Full Paper

Effect of Aripiprazole on 5-HT₂ Receptor-mediated Wet-Dog Shake Responses and Disruption of Prepulse Inhibition in RatsShuntaro Kohnomi¹, Katsuya Suemaru^{2,*}, Hiromu Kawasaki¹, and Hiroaki Araki²¹*Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan*²*Department of Clinical Pharmacology and Pharmacy, Ehime University Graduate School of Medicine, Shitsukawa Toon, Ehime 791-0295, Japan*

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Abstract. Aripiprazole, an atypical antipsychotic drug, is a D₂ dopamine-receptor partial agonist, but also has affinity to several serotonin receptors (5-HT_{1A,2A,2C,7}). However, little is known about the contribution of serotonin receptors in the action of aripiprazole. The present study investigated the effects of aripiprazole on 5-HT_{2A} receptor-mediated behaviors and compared them with the effects on dopamine receptor-mediated behavior in rats. Aripiprazole (10, 30 mg/kg, p.o.) inhibited the stereotyped behavior induced by apomorphine (1 mg/kg, s.c.), a dopamine-receptor agonist, and the wet-dog shake responses induced by DOI (2,5-dimethoxy-4-iodoamphetamine, 2 mg/kg, s.c.), a 5-HT_{2A}-receptor agonist. Moreover, aripiprazole improved the disruption of prepulse inhibition induced by both apomorphine and DOI significantly. These data suggest that not only the dopaminergic system, but also the serotonergic system are involved in the antipsychotic effect of aripiprazole.

Keywords: aripiprazole, 5-HT_{2A} receptor, 2,5-dimethoxy-4-iodoamphetamine (DOI), apomorphine, prepulse inhibition

Introduction

Atypical antipsychotic drugs are now commonly used in the treatment of schizophrenia, due to the fact that these can more effectively treat the negative symptoms of schizophrenia, while demonstrating less extrapyramidal side effects than typical drugs. Aripiprazole, an atypical antipsychotic drug, is a partial agonist of D₂ receptors ($K_i = 0.74$ nM for D_{2L} receptor), and it has moderate affinity for several serotonin receptors (5-HT_{1A,2A,2C,7}), such as being a 5-HT_{1A}-receptor partial agonist ($K_i = 5.6$ nM) and 5-HT_{2A}-receptor antagonist ($K_i = 22$ nM) (1). Above all, the 5-HT_{2A} serotonin receptor is known to be involved with the negative symptoms of schizophrenia, which is one of the targets of serotonin-dopamine antagonists (SDAs). This raises the possibility that the antipsychotic effects of aripiprazole may also be involved with the 5-HT_{2A} serotonin

receptor. However, little is known about the contribution of serotonin receptors including the 5-HT_{2A} receptor in the action of aripiprazole. Therefore, in this study, we investigated the involvement of the 5-HT_{2A} serotonin receptor on the antipsychotic effects of aripiprazole in Wistar rats.

Prepulse inhibition (PPI) is a phenomenon in which the amplitude of the acoustic startle response is reduced by a preceding weak stimulation. PPI is thought to reflect the operation of a sensorimotor gating system in the brain (2, 3). It is known that PPI tends to be deficient in patients with schizophrenia (4, 5) and in animals treated with a dopamine-receptor agonist (6, 7), a 5-HT_{2A}-receptor agonist (8), or NMDA-receptor antagonist (9) and that these deficiencies of the PPI are improved by various antipsychotic drugs (10, 11). Therefore, the disruption of the PPI in these animals is thought to be useful for evaluating the effect of antipsychotic drugs. On the other hand, it is well known that DOI, a 5-HT_{2A/2C}-receptor agonist, produces the disruption of PPI or wet-dog shaking behavior, which is mediated via the activation of the 5-HT_{2A} receptor in rats

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(8). These behavioral models are useful for studying the effects of antipsychotic drugs on the function of 5-HT_{2A} receptors.

In the present study, first of all, in order to confirm the antagonism against dopamine and 5-HT_{2A} receptors in vivo, the effect of aripiprazole on dopamine and 5-HT_{2A} receptor-mediated behaviors were examined. For dopaminergic antagonism, the effect on the stereotyped behavior induced by the dopamine-receptor agonist apomorphine was investigated, and for serotonergic antagonism, the effect on the wet-dog shake responses induced by DOI was examined. Next, the effects of aripiprazole on the PPI disruption, induced by apomorphine and DOI were investigated to clarify the antipsychotic effects of aripiprazole via 5-HT_{2A} receptors.

Materials and Methods

Animals

Male Wistar strain rats (at 8–10 weeks of age) were obtained from Charles River Laboratories (Atsugi). All animals were housed in an animal room maintained at $22 \pm 2^\circ\text{C}$ under a 12 h / 12 h light/dark cycle with lights on from 7:00 a.m. Food and water were available ad libitum. Animal experiments were performed in compliance with the Guidelines for Animal Experimentation, Ehime University School of Medicine. We used 5–9 animals per group in the stereotyped behavior test and wet-dog shake response test and 10 animals per group in PPI test.

Drugs

Apomorphine hydrochloride (Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in saline containing 0.1% ascorbic acid and the solution was kept on ice in the dark to protect against oxidative degradation. DOI (Sigma-Aldrich) was dissolved in saline. All drugs were expressed as a free base. Each drug was administered subcutaneously in a volume of 0.2 ml/100 g body weight. The appropriate doses of apomorphine and DOI were determined in our previous study (12). Aripiprazole was suspended in 0.5% methylcellulose in a volume of 0.5 ml/100 g body weight, and administered orally.

Stereotyped behavior test

Stereotyped behavior induced by apomorphine was observed in the individual wire mesh cages (20 × 15 × 15 cm) and scored. The animals were allowed to become accustomed to their cages over a 30-min period, and then aripiprazole or vehicle (0.5% methylcellulose in distilled water) was administered orally. One hour after the administration of aripiprazole or vehicle, apomorphine (1 mg/kg) was administered subcutaneously. Immediately

after the injection of apomorphine, the stereotyped behavior was scored every 5 min for 90 min. The degree of stereotyped behavior was measured by the following scoring system: 0, no stereotype; 1, discontinuous sniffing; 2, continuous sniffing; 3, continuous sniffing and discontinuous licking or biting; 4, continuous sniffing and continuous licking or biting. The scores were plotted against time after the apomorphine administration, and AUCs were calculated.

Wet-dog shake responses

The wet-dog shake responses induced by DOI were observed in individual clear polycarbonate cages (22 × 38 × 18 cm). Aripiprazole or vehicle (0.5% methylcellulose in distilled water) was administered orally 1 h before DOI administration. Then DOI (2 mg/kg) was administered subcutaneously, and immediately after the administration, the number of wet-dog shake responses was counted for 30 min.

Prepulse inhibition

One startle chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA) was used. The chamber consisted of a Plexiglas cylinder (8.9 cm in diameter × 20 cm long) resting on a Plexiglas frame in a sound-attenuated lighted, ventilated enclosure. Background noise and acoustic noise bursts were presented via a loudspeaker mounted 12-cm above the cylinder. Startle responses, reflecting the motion of animals in the cylinder following the acoustic stimulus, were detected by a piezoelectric transducer mounted below the frame. Stabilimeter readings were rectified and recorded by a microcomputer and interface ensemble (San Diego Instruments). After a 5-min acclimation period, the PPI test was started. Throughout both the preliminary period and the test session, the chamber light was on, and the background noise was set at 68 dB; all sounds were given as white noise. The test session included five initial startle stimuli (120 dB of 20-ms duration) to accustom the rats to the startle stimuli, followed by three different types of 10 trials (total 30 trials) presented in random order: a pulse (P) trial (120 dB of 20-ms duration) and two prepulse and pulse (PP-P) trials, which involved a prepulse (80 or 83 dB of 20-ms duration) followed by the same pulse as in the P trial 100 ms later. The average intertrial interval was 40 s (range: 20–60 s), and this interval was randomized. The startle responses were measured for 100 ms at every trial, and the maximum value was defined as the startle amplitude. The animals' startle amplitudes in response to repetition of each trial type were averaged across the session. The experimental schedule was controlled by a microcomputer. The PPI in PP80-P or PP83-P trial was

calculated as a percent inhibition of the startle amplitude in the P trial according to the following formula: [startle amplitude (P trial) – startle amplitude (PP-P trial)] / startle amplitude (P trial) × 100. The five initial startle stimuli were excluded from the statistical analysis.

Aripiprazole or vehicle (0.5% methylcellulose in distilled water) was administered orally 75 min before the measurement, and apomorphine, DOI, or vehicle (saline containing 0.1% ascorbic acid or normal saline, respectively) was administered subcutaneously 15 min before the measurement.

Data analyses

In the stereotyped behavior experiments, the calculated AUCs were analyzed using a one-way analysis of variance (ANOVA), and the *post hoc* comparisons were performed with Dunnett's test. The number of wet-dog shake responses were analyzed using one-way ANOVA, and *post hoc* comparisons were performed with Tukey's test. In the PPI experiments, the disrupting effects of apomorphine or DOI were analyzed using Student's *t*-test, and repeated measures were analyzed using an analysis of variance (two-way ANOVA) with drug treatment as a between-subject factor and pulse intensity as a within-subject factor. The *post hoc* individual comparisons were performed with Dunnett's test. The startle amplitude of the P-trial was analyzed using one-way ANOVA, and *post hoc* comparisons were performed with Dunnett's test. The significance level was set at $P < 0.05$ in all experiments.

Results

Aripiprazole (10, 30 mg/kg, p.o.) significantly inhibited the stereotyped behavior induced by apomorphine (1 mg/kg, s.c.) ($F_{(2,12)} = 14.91$, $P < 0.05$) in a dose-dependent manner (Fig. 1: a, b). Furthermore, aripiprazole also significantly inhibited the wet-dog shake responses induced by DOI (2 mg/kg, s.c.) ($F_{(2,17)} = 11.46$, $P < 0.05$) (Fig. 2). These data confirmed that aripiprazole antagonized both dopaminergic and serotonergic action.

Figure 3 shows the effect of aripiprazole on the disruption of the PPI. Aripiprazole alone (10, 30 mg/kg, p.o.) did not affect the PPI. Next, the effect of aripiprazole on the disruption of the PPI induced by apomorphine (Fig. 4) or DOI (Fig. 5) was investigated. Apomorphine and DOI disrupted the PPI at both prepulse intensities (apomorphine: $P < 0.01$ at 80 dB and $P < 0.05$ at 83 dB, DOI: $P < 0.01$ at both 80 and 83 dB). Aripiprazole (10, 30 mg/kg) improved the disruption of the PPI induced by apomorphine significantly at both prepulse intensities ($F_{(2,27)} = 4.549$, $P < 0.05$). There was

Fig. 1. The effect of aripiprazole on stereotyped behavior induced by apomorphine. a: The time course of score values after administration of apomorphine (APO, 1 mg/kg, s.c.). Score values (mean $\pm$ S.E.M.) were plotted against the time after administration of apomorphine. b: AUC for the score value of stereotyped behavior. Each column represents the mean $\pm$ S.E.M. of the AUC. * $P < 0.05$, ** $P < 0.01$, compared with the vehicle group (one-way ANOVA followed by Dunnett's test).

Fig. 2. The effects of aripiprazole on wet-dog shake behavior induced by DOI. Each column represents the mean $\pm$ S.E.M. (n given in parentheses). * $P < 0.05$, ** $P < 0.01$, compared with vehicle group (one-way ANOVA followed by Tukey's test).

no significant dose $\times$ pulse intensity interaction ($F_{(2,54)} = 0.043$, not significant). In addition, the disruption of the PPI induced by DOI was improved at the prepulse amplitude of 83 dB at the dose of 30 mg/kg ($F_{(2,27)} = 3.301$, $P > 0.05$). There was no significant dose $\times$ pulse intensity interaction as well ($F_{(2,54)} = 0.046$, not significant).

Fig. 3. The effect of aripiprazole on the prepulse inhibition (PPI). Each column represents the mean $\pm$ S.E.M. ($n = 10$). PP80 and PP83 stand for 80-dB and 83-dB prepulse, respectively, followed by 120-dB pulse.

Fig. 4. The effect of aripiprazole on the disruption of the prepulse inhibition (PPI) induced by apomorphine. Each column represents the mean $\pm$ S.E.M. ($n = 10$). PP80 and PP83 stand for 80-dB and 83-dB prepulse, respectively, followed by 120-dB pulse. * $P < 0.05$, ** $P < 0.01$, compared with vehicle group (Student's t -test). # $P < 0.05$, compared with apomorphine-alone group (two-way ANOVA followed by Dunnett's test).

Table 1 shows the effect on the startle amplitude in rats. Aripiprazole (10, 30 mg/kg, p.o.) had no significant effect on the startle amplitude by itself. Apomorphine (1 mg/kg, s.c.) also had no significant effect, but DOI (2 mg/kg, s.c.) significantly decreased it ($P < 0.01$). In the group of apomorphine + aripiprazole (30 mg/kg), startle amplitude of the P-trial was significantly ($P < 0.01$) reduced compared with the group of only apomorphine administration. On the other hand, no significant difference was observed between DOI and DOI + aripiprazole (10 and 30 mg/kg).

Discussion

In this experiment, aripiprazole was shown to antago-

Fig. 5. The effect of aripiprazole on the disruption of the prepulse inhibition (PPI) induced by DOI. Each column represents the mean $\pm$ S.E.M. ($n = 10$). PP80 and PP83 stand for 80-dB and 83-dB prepulse, respectively, followed by 120-dB pulse. ** $P < 0.01$, compared with the vehicle group (Student's t -test). # $P < 0.05$, compared with DOI-alone group (two-way ANOVA followed by Dunnett's test).

Table 1. Startle amplitude (V_{max}) of single pulse trial

Drugs (dose mg/kg)	Startle amplitude (V_{max})
Vehicle	1656.25 $\pm$ 323.04
Aripiprazole (10)	1016.00 $\pm$ 169.93
Aripiprazole (30)	1222.82 $\pm$ 320.11
Vehicle	1970.78 $\pm$ 351.39
Apomorphine (1)	1727.87 $\pm$ 266.18
Aripiprazole (10) + Apomorphine (1)	1263.18 $\pm$ 130.71
Aripiprazole (30) + Apomorphine (1)	922.21 $\pm$ 141.14 [#]
Vehicle	2341.21 $\pm$ 337.03
DOI (2)	791.69 $\pm$ 96.62**
Aripiprazole (10) + DOI (2)	793.94 $\pm$ 71.35
Aripiprazole (30) + DOI (2)	661.53 $\pm$ 115.89

Each column represents the mean $\pm$ S.E.M. ($n = 10$). ** $P < 0.01$, compared with vehicle group (Student's t -test). # $P < 0.05$, compared with apomorphine-alone group (two-way ANOVA followed by Dunnett's test).

nize both dopaminergic and serotonergic action in vivo. Aripiprazole inhibited the stereotyped behavior induced by apomorphine and the wet-dog shake responses induced by DOI. These results are consistent with those of a previous report in which aripiprazole demonstrated affinity for both D_2 receptors and 5-HT_{2A} receptors (1). Moreover, aripiprazole (10 and 30 mg/kg) improved the disruption of the PPI induced by apomorphine and DOI dose-dependently. The effect of aripiprazole on the PPI disruption induced by apomorphine was previously reported by other researchers (13). In their investigation, not only high dose of aripiprazole (10 mg/kg), but also

low dose (0.16 mg/kg) was effective. However, in their research, no dose-dependent effect was seen. Although investigations at the lower doses may be needed, the results of the present study indicated that the antipsychotic action of aripiprazole is, at least partially, associated with 5-HT_{2A} receptors as well as D₂ receptors.

Aripiprazole has moderate affinity for several serotonin receptors (5-HT_{1A,2A,2C,7}), that is, 5-HT_{1A}-receptor partial agonist and 5-HT_{2A}-receptor antagonist (1). In the binding study, aripiprazole had a higher affinity to the D_{2L} receptor ($K_i = 0.74$ nM for D_{2L} receptor) than the 5-HT_{2A} receptor ($K_i = 22$ nM) (1). Moreover, based on the cAMP accumulation assay, aripiprazole was shown to retain the weak D_{2L}-receptor agonistic action over 10^{-6} M (14). Therefore, in our experimental condition, aripiprazole is thought to act as a 5-HT_{2A}-receptor antagonist and a partial agonist for the D₂ receptor.

DOI, a 5-HT_{2A/2C}-receptor agonist, has been shown to disrupt the PPI in rats (15), and the DOI-induced disruption was antagonized by the selective 5-HT_{2A}-receptor antagonist M100907, but not by either a selective 5-HT_{2C}-receptor antagonist or a non-selective 5-HT₁-receptor antagonist according to the previous studies (8, 16). Taken together, our study suggested that the 5-HT_{2A} receptor is responsible for the ameliorating effect of aripiprazole in the disruption of the PPI produced by DOI.

Several studies have suggested the involvement of 5-HT_{1A} receptors in disruption of the PPI. Recently, Buuse et al. (17) reported that aripiprazole (5 mg/kg, i.p.) had not effect on the PPI by itself and the disruption of the PPI induced by 8-OH-DPAT as well, under experimental conditions similar to those used in our study (prepulse intensities of 48 and 116 dB above background and the prepulse interval of 100 ms). Considering these observations, it is speculated that the ameliorating effect of aripiprazole on the PPI disruption observed in our study was not involved in that receptor. On the other hand, an in vivo brain microdialysis study has shown that aripiprazole modulates 5-HT and DA release in the medial prefrontal cortex through the activation of 5-HT_{1A} receptors (18). The other antipsychotic effect of aripiprazole may be involved in the 5-HT_{1A} receptor.

In conclusion, these experiments have shown that aripiprazole improved the part of the disruption of the sensorimotor gating system induced by not only dopamine-receptor agonists, but also 5-HT_{2A}-receptor agonists. These data suggest the possibility that the antipsychotic effects of aripiprazole were partially exerted via the 5-HT_{2A} receptor. The effectiveness of the atypical antipsychotics for the negative symptoms of schizophrenia is known to be involved in the antagonism

against the 5-HT_{2A} receptor. Therefore, our results supplied pharmacological data suggesting that aripiprazole is also effective for the treatment of the negative symptoms associated with schizophrenia.

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