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Effects of aripiprazole on MK-801-induced prepulse inhibition deficits and mitogen-activated protein kinase signal transduction pathway

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ABSTRACT

Based on NMDA hypofunction hypothesis for negative symptoms and cognitive deficits in schizophrenia, MK-801-induced animal models of schizophrenia may help us understand the different effects between typical and atypical antipsychotics. On the other hand, the mitogen-activated protein kinase (MAPK) signaling pathways may participate in antipsychotic actions. The aim of this study was to investigate the effects of aripiprazole on MK-801-induced prepulse inhibition (PPI) disruption and MAPK phosphorylation in mice. To clarify the effects of aripiprazole on MK-801-induced PPI disruption, we measured PPI of 51 ddY male mice after aripiprazole was administered 15 min prior to the injection of MK-801, and measured activation of cytosol and nuclear MAPK phosphorylation by western blotting. Aripiprazole (4.0 mg/kg) significantly reversed the MK-801 (0.15 mg/kg)-induced PPI deficits. Pretreatment of aripiprazole (40 mg/kg) had a tendency to suppress MK-801 (1.0 mg/kg)-induced pMEK/MEK (Ser218/222) activation. In addition, aripiprazole treatment showed a significant decrease of pERK/ERK. Our data suggested that aripiprazole may reverse MK-801-induced PPI deficits through regulation of MAPK phosphorylation in the same way as the atypical antipsychotic drug, clozapine.

Prepulse inhibition (PPI) is one of the few and major paradigms for investigating sensorimotor gating systems in humans and rodents in similar fashion. PPI deficits are observed in patients with schizophrenia [2,8,28]. Several lines of evidence showed that both the dopamine and the NMDA system may take part in PPI [7,27]. In rodents, typical antipsychotics (e.g. haloperidol) reverse PPI deficits induced by dopamine agonists, but not those induced by NMDA antagonists (e.g. phencyclidine or MK-801), whereas atypical antipsychotics (e.g. clozapine) reverse PPI deficits induced by dopamine agonists and NMDA antagonists [17,28,31]. Based on NMDA hypofunction hypothesis for negative symptoms and cognitive deficits in schizophrenia [9,11–12], MK-801-induced animal models [20] may help us understand the different effects between typical and atypical antipsychotics [17,31]. Aripiprazole (OPC-14597) is an antipsychotic with a unique pharmacological profile as a dopamine D2 receptor partial agonist, which has been demonstrated to reduce symptoms of the schizophrenia. In addition to NMDA hypofunction model, another hypothesis of schizophrenia model is that the disease is characterized by abnormally low dopamine activity in mesocortical dopaminergic neurons that results in negative and deficit syndromes [5]. On the other hand, excessive dopamine in mesolimbic dopaminergic neurons in the same brain may result in positive symptoms. Aripiprazole displays properties of an agonist and antagonist in animal models of dopaminergic hypoactivity and hyperactivity, respectively [6,15]. From the viewpoint of the possible co-occurrence of high and low dopamine activity in the schizophrenic brain, partial dopamine agonism, which results in antagonistic effects by high dopamine concentrations and agonistic effects by low dopamine concentrations, plays a pivotal role for the effective treatment of positive and negative symptoms of psychosis [14,26].

Previous studies have implicated the dopamine D1/2 receptor agonist, apomorphine-induced PPI deficit was restored by aripiprazole [18]. However, to the best of our knowledge, there is only one report about no significant effects of aripiprazole on NMDA receptor antagonist-induced PPI deficits in rats [19]. To use mice instead of rats, we examine whether aripiprazole can reverse MK-801-induced PPI deficits as an atypical antipsychotic drug.

Extracellular signal-regulated kinases (ERK), isoforms of mitogen-activated protein kinases (MAPK), have been characterized to respond to extracellular stimuli and to regulate cell proliferation and differentiation [24,25]. MAP kinase kinase (MEK) is a dual-specific kinase that phosphorylates the tyrosine and threonine residues on ERK 1 and 2 for their activation. MAPK pathway

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is known to be a downstream signal transduction system that is common to the dopamine system and the NMDA system, and mediates short- and long-term effects of intracellular signaling in neurons. Clozapine, the prototypic atypical antipsychotic drug, has antagonistic effects at a number of receptor systems, notably dopamine and serotonin [21,23]. The antipsychotic agent, clozapine, selectively activates the MEK/ERK MAPK pathway [3]. In addition, aripiprazole also partially activated the MAPK pathway in Chinese hamster ovary cells (CHO) cells [30]. However, it is still unclear whether aripiprazole may act on MEK/ERK-mediated cascades in animal models.

In the current study, to examine atypical antipsychotic characteristics of aripiprazole, we investigated effects of aripiprazole on MK-801-induced PPI deficits, and on activation of MEK and ERK pathways in mice.

Eighty-one male ddY mice (10–12-week old) were purchased from Nihon SLC (Hamamatsu, Shizuoka, Japan). The animals were kept for at least one week in the animal colony at our laboratory prior to the beginning of behavioral testing. The mice were housed 5–6 per cage kept at a controlled temperature (23 ± 1 °C) and on a 12-h light/dark cycle (light on at 07:00 h). The animals were provided food and water ad libitum. All behavioral testing was conducted between 09:00 and 17:00 h. The research was carried out according to the Guide for Animal Experimentation of the Chiba University Graduate School of Medicine.

Aripiprazole (Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) was prepared as a suspension in aqueous Tween 80 (two drops/10 ml distilled water). Clozapine (Novartis Pharmaceuticals, Ltd., Basel, Switzerland) and MK-801 (Sigma–Aldrich, Steinheim, Germany) was dissolved in physiological saline. All chemicals were injected intraperitoneally (i.p.) into the animals.

The mice were tested to assess their acoustic startle reactivity (ASR) in two startle chambers (SR-LAB, San Diego Instruments, CA) using standard methods described by previous study [29]. Background noise was set at 65 dB. Four trial types were used. Pulse-alone trials (P) consisted of a single white-noise burst (120 dB, 40 ms). The prepulse + pulse trails (PP69P, PP73P, PP77P, and PP81P) consisted of a prepulse of noise (20 ms at 69, 73, 77, or 81 dB, respectively), which was followed by 100 ms after the prepulse onset by a startle pulse (120 dB, 40 ms). No-stimulus (NS) trials consisted of the background noise only. Sessions were structured as follows: (1) 15-min acclimation at background noise level; (2) five P trials; (3) 10 blocks, each containing all 10 trials (P, PP69P, PP73P, PP77P, PP81P, and NS) in pseudorandom order; and (4) five P trials. Intertrial intervals were distributed between 7 and 23 s. The average percent reduction in startle intensity between pulse and prepulse + pulse trials at all four prepulse levels was defined as the PPI level. The percentage PPI induced by each prepulse intensity was calculated as follows: [1 − (startle amplitude on prepulse trial)/(startle amplitude on pulse alone)] × 100%. Startle magnitude in this formula was calculated as the average response to all of the P trials, excluding the first and last blocks of five P trials.

MK-801 (0.15 mg/kg) was administered 30 min before the start of the recording. For the effects of antipsychotic drugs on PPI, aripiprazole (0.04, 0.4, 1.0, 4.0 and 10.0 mg/kg) was administered 15 min prior to the injection of MK-801.

The frontal cortex was quickly dissected from the brain on ice after decapitation, after MK-801 treatment (0.15, 0.3, 1.0 and 3.0 mg/kg, i.p.) or no treatment (control). Single administration of clozapine (10 mg/kg) or aripiprazole (40 mg/kg), and combined treatment of clozapine (10 mg/kg) or aripiprazole (40 mg/kg) 15 min prior administration of MK-801 (1 mg/kg) were done. The tissues were homogenized in a lysis buffer (50 mM Tris–HCl, pH 7.5; 50 mM NaCl; 1% Triton X-100; 5 mM EDTA 2Na; 50 mM NaF; 1 mM Na_3VO_4) containing protease inhibitor cocktail (Complete Mini, Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instruction. The homogenates were placed at 4 °C for 30 min and centrifuged to remove insoluble material (10,000 × *g*, 30 min at 4 °C). The protein concentration of the supernatant was determined using a D.C. Protein Assay kit (Bio-Rad Laboratories, Hercules, CA). Ten micrograms of total protein were separated on 10% or 12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Amersham Bioscience, Buckinghamshire, UK). The membranes were blocked with 5% non-fat dry milk and incubated with an anti-rabbit phosphospecific-ERK1/2 antibody (Cell Signaling Technology Inc., Danvers, MA) according to the manufacturer's instructions. After washing three times with TBST (0.1% Tween-20), the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Amersham Bioscience, Buckinghamshire, UK) for 1 h at room temperature. Following the three washes with TBST, the membranes were visualized with an ECL plus chemiluminescence system (Amersham Bioscience, Buckinghamshire, UK) and Image Reader LAS-3000 (Fuji Photo Film Co. Ltd., Tokyo, Japan). The membranes immunoblotted with phosphospecific antibodies were stripped in a stripping buffer (2% SDS, 62.5 mM Tris–HCl and 100 mM mercaptoethanol, pH 6.7) at 50 °C for 30 min with shaking, washed with TBST and re-immunoblotted with a total anti-mouse ERK antibodies (BD Biosciences, San Jose, CA). Measurements of phosphorylated ERK levels were normalized with total ERK levels and quantified using Multi-Gauge ver.3 software (Fuji Photo Film Co. Ltd., Tokyo, Japan).

The percentage of inhibition of startle and basic startle response for different trials was analyzed by two-way ANOVA, where drug group was included as a between-subject factor, and prepulse intensity as a repeated measurement factor. Bonferroni's correction was used for *post hoc* comparisons when ANOVA revealed statistically significant differences between the drug groups. The immunoreactivity data were analyzed using non-repeated measures ANOVA followed by Bonfferoni *post hoc* comparisons at the $p < 0.05$ level of significance. Data in the text are mean ± SEM.

In the analysis of the effects of MK-801 (0.15 mg/kg) on each prepulse intensity (Fig. 1a), the two-way repeated ANOVA indicated significant differences among drug-treatment groups ($F(6,51) = 5.119$, $p < 0.001$). The *post hoc* Bonferroni's test revealed that the prepulse intensities in vehicle + MK-801 group were significantly lower than those in the vehicle + saline group (PP69, 73, 77, $p < 0.05$; PP81, $p < 0.01$). Moreover, the prepulse intensities in the aripiprazole 4.0 mg/kg + MK-801 group were significantly higher than those in the vehicle + MK-801 group (PP81, $p < 0.01$). Especially, aripiprazole (4.0 mg/kg) significantly reversed the MK-801 (0.15 mg/kg)-induced PPI deficit (Fig. 1c). The *post hoc* Bonferroni's test revealed that aripiprazole (4.0 mg/kg) had a statistically significant effect on a prepulse intensity (APZ 4.0 + MK 0.15 group compared to Veh + MK 0.15 group, PP81, $p < 0.01$). Therefore, the present findings suggested that aripiprazole at a dose of 4.0 mg/kg led to an improvement of prepulse inhibition disrupted by MK-801.

In startle magnitudes (Fig. 1b, inset), one-way ANOVA revealed significant differences among the four drug groups ($F(3,42) = 6.568$, $p < 0.001$). The *post hoc* Bonferroni's test revealed that startle magnitudes were significantly higher in the vehicle + MK-801 group ($p < 0.05$) than in the vehicle + saline group. No significant differences between vehicle + saline group and APZ 4.0 + MK0.15 group were found in the startle magnitude without prepulse.

We examined the effect of MK-801 (0.15, 0.3, 1.0 and 3.0 mg/kg) and pretreatment of both clozapine (10 mg/kg) and aripiprazole (40 mg/kg) in the frontal cortex. In pMEK/MEK (Ser218/222) ratio of the frontal cortex, analysis of variance (non-repeated measures ANOVA, $F(8,18) = 4.521$, $p < 0.005$) followed by Bonferroni *post hoc* comparisons indicated that MK-801 (0.15, 0.3, 1.0 and 3.0 mg/kg) produced a bell-shaped curve with a peak at 1.0 mg/kg ($p < 0.05$) (Fig. 2a). The significant effects of pMEK/MEK by MK-801 were