., Ltd., Tokyo, Japan, 5 mg/ml) was diluted with saline to make 0.2 mg/ml haloperidol, and bulk powder of methamphetamine (Dainippon Sumitomo) was diluted with saline to make 1.0 mg/ml methamphetamine.

2.2. Experiment 1

In experiment 1, the effect of 0.2 mg/kg haloperidol and 0.1, 0.3, and 3.0 mg/kg aripiprazole on methamphetamine-induced (1.0 mg/kg) disruption of LI was examined. Sixty rats were randomly assigned to twelve experimental groups. To obtain a standard suppression ratio, we prepared five PE rats and five NPE rats without any drug treatment (naïve group). The remaining fifty rats were assigned to ten methamphetamine-treated groups in a 2 × 5 factorial arrangement with the main factors being preexposure (PE, NPE) and drug (saline (SAL), 0.2 mg/kg haloperidol (Hal 0.2), and 0.1, 0.3, 3.0 mg/kg aripiprazole (Ari 0.1, Ari 0.3, Ari 3.0, respectively)). They were injected with aripiprazole (0.1, 0.3, 3.0 mg/kg, i.p.), haloperidol (0.2 mg/kg, i.p.), or saline (1.0 ml/kg, i.p.) 60 min before the conditioning, and 45 min after these injections, methamphetamine was injected (1.0 mg/kg, i.p.).

2.3. Experiment 2

In experiment 2, the expression patterns of c-Fos in the prefrontal cortex (PFC), nucleus accumbens core area (AcbC), nucleus accumbens shell area (AcbSh), and basolateral area of the amygdala (BLA) were examined. These patterns could be related to the ameliorating effects of 0.2 mg/kg haloperidol and 0.3 mg/kg aripiprazole on the methamphetamine-induced disruption of LI in experiment 1. Fifteen PE rats were randomly assigned to three groups for 1.0 ml/kg saline i.p. injection (SAL), 0.2 mg/kg haloperidol i.p. injection (Hal 0.2), or 0.3 mg/kg aripiprazole i.p. injection (Ari 3.0) 60 min before conditioning. They were also injected with methamphetamine (1.0 mg/kg, i.p.) 15 min before conditioning. The rats were then deeply anesthetized with sodium pentobarbital 90 min after the end of the conditioning session and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Their brains were removed immediately, postfixed at 4°C for 1 h in the above fixative, immersed in

0.1 M PB with 10% sucrose at 4°C for 1 h, and then cryoprotected at 4°C overnight in the same buffer with 30% sucrose. The samples were subsequently cut on a freezing microtome into 50 μm coronal sections. An immunohistochemical examination of c-Fos expression was performed in accordance with the manufacturer's instructions on the use of a streptavidin-biotin system (Histofine SAB-PO(R) kit, Nichirei, Tokyo, Japan). Briefly, biotinylated secondary antibodies were coupled with streptavidin-biotinylated horseradish peroxidase, and the reaction was visualized using diaminobenzidine as a chromogen enhanced by cobalt chloride. Between each incubation step, the free-floating sections were thoroughly rinsed with phosphate-buffered saline. The antibodies for c-Fos (1:5000) were rabbit polyclonal antisera (sc-52; Santa Cruz Biotechnology, CA, USA). Sections through the PFC, AcbC, AcbSh (approximately 1.70 mm rostral to the bregma), and BLA (approximately 2.80 mm caudal to the bregma) were selected (**Figure 1**) [10]. c-Fos immunoreactivity was analyzed using light microscopy. Four sections were taken through each structure of each animal. They were taken at the same level along the animal's antero-posterior axis to avoid variance of the level of the structure among the animals. The expression of c-Fos was quantified by counting the number of cells immunopositive for c-Fos in 0.25 × 0.25 mm squares using a 20 × microscope objective. c-Fos immunopositive cells were counted bilaterally and averaged per structure/animal.

3. Results

In experiment 1, the naïve PE group showed a significantly lower suppression ratio than the naïve NPE group (t-test, $p < 0.01$) (**Figure 2**). Two-way ANOVA with main factors of preexposure (PE, NPE) and drug (SAL, Hal 0.2, Ari 0.1, Ari 0.3, Ari 3.0) carried out on the suppression ratios yielded the significant main effects of preexposure [$F(1,40) = 10.92$, $p < 0.005$], drug [$F(4,40) = 5.28$, $p < 0.005$], and their interaction [$F(4,40) = 3.38$, $p < 0.05$]. **Figure 2** shows the mean suppression ratios of the PE and NPE groups for the five drug treatments. The simple main effect was significant for PE [$F(4,40) = 7.77$, $p < 0.001$] but not for NPE. For PE, a post hoc Bonferroni test revealed that the suppression ratios of Hal 0.2 and Ari 0.3 were significantly lower than that of SAL ($p < 0.01$ for both).

On the other hand, the simple main effects were significant for Hal 0.2 [$F(1,40) = 8.99$, $p < 0.01$] and Ari 0.3 [$F(1,40) = 14.57$, $p < 0.001$] but not for SAL, Ari 0.1, and Ari 3.0.

In experiment 2, the number of c-Fos-immunopositive cells counted in the PFC, AcbC, AcbSh, and BLA sec-

tions for the animals in the SAL, Hal 0.2, and Ari 0.3 groups, which were all preexposed to the conditioned stimulus (tone), were used for statistical analysis. Two-way ANOVA with main factors of region (PFC, AcbC, AcbSh, BLA) and drug (SAL, Hal 0.2, Ari 0.3) yielded significant main effects of region [$F(3,48) = 12.09$, $p < 0.001$] and region x drug interaction [$F(6,48) = 4.01$, $p < 0.005$], but not of drug [$F(2,48) = 2.55$, $p < 0.1$]. Significant simple main effects were detected for AcbSh ($p < 0.001$) but not for AcbC ($p > 0.1$), PFC ($p > 0.1$), or BLA ($p > 0.1$). A post hoc Bonferroni test carried out for an AcbSh section revealed that the number of cells in the Hal 0.2 was significantly greater than that in the Ari 0.3 and SAL ($p < 0.001$ for both) (**Table 1**).

4. Discussion

These results showed that the disruption of LI induced by methamphetamine was significantly ameliorated by administration of haloperidol (0.2 mg/kg) and by a moderate dose of aripiprazole (0.3 mg/kg) but not by a relatively low or high dose of aripiprazole (0.1 or 3.0 mg/kg).

It has been reported that amphetamine-induced disruption of LI is ameliorated by APDs, olanzapine [3], chlorpromazine [4], haloperidol [5], and clozapine [6]. Even though the receptor binding profiles (D_2 and D_3 partial agonistic, $5HT_{1A}$ agonistic, and $5HT_{2A}$ antagonistic [7, 11]) of aripiprazole differ from those of other APDs, its effect on disrupted LI induced by injection of a dopamine-releasing agent was suggested to be equivalent.

Since lower and higher doses of aripiprazole did not have an ameliorating effect on disrupted LI, simple dose dependency was not confirmed. It is reported that aripiprazole has a functional D_2 partial agonist effect at low

**Table 1. Number of c-Fos-immunopositive cells/0.0625 mm²
in SAL, Hal 0.2, and Ari 0.3 groups. The brain sample was
obtained 90 min after conditioning. Animals had preexposure to the conditioned stimulus (tone) one day before conditioning. Saline, haloperidol 0.2 mg/kg, or aripiprazole 0.3 mg/kg was injected 60 min before conditioning, and all groups had an injection of methamphetamine 1.0 mg/kg 15 min before conditioning.**

Area	Group		
	SAL	Hal 0.2	Ari 0.3
PFC	15.1 ± 1.7	8.9 ± 1.4	12.2 ± 2.8
AcbC	20.7 ± 4.2	24.1 ± 2.8	17.5 ± 2.0
AcbSh	17.2 ± 3.1	33.3 ± 5.9*	16.8 ± 2.0
BLA	13.9 ± 1.4	9.8 ± 1.0	11.9 ± 0.8

Value represents mean number ± SEM. PFC: prefrontal cortex, AcbC: nucleus accumbens core area, AcbSh: nucleus accumbens shell area, BLA: basolateral area of amygdala. *Number of c-Fos-positive cells was significantly higher for Hal 0.2 than Ari 0.3 and SAL in AcbSh ($p < 0.001$ for both).
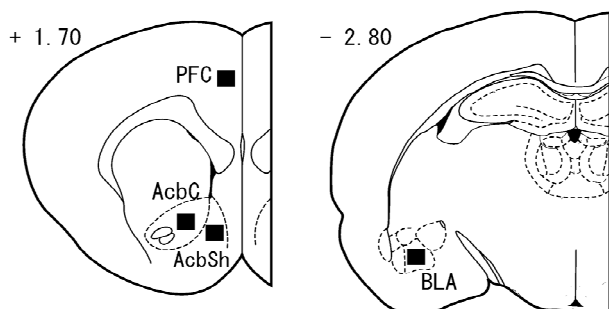
Figure 1. Schematic representation of anatomical regions in which the number of c-Fos-immunopotive cells was evaluated (drawn on the atlas of Paxinos & Watson, [10]). The number on the side of each section indicates the distance (mm) anterior (+) or posterior (–) to the bregma.
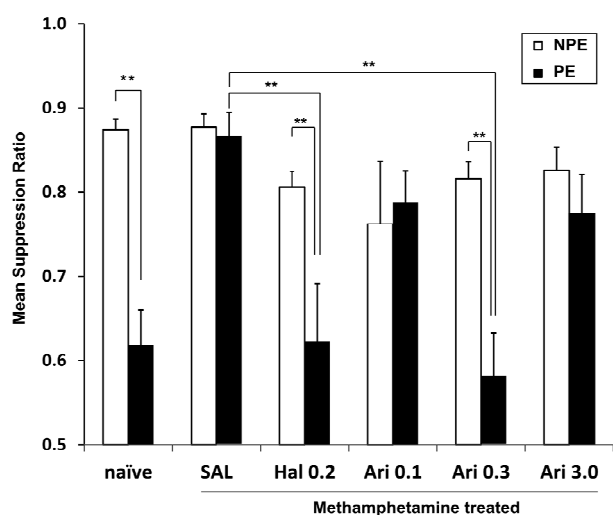

Figure 2. Mean and standard error of suppresion ratio for non-preexposed (NPE) and preexposed (PE) groups under naïve and five drug teatments. **p < 0.01.

doses and a functional D_2 blockade at high doses levels. Possibly relating to this profile, tri-phasic dose-response of aripiprazole on hyperactivity of the ckr mouse [12], and more efficient effect of a lower dose than higher dose of aripiprazole on impulsivity [13], and increase of DOPAC in the striatum after low dose, but not high dose of aripiprazole [14], were reported. In the present study, similar tri-phasic dose-response and effects within only a marginal therapeutic window were found.

Immunohistochemical examination showed a significantly increased number of c-Fos-labeled cells in the AcbSh after administration of haloperidol (0.2 mg/kg) but not after aripiprazole (0.3 mg/kg), compared to saline injection. It is reported that aripiprazole which is lower than 10.0 mg/kg did not produce c-Fos expression in AcbSh [15]. In the present study, various contributing factors, the dopamine-releasing agent used, the APDs used, and the conditioning procedure, are included and it

is difficult to compare directly the results, but 0.3 mg/kg aripiprazole might be insufficient to induce c-Fos expression in AcbSh.

It is suggested that aripiprazole has an ameliorating effect on methamphetamine-induced disruption of latent inhibition within only a marginal therapeutic window, although it's neural mechanisms have not been revealed.

5. Acknowledgements

6. References

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