*Full Paper***Effect of Aripiprazole on Anxiety Associated With Ethanol Physical Dependence and on Ethanol-Induced Place Preference**Masahiro Shibasaki^{1,†}, Kazuhiro Kurokawa¹, Koji Mizuno¹, and Seitaro Ohkuma^{1,*}¹Department of Pharmacology, Kawasaki Medical School, 577 Matsushima, Kurashiki 701-0192, Japan

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Abstract. In the present study, we investigated the effect of aripiprazole, a dopamine system stabilizer, on ethanol-induced psychological and physiological dependence and anxiety-like behavior. First we determined the effect of aripiprazole, a dopamine system stabilizer, on the development and expression of ethanol-induced place preference. Both the development and expression of ethanol-induced place preference was significantly suppressed by treatment of aripiprazole. Next, the withdrawal score gradually increased with increasing duration after the withdrawal from ethanol for 6 days in vehicle-treated mice and the maximal score was observed 10 h after the ethanol withdrawal. Aripiprazole caused no changes in the withdrawal score as compared to vehicle-treated mice. Under these conditions we investigated the effect of aripiprazole on the anxiety-like behavior of ethanol physical dependent mice, which were animals subjected to ethanol vapor for 6 days. The significant decrease of time spent in the open arms and number of open arm entries characterize the anxiety-like behavior in ethanol physical dependent mice, compared to control mice. These decreases were reversed by treatment of aripiprazole, which were inhibited by WAY100635, a serotonin 5-HT_{1A} receptor antagonist. The present findings suggest that aripiprazole was efficient for reversing ethanol-induced place preference and anxiety-like behavior.

Keywords: aripiprazole, alcohol dependence, anxiety, place preference

Introduction

The emotional behavior changes in ethanol-dependent mice have been documented in only a small number of reports, although, many studies have demonstrated that both acute and chronic alcohol (ethanol) consumption modifies a multitude of molecular events, including membrane fluidity, neurotransmitter turnover, function of neurotransmitter receptors, and intracellular signal transduction systems coupled to neurotransmitter receptors, and biochemical processes, and that the process of ethanol dependence may result from adaptive changes in the central nervous systems (1, 2). Among the emotional behaviors and emotion-dependent behaviors that are considered to be modified by ethanol, the degree of anxiety

and anxiety-related behaviors have been well reported as an emotional change by ethanol (3, 4). Furthermore, functional relationship between alcohol dependence and depression has been supposed (5), and several investigators also demonstrated the co-morbidity of lifetime psychiatric disorder among alcoholic patients (6). An anxiety-like effect has been observed in laboratory animals following withdrawal from chronic ethanol exposure (7 – 10) and it is also one of the withdrawal signs in alcoholics (11). Thus, it is likely that ethanol physical dependence is associated with anxiety.

Numerous studies conducted to find possible neural substrates inducing ethanol reward and reinforcement have implicated the mesolimbic dopaminergic system as a primary candidate (12, 13). Dopaminergic (DA) neurons in the ventral tegmental area (VTA) and their projections to the limbic forebrain regions have been implicated to mediate the effects of drugs of abuse, including ethanol (14, 15). Administration of ethanol activates the mesolimbic DA system and increases extracellular DA levels in the nucleus accumbens (16, 17). Electrophysiological studies using slice culture or in vivo experiments

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showed that both exposure (20–320 mM) of slices to and intravenous injection of ethanol (125–1000 mg/kg) stimulated DA neurons in the VTA (18–21). In addition, intra-VTA microinjections of ethanol increased DA release in the nucleus accumbens (22).

Serotonergic neurons within the dorsal raphe nucleus (DRN) project to the brain regions which are associated with stress- and anxiety-related physiological and behavioral responses (23). Collateral projections of serotonergic neurons to the nucleus accumbens and medial prefrontal cortex have been immunohistochemically identified and shown to originate almost exclusively from the DRN (24). Anterograde tracing and retrograde tracing studies have demonstrated relatively large numbers of projections from the DRN to the basolateral amygdaloid nucleus (25, 26), central amygdaloid nucleus (26–28), and bed nucleus of the stria terminalis (29). As far as the functional relationship between the serotonergic system and anxiety, serotonin 1A receptor (5-HT_{1A}), which is a key mediator of serotonergic signaling in the central nervous system, was reported to be possibly involved in the expression of anxiety-like behavior (30).

Aripiprazole 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-2(1H)-quinolinone, a drug that has been approved by the Food and Drug Administration (FDA) for the treatment of schizophrenia (31, 32) and shows good efficacy in bipolar disorder and major depressive disorder (33), has a unique pharmacological property. A number of studies have confirmed that aripiprazole acts as a dopamine system stabilizer alone (34) and also may have potential to “stabilize” dopamine–serotonin balance (35). In animals, aripiprazole at lower doses increases dopamine release in precortical areas, but at slightly higher doses, reduces dopamine release in the nucleus accumbens (36). Previous reports provide in vivo evidence of aripiprazole-induced changes in forebrain dopaminergic and serotonergic function that may reflect its partial agonistic activity at presynaptic dopamine D₂ and 5-HT_{1A} receptors and antagonistic activity at 5-HT_{2A} receptors (37–41). As ethanol modifies the emotional state, especially the pathophysiological state such as anxiety and depressive conditions, as mentioned above, it is supposed that aripiprazole may have therapeutic efficacy to reduce anxiety and depressive conditions associated with alcohol physical dependence.

In the present study, we therefore investigated whether aripiprazole has pharmacological potential to reduce or abolish anxiety-like behaviors, conditioned place preference, and withdrawal behavior in association with ethanol-induced dependence.

Materials and Methods

Animals

Male ddY mice (Japan SLC, Hamamatsu) were used in the present study. Animals were kept in a room with an ambient temperature of 22°C ± 1°C and a 12-h light-dark cycle (lights on 08:00–20:00 h). Food and water were available ad libitum.

All experiments presented in this study were approved by the Animal Research Committee of Kawasaki Medical School and conducted according to the “Guide for Care and Use of Laboratory Animals” of Kawasaki Medical School that is based on the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

Procedure for ethanol inhalation

The procedure for ethanol vapor inhalation of mice was described previously with a minor modification (42). In brief, mice with initial body weight of 40 g were exposed to ethanol vapor (concentrations of 11 mg/l) or air in the chamber (52 × 34 × 27 cm) with food and drinking water for 6 days. In order to stabilize blood alcohol levels, mice were given daily intraperitoneal injections of pyrazole (1.0 mmol·kg⁻¹·day⁻¹), an inhibitor of liver alcohol dehydrogenase. At the end of ethanol exposure on the 7th day, mice were removed from the chamber and withdrawal signs such as tonic-clonic convulsions were evaluated by scoring the degree of tonic-clonic convulsions according to the previous method (43) as mentioned below. The blood concentration of ethanol determined immediately after the withdrawal from ethanol vapor was approximately 250 mg/dl (42), which was in good agreement with the data reported by Goldstein (43). In addition, mice with this blood ethanol concentration showed sedation. In this study, we used animals exposed to air instead of ethanol vapor with concomitant administration of pyrazole as the control.

Measurement of withdrawal signs

The measurement of tonic-clonic convulsions was carried out according to the previously reported method (43) and represented as the convulsion score. Scores of 1 to 4 for convulsions were elicited by lifting a mouse by the tail: Score 1, tonic convulsion when the mouse is lifted and given a gentle 180° turn; Score 2, tonic-clonic convulsion elicited by the gentle spin, or tonic convulsion when lifted without turning; Score 3, tonic-clonic convulsion not requiring any spin; Score 4, violent tonic-clonic convulsion, often continuing after release of the mouse (43).

In order to investigate effects of aripiprazole on the expression of withdrawal signs after discontinuation of

ethanol vapor inhalation, mice were divided into two groups. After all mice in both groups continuously inhaled ethanol vapor for 6 days, mice were given aripiprazole (3 mg/kg, p.o.) or vehicle 1 h before the withdrawal of ethanol vapor inhalation.

Place conditioning

Place conditioning studies were conducted using an apparatus consisting of a shuttle box made of an acrylic resin board (44). This shuttle box was divided into two equal-sized compartments, and one compartment was white with a textured floor and the other was black with a smooth floor to create equally inviting compartments.

Place conditioning schedule consisted of three phases (pre-conditioning test, conditioning, and post-conditioning test). A) Pre-conditioning test: The partition separating the two compartments was raised to 7-cm above the floor, which makes a state of ambulation free, and mice that had not been treated with either drugs or saline were then placed on the border of the two compartments. The time spent in each compartment during a 900-s session was automatically recorded with an infrared beam sensor (BS-CPP-MS; BrainScienceIdea Co., Ltd., Osaka). B) Conditioned session: Conditioning sessions (4 for ethanol and 4 for saline) were started the next day after the pre-conditioning test and conducted once daily for 8 days. Mice were placed for 30 min in the opposite compartment in which they had spent most time in the pre-conditioning test immediately after intraperitoneal (i.p.) injection of ethanol (2 mg/kg). On alternate days, these animals received vehicle and were placed in the other compartment for 30 min. C) Post-conditioning test: On the day after the final conditioning session, a post-conditioning test that was identical to the pre-conditioning test was carried out. The data of place conditioning presented in the y-axis were calculated by “spent time in the post-conditioning test – spent time in the pre-conditioning test” and were represented as “preference for drug-paired place (s)”.

In the combination study, aripiprazole {7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-2(1*H*)-quinolinone; 0.3, 1, 3 mg/kg} were p.o. administered 1 h before i.p. treatment with ethanol in the conditioning session for the development experiment and in post-conditioning test session for the expression experiment.

Locomotor assay

The locomotor activity of mice was measured by an animal movement analyzing system (Actimo-100 system; Shintech, Ltd., Fukuoka), which consists of a rectangular enclosure (30 × 20 cm) with a side wall equipped with photo sensors at 2-cm intervals. Each pair of photo

sensors scanned animal movement at 0.5-s intervals. Total activity counts were automatically recorded for 10 min. Aripiprazole was intragastrically administered 1 h before the measurement of total activity counts.

Elevated plus-maze test

We also used the elevated plus-maze test to measure anxiety. This test has been used extensively to identify novel anxiolytic agents and to investigate physiological and neurochemical basis of anxiety (45). Briefly, the elevated plus-maze consists of two opposing open and closed arms joined by a common central platform. The maze was elevated 40-cm above the floor. The open and closed arms and the central platform were subjected to approximately equal illumination. Mice were placed on the central square facing the open arms at the beginning of the test and were allowed to explore the maze for 5 min. The results were calculated as the mean ratios of the time mice spent in the open arms to time mice spent in both the open and closed arms and of the number of entries of mice into the open arms to that of entries into both the open and closed arms.

Aripiprazole (0.3, 1, 3 mg/kg), ketanserin (10 mg/kg), and tandospirone (20 mg/kg) were orally administered 1 h before withdrawal from ethanol vapor exposure for 6 days. WAY100635 (0.03, 0.1 mg/kg, i.p.) was pre-treated 30 min before aripiprazole administration.

Statistical analyses

Each of the data was expressed as the mean ± S.E.M. The statistical significance was assessed by the methods described in each figure legend following the application of the one-way or two-way ANOVA with Bonferroni post hoc test (Prism 5; GraphPad Software, Inc., La Jolla, CA, USA).

Drugs

Aripiprazole was provided by Otsuka Pharmaceutical Co., Ltd. (Tokyo) and was suspended in a 5% arabic gum–distilled water.

WAY100635, ketanserin, and tandospirone were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and were dissolved in physiological saline. The other agents used were of analytical grade and available locally.

Results

Effects of aripiprazole on the development and expression of ethanol-induced place preference

Effect of aripiprazole, a dopamine system stabilizer, on the development and expression of ethanol-induced place preference was examined. As shown in Fig. 1, the

Fig. 1. Effect of aripiprazole on the development of ethanol (EtOH)-induced place preference mice. Mice were conditioned by ethanol (2 g/kg, i.p.) or saline (SAL) after 1 h of pre-treatment with aripiprazole (ARI: 0, 0.3, 1, and 3 mg/kg, p.o.). The data represent the mean $\pm$ S.E.M. obtained from eight mice. * P < 0.05, ** P < 0.01 vs. group (Bonferroni's test).

conditioning by ethanol significantly induced place preference. This development of ethanol-induced place preference was suppressed by aripiprazole simultaneously treated on ethanol conditioning in a dose-dependent manner and aripiprazole alone did not show any effects on place preference (Fig. 1). In order to investigate effect of aripiprazole on the expression of ethanol-induced place preference, aripiprazole was administered in the post-conditioning test. Aripiprazole administered in the post-conditioning test also significantly suppressed the expression of ethanol-induced place preference (Fig. 2), which suggests that this agent has a potential to suppress sufficiently the expression of place preference even after ethanol-induced place preference.

Effect of aripiprazole on withdrawal signs in mice after termination of continuous vapor inhalation

Mice that continuously inhaled ethanol vapor for 6 days were divided into two groups; vehicle- and aripiprazole-challenged groups. The degree of tonic-clonic convulsions is measured as the withdrawal score. After the cessation of ethanol inhalation, the withdrawal score gradually increased with increasing duration after withdrawal from ethanol in both vehicle (for dissolving aripiprazole)- and aripiprazole-treated mice, and the maximal scores were observed 10 h after ethanol withdrawal in these two groups of ethanol-treated mice with vehicle or aripiprazole (Fig. 3). In mice without ethanol

Fig. 2. Effect of aripiprazole on the expression of ethanol (EtOH)-induced place preference mice. Mice were conditioned by ethanol (2 g/kg, i.p.) or saline (SAL). Post-conditioning test was performed after 1 h of treatment with aripiprazole (ARI: 0 and 3 mg/kg, p.o.). The data represent the mean $\pm$ S.E.M. obtained from eight mice. * P < 0.05, ** P < 0.01 vs. group (Bonferroni's test).

Fig. 3. Effects of aripiprazole on withdrawal score after withdrawal from continuous ethanol inhalation for 6 days. After mice inhaled ethanol vapor (EtOH: closed circle and open square) or air (Cont: closed triangle and open diamond) for 6 days, aripiprazole (3 mg/kg, p.o.) (Ari: open diamond and open square) or vehicle (Veh: closed triangle and closed circle) was administered 1 h before the withdrawal from ethanol vapor. Each value represents as the mean $\pm$ S.E.M. of 9–11 mice.

vapor inhalation, aripiprazole alone showed no changes in the withdrawal score as compared to vehicle-treated mouse groups.

Effect of aripiprazole on anxiety-like behavior and locomotor activity in mice with ethanol physical dependence

We investigated anxiety-related behavior in mice with ethanol physical dependence. Figure 4 shows the significant decrease of time spent in open arms (Fig. 4A) and of the number of open arm entries (Fig. 4B) in mice with ethanol physical dependence when compared with control mice, which suggests that mice with ethanol physical dependence exhibit significantly enhanced anxiety-like behavior compared to control mice.

In the control mice, the treatment with aripiprazole dose-dependently reduced the time spent in open arms with no changes of the number of open arm entries in control mice (Fig. 4: A and B). To determine whether or not aripiprazole has some effect on motor activity of the mice, we examined its effect on locomotor activity. Therefore, we examined the effect of aripiprazole on locomotor activity to check this possibility. As shown in Fig. 5, the treatment with aripiprazole significantly reduced locomotor activity both in control mice and mice with ethanol physical dependence. Thus, such reduction of locomotor activity by aripiprazole in control mice may partially induce the decrease of the time spent in open arms in the control mice.

Under such conditions, we further investigated the effect of aripiprazole on anxiety-like behavior in mice with ethanol physical dependence. The decrease of the time spent in open arms and the number of open arm entries as anxiety-like behavior observed in mice with ethanol physical dependence were dose-dependently reversed by aripiprazole (Fig 4: A and B).

As shown in Fig. 5, in groups of mice without aripiprazole treatment, the locomotor activity of mice on the

termination of ethanol vapor inhalation for 6 days showed no statistically significant changes when compared to that of mice without ethanol vapor exposure, although the former tended to be lower than the latter. The treatment of mice with or without the exposure of ethanol vapor significantly decreased locomotor activity (Fig. 5). Therefore, it is reasonable to suppose that the enhancement of decreased anxiety-like behavior in mice with ethanol physical dependence by aripiprazole is due to the pharmacological potential of this agent, different from

Fig. 5. Effect of aripiprazole on locomotor activity in ethanol-physical dependent mice (EtOH-PD) and control mice. After mice inhaled ethanol vapor or air for 6 days, aripiprazole (ARI: 3 mg/kg, p.o.) was administered 1 h before the withdrawal from ethanol vapor. Each value represents the mean $\pm$ S.E.M. of 8 mice. * P < 0.05, *** P < 0.001 vs. group (Bonferroni's test). n.s.: not significant.

Fig. 4. Effect of aripiprazole (3 mg/kg, p.o.) in the ethanol-physical dependent mice (EtOH-PD) or control mice on the percentage of time spent in open arms (A) and number of open arm entries (B) after 1 h of pre-treatment with aripiprazole (ARI: 0, 0.3, 1, and 3 mg/kg, p.o.). Each value represents the mean $\pm$ S.E.M. of 8 mice. * P < 0.05, ** P < 0.01 vs. group (Bonferroni's test).

that to reduce locomotor activity.

In order to examine which mechanisms works in aripiprazole-induced facilitation of decreased anxiety-like behavior in mice with ethanol physical dependence, we examined effects of several ligands for 5-HT receptor subtypes on ethanol-induced anxiety-like behavior because aripiprazole had been reported to have multiple actions on dopamine D₂ receptors and 5-HT receptor subtypes (37–41). The suppression by aripiprazole on anxiety-like behavior in mice with ethanol physical dependence mentioned above was inhibited by WAY100635, a serotonin 5-HT_{1A} receptor antagonist, in a dose-dependent manner (Fig. 6: A and B). Effects of tandospirone, a 5-HT_{1A} receptor agonist, and ketanserin, a 5-HT_{2A} antagonist, on anxiety-like behavior induced by

ethanol physical dependence were also examined. Although ketanserin caused no changes in the time spent in open arms and the number of open arm entries in mice with ethanol physical dependence, tandospirone significantly reversed the decrease of these two parameters showing anxiety-like behavior in mice with ethanol physical dependence (Fig. 7: A and B). On the other hand, in control mice, tandospirone increased the time spent in open arms and showed no effects on the number of open arm entries (Fig. 7: A and B).

Discussion

In the present study, we demonstrated the effects of aripiprazole on the development and expression of etha-

Fig. 6. Effect of WAY100635 (WAY: 0.03 and 1 mg/kg, i.p.) with aripiprazole on ethanol physical dependent mice (EtOH-PD) or control mice on percentage of time spent in open arms (A) and number of open arm entries (B). Each value represents the mean $\pm$ S.E.M. of 8 mice. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. group (Bonferroni's test).

Fig. 7. Effect of ketanserin (Ket: 10 mg/kg, p.o.) and tandospirone (Tan: 20 mg/kg, p.o.) in the ethanol physical dependent mice (EtOH-PD) or control mice on percentage of time spent in open arms (A) and number of open arm entries (B). Each value represents the mean $\pm$ S.E.M. of 8 mice. ** P < 0.01, *** P < 0.001 vs. group (Bonferroni's test).

nol-induced place preference and the anxiety-like behavior under the conditions with ethanol physical dependence.

Previous reports have demonstrated the involvement of the dopaminergic system in ethanol-induced place preference. That is, the reduction of ethanol-conditioned place preference in dopamine D₂ receptor-deficient mice (46) and involvement of dopamine D₁ and D₂ receptors in the ethanol-associated place preference in rats exposed to conditioned fear stress (47) were reported. Similarly, activation of dopamine D₂ receptors by cocaine self-administration produces both reinforcement of drug-seeking behavior and locomotor sensitization (48). Blockade of amphetamine sensitization by dopamine D₂ receptor antagonists such as YM-09151-2, nemonapride, haloperidol, and clozapine, but not pimozide, Ro-22-2586, eticlopride, and spiperone, has been shown (49), although studies with sulpiride have yielded mixed results. Taken together these findings suggests that enhanced sensitivity of dopamine D₂ receptors may play an important role in mechanisms via which drug dependence and changes of locomotor activity associated with drug dependence are mediated.

The antipsychotic aripiprazole antagonizes ethanol- and amphetamine-induced locomotor stimulation in mice (50) and significantly suppresses locomotor activity in naïve animals as reported in a previous investigation (51) and this study. Moreover, other experimental results suggest the possible therapeutic application of aripiprazole to reduce the severity of morphine-induced adverse effects relating to dopaminergic neuronal activity such as morphine-induced hyperlocomotion and reward (52). These data on the effects of aripiprazole described above and the experimental findings demonstrating the attenuation of development and expression of ethanol-induced place preference by aripiprazole as demonstrated here are considered to strongly suggest the involvement of the dopamine D₂ receptor system in the pharmacological properties of aripiprazole. However, it is reported that 5-HT_{1A} receptor antagonists reduce the ethanol-induced locomotor activity and rewarding and reinforcing effects (53–55). Thus, the inhibition of locomotor activity and place preference by aripiprazole are supposed to be regulated by not only dopamine D₂ receptors but also 5-HT_{1A} receptors.

The present study shows that aripiprazole does not affect the ethanol withdrawal score after discontinuation of ethanol inhalation. It has been reported that the locus coeruleus, from which noradrenergic neurons project to various regions of the brain, play an important role in the precipitation of the physical signs of opiate withdrawal (56, 57), although which brain regions are involved in expression of withdrawal syndrome by discontinuation

of ethanol has not been clarified. Based on the pharmacological properties of aripiprazole, classified as a dopamine and serotonin stabilizer, with no potential on expression of withdrawal syndrome by discontinuation of ethanol as presented here, it is considered to be reasonable that the expression of withdrawal syndrome in ethanol physical dependence may be modified by the noradrenergic system originating from the locus coeruleus. However, the exact mechanisms remain to be elucidated.

It has been reported that chronic ethanol exposure enhances dopamine uptake in the nucleus accumbens and caudate putamen of rats (58). These enhancements of dopamine uptake is suggested to be a compensatory response to increased dopamine release by repeated alcohol exposure, which down-regulates in turn extracellular dopamine levels. The sensitivity of terminal release-regulated dopamine D₂ receptors in alcohol-exposed rats is considered to be not different from that in alcohol-naïve animals. Thus, it is suggested that locomotor activity in mice with ethanol physical dependence may be also decreased by aripiprazole as well as that in control mice. These data therefore implicate that decrease of locomotor activity as behavioral suppression by aripiprazole in both mice with ethanol physical dependence and control mice may be related to the partial agonistic effect of aripiprazole on dopamine D₂ receptors. Moreover, this behavioral suppression may be, in part, involved in the decrease of time spent in open arms in the elevated-plus maze.

This study demonstrated that ethanol physical dependence induced decrease of the time spent in open arms and the number of open arm entries as anxiety-like behavior, suggesting that animals with ethanol physical dependence are under conditions of anxiety. Several investigations have reported that long-term ethanol administration alters anxiety sensitivity in animals (3, 4) and induces depression in humans (5). 5-HT_{1A} receptors are classified as G-protein-coupled receptors that are widely distributed in brain regions such as the frontal cortex, septum, amygdala, hippocampus, and hypothalamus, which receive serotonergic input from the raphe nuclei (59, 60); and serotonergic activity modulates the diverse behavioral functions such as cognition and emotion through 5-HT_{1A} receptors (61). The data shown in Fig. 7, that is, tandospirone, a 5-HT_{1A} receptor agonist, not but ketanserin, a 5-HT_{2A} receptor antagonist, reversed anxiety-like behavior in ethanol physical dependent mice, are considered to be in good agreement with the theory mentioned above.

In mice under conditions of anxiety, the anxiety-like behavior was reversed by the treatment with aripiprazole and such effects were inhibited by WAY100635, a serotonin 5-HT_{1A} receptor antagonist. Several investigators

reported the decrease of 5-HT_{1A} receptors mRNA in rats with chronic ethanol administration (62). Previous clinical studies have also shown the co-morbidity of lifetime psychiatric disorder among alcoholic patients (6). Local injection of an agonist for 5-HT_{1A} receptors, flesinoxon, into the dorsal raphe attenuates social defeat in animals, while WAY100635, an antagonist for 5-HT_{1A} receptors, injected into the dorsal raphe enhances defeat-related conditioning (61). Similarly, injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the dorsal raphe alleviated anxiety of rats when examined with a maze task (63) and reduced the efficacy of fear conditioning using inescapable shock (64, 65). Thus, the ability of aripiprazole to reverse anxiety-like behavior in mice with ethanol physical dependence may involve 5-HT_{1A} receptors. These data may also implicate that anti-anxiety-like behavior by aripiprazole is considered to be due to agonistic effects of aripiprazole on 5-HT_{1A} receptors. On the other hand, it is reported that the dopamine D₂ receptors are related to anxiety-like behavior (66). Thus, the reverse of anxiety-like behavior following the ethanol dependence by aripiprazole may be due to the involvement of not only 5-HT_{1A} receptors but also dopamine D₂ receptors.

The present study demonstrated that aripiprazole suppressed the anxiety-like behavior in ethanol physical dependence, which is confirmed by the experimental results that aripiprazole reversed the decrease in the number of open arm entries in ethanol physical dependent mice, whereas it decreases anxiety-like behavior in control mice. Although such differences in the effects of aripiprazole on anxiety-like behavior between mice with normal and pathophysiological conditions including ethanol physical dependence may due to the differences of changes in various neurotransmission systems, the exact reasons for such a difference are not clear at present.

It is reported that alcoholism causes co-morbidity of lifetime psychiatric disorders like anxiety disorder and depression (5, 6). Especially, 5-HT has been known to be associated with depression and alcoholism. Decreased cerebrospinal fluid levels of the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA) are observed in individuals with early onset alcoholism (67). Postmortem studies report reduced binding sites of 5-HT_{1A} receptors in the prefrontal cortex of alcoholic suicides and alcoholic non-suicides (68).

Clinical studies demonstrate that aripiprazole may be useful in the treatment of bipolar depression, major depressive disorders, treatment-resistant depression, and possibly anxiety disorders (69–71). Moreover, a randomized, multicenter, double-blind, placebo-controlled study of the efficacy and safety of aripiprazole for the treatment of alcohol dependence revealed no difference between aripiprazole and placebo on the % of days ab-

sent, although aripiprazole reduces craving of ethanol (72). Another study provides both novel and valuable information regarding the effect of aripiprazole on cue-induced brain activation and voluntary drinking during treatment (73). Similar to the data obtained from the clinical research described above, the data presented in this study suggest the possible therapeutic effect of aripiprazole on anxiety induced by ethanol physical dependence.

In conclusion, the development and expression of ethanol-induced place preference was suppressed by aripiprazole treatment during ethanol conditioning. Moreover, the decrease of time spent in open arms and number of open arm entries as anxiety-like behavior in ethanol physical dependence was found when compared with control mice. These effects of aripiprazole on anxiety-like behavior were inhibited by WAY100635, a serotonin 5-HT_{1A} receptor antagonist. The findings presented here suggest that aripiprazole was efficient for reversing ethanol-induced place preference and anxiety-like behavior.

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