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Role of endocannabinoid and glutamatergic systems in DOI-induced head-twitch response in mice

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ABSTRACT

We previously reported that systemic administration of the endocannabinoid anandamide inhibited the head-twitches induced by the hallucinogenic drug 2,5-dimethoxy-4-iodoamphetamine (DOI) in mice, which is mediated via the activation of 5-HT_{2A} receptors. Endocannabinoid and glutamatergic systems have been suggested to modulate the function of 5-HT_{2A} receptors. In the present study, we further investigated the role of endocannabinoid and glutamatergic systems in DOI-induced head-twitch response in mice. An anandamide transport inhibitor AM404 (0.3–3 mg/kg, i.p.), a fatty acid amide hydrolase inhibitor URB597 (0.1–10 mg/kg, i.p.), a glutamate release inhibitor riluzole (0.3 and 1 mg/kg, i.p.), a natural glutamate analog L-glutamylethylamide (theanine, 1 and 3 mg/kg, p.o.) and an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist NBQX (0.01–0.3 mg/kg, i.p.) significantly inhibited DOI-induced head-twitch response. The AMPA receptor positive modulator aniracetam (30 or 100 mg/kg, p.o.) reversed inhibition of head-twitch response by NBQX and URB597. These findings indicated that endocannabinoid and glutamatergic systems participate in the mechanism of action of DOI to induce head-twitch response.

1. Introduction

Endocannabinoid system is associated with schizophrenia. An endogenous cannabinoid anandamide (arachidonylethanolamide) has been shown to be elevated in the cerebrospinal fluid (CSF) and blood of schizophrenic patients (De Marchi et al., 2003; Giuffrida et al., 2004; Leweke et al., 1999). The density of CB_1 cannabinoid receptors was increased in the anterior cingulate cortex and dorsolateral prefrontal cortex in schizophrenic patients (Dean et al., 2001; Zavitsanou et al., 2004). Genetic studies revealed that the CNR1 gene, which encodes the CB_1 receptor, is associated with schizophrenia (Ujike and Morita, 2004). Interestingly, a selective anandamide transporter inhibitor *N*-(4-hydroxyphenyl)arachidonylamide (AM404) attenuates spontaneous hyperlocomotion in dopamine transporter knockout mice, which is an animal model of neurobiological alterations associated with hyperdopaminergia relevant to schizophrenia and attention-deficit/hyperactivity disorder (ADHD) (Tzavara et al., 2006). Furthermore, cyclohexylcarbamic acid 3-carbamoylbiphenyl-3-yl ester (URB597), a selective inhibitor of the enzyme fatty acid amide hydrolase (FAAH), which catalyzes the intracellular hydrolysis of the anandamide, improves social withdrawal (negative symptom) in rats treated with subchronic administration of phencyclidine (PCP), a well-established pharmacological model of schizophrenia (Seillier et al., 2009). In addition, AM404 and URB597 exert anxiolytic- and antidepressant-like properties in mice and rats (Adamczyk et al., 2008; Bortolato et al., 2006; Braida et al., 2007; Gobbi et al., 2005; Hill and Gorzalka, 2005; Moreira et al., 2008; Patel and Hillard, 2006; Rubino et al., 2008).

Head-twitches (mice) and wet-dog shakes or head-shakes (rats), induced by drugs such as a serotonin 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) and its structural analogs, are thought to be mediated via central 5-HT_{2A} receptors, and these models have been used as *in vivo* tests of 5-HT_{2A} receptor pharmacology (Barnes and Sharp, 1999). Indeed, DOI-induced head-twitch response (HTR) is completely antagonized by the 5-HT_{2A} receptor antagonists ketanserin, MDL100907 and EMD281014 (Bartoszyk

Abbreviations: ADHD, Attention-deficit/hyperactivity disorder; AM281, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; AM404, *N*-(4-hydroxyphenyl)arachidonylamide; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; Anandamide, Arachidonylethanolamide; ANOVA, Analysis of variance; BINA, Biphenyl-indanone; CB_1, Cannabinoid receptor type 1; CB_2, Cannabinoid receptor type 2; CSF, Cerebrospinal fluid; DOB, 2,5-dimethoxy-4-bromoamphetamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; FAAH, Fatty acid amide hydrolase; 5-HT, Serotonin; HTR, Head-twitch response; LSD, Lysergic acid diethylamide; mGlu, Metabotropic glutamate; MK-801, Dizocilpine; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide; NMDA, *N*-methyl-D-aspartate; PCP, Phencyclidine; THC, Δ^9-tetrahydrocannabinol; Theanine, L-glutamylethylamide; TRPV1, transient receptor potential vanilloid 1; URB597, Cyclohexylcarbamic acid 3-carbamoylbiphenyl-3-yl ester.

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et al., 2003; Darmani et al., 1990; Egashira et al., 2004b. Moreover, the effects of hallucinogenic drugs, such as DOI and lysergic acid diethylamide (LSD), require the 5-HT_{2A} receptors (Glennon, 1990; González-Maeso et al., 2003, 2007; Vollenweider et al., 1998) and resemble some of the core symptoms of schizophrenia (Colpaert, 2003; Gouzoulis-Mayfrank et al., 2005; Vollenweider et al., 1998). Some antipsychotic drugs were identified by their high affinity for 5-HT_{2A} receptors (Lieberman et al., 1998; Miyamoto et al., 2005). Recently, the DOI-induced HTR and head bobs has been reported to be modulated by 5-HT_{2C} receptor activity in mice and rabbits (Canal et al., 2010; Scarlota et al., 2011), suggesting that receptors other than 5-HT_{2A} receptors affect the DOI-induced HTR. Drugs that interact with metabotropic glutamate (mGlu) receptors also have potential for the treatment of schizophrenia (Conn et al., 2009; Patil et al., 2007), and modify the DOI-induced HTR and head-shakes in mice and rats (Gewirtz and Marek, 2000; Kłodzinska et al., 2002). We previously reported that systemic anandamide administration inhibited DOI-induced HTR in mice (Egashira et al., 2004b). Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as MK-801 (dizocilpine), ketamine and dextrophan have been reported to enhance HTR induced by intracerebroventricular 5-HT administration in mice (Kim et al., 1998). These findings suggest that endocannabinoid and glutamatergic systems may modulate the DOI-induced HTR. In the present study, we sought further clarification of the effects of AM404 and URB597 on the DOI-induced HTR in mice. We also examined the effects of glutamate-related drugs such as riluzole, L-glutamylethylamide (theanine), MK-801 and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide (NBQX) on these responses. Furthermore, to investigate the involvement of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor, we examined the effects of aniracetam on inhibition of DOI-induced HTR by NBQX and URB597. To investigate the involvement of CB_1 receptor, we examined the effect of *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM281) on inhibition of DOI-induced HTR by URB597.

2. Materials and methods

2.1. Animals

Male ddY mice (Kyudo, Saga, Japan), aged 4 weeks and weighing 20–25 g, were housed in groups of five in a temperature-controlled room (23 ± 2 °C) on a 12-h light–dark cycle (lights on 7:00–19:00 h), with food and water available *ad libitum*. All procedures regarding animal care and use were carried out based on the regulations established by the Experimental Animal Care and Use Committee at Fukuoka University (Japan), and we followed the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Drugs

DOI, MK-801, AM404, riluzole and NBQX were purchased from Sigma-Aldrich (St. Louis, MO, USA). URB597 was purchased from Cayman Chemical Co. (Michigan, USA). AM281 was purchased from Tocris Bioscience (Bristol, UK). Aniracetam was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). DOI and MK-801 were dissolved in saline. AM404 was dissolved in emulphor vehicle (18:1:1, saline: emulphor: ethanol). Riluzole, NBQX and aniracetam were dissolved in 0.5% CMC-Na. AM281 and URB597 were dissolved in 1% Tween 80 solution. Theanine was purchased from Tokyo Chemical industries (Tokyo, Japan) and was dissolved in distilled water.

2.3. HTR measurement and drug treatments

HTR is a distinctive twitching behavior of the head. The procedure followed was that previously described by Egashira et al. (2004b). DOI-induced HTR was observed in a plastic container (10 × 30 × 30 cm). AM404, AM281 and URB597 were injected intraperitoneally (i.p.) 60 min before the number of head-twitches was counted. Riluzole, MK-801 and NBQX were administered i.p. 30 min before the number of head-twitches was counted. Theanine and aniracetam were injected orally 60 min before the number of head-twitches was counted. We performed oral injection using plastic syringe with a stainless tube for oral injection. Five minutes after injection of DOI (5 mg/kg, i.p.), the number of head-twitches was counted for a 5-min period. Control animals received injections with vehicle via the same route. All drugs were administered at a volume of 0.1 mL/10 g of body weight. The number of head-twitches was scored using a tally counter by three observers who did not know what agent was being tested. The doses of AM404, URB597, riluzole, theanine, MK-801, NBQX, aniracetam and AM281 were chosen based on previous reports (Braida et al., 2007; Egashira et al., 2004a, 2004b, 2008; Fegley et al., 2005; Kim et al., 1998; Nakamura et al., 2000; Rutkowska et al., 2006; Saber-Tehrani et al., 2010; Tzavara et al., 2006; Zhang and Marek, 2008). URB597 was administered 1 h before testing to coincide with previous findings of peak anandamide elevations at this time point (Fegley et al., 2005). Similarly, the administration schedule of AM404 was determined based on a previous report (Tzavara et al., 2006). To antagonize on the CB_1 receptors, AM281 was administered 1 h before testing. Moreover, the administration schedules of riluzole, MK-801, NBQX and aniracetam were determined based on previous studies (Egashira et al., 2008; Kim et al., 1998; Nakamura et al., 2000; Zhang and Marek, 2008). In addition, the injection route of theanine was p.o., it was decided that the injection time prior testing of theanine is 1 h.

2.4. Statistical analysis

The results from the HTR measurement were analyzed by one-way analysis of variance (ANOVA), followed by Tukey–Kramer's post-hoc test to determine differences among the groups. The criterion for statistical significance was considered to be $p<0.05$. Values are expressed as the mean ± SEM.

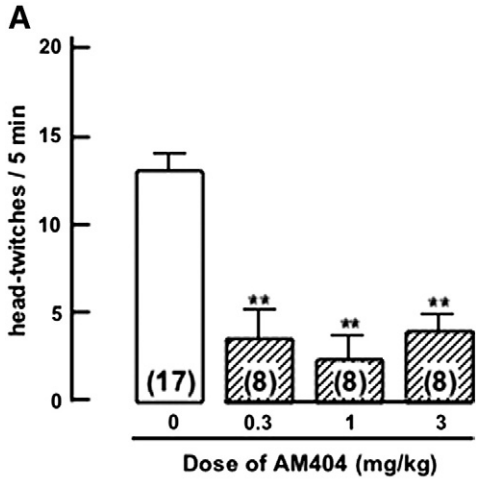
3. Results

3.1. Effects of AM404 and URB597 on DOI-induced HTR

No HTR in control animals was observed. The 5-$HT_{2A/2C}$ receptor agonist DOI (5 mg/kg, i.p.) caused a HTR in mice. The anandamide transport inhibitor AM404 (0.3–3 mg/kg, i.p.) significantly inhibited this response [$F(3,37)=21.98$, $p<0.001$ by one-way ANOVA; DOI 5 mg/kg + AM404 0.3–3 mg/kg: $p<0.01$ by Tukey–Kramer's post-hoc test; Fig. 1A]. Similarly, the FAAH inhibitor URB597 (0.1–10 mg/kg, i.p.) significantly inhibited the DOI-induced HTR [$F(3,27)=5.689$, $p<0.01$ by one-way ANOVA; DOI 5 mg/kg + URB597 0.1–1 mg/kg: $p<0.05$, DOI 5 mg/kg + URB597 10 mg/kg: $p<0.01$ by Tukey–Kramer's post-hoc test; Fig. 1B]. In addition, AM404 (0.3–3 mg/kg, i.p.) or URB597 (0.1–10 mg/kg, i.p.) alone had no effect on HTR.

3.2. Effects of riluzole and theanine on DOI-induced HTR

The glutamate release inhibitor riluzole (0.3 and 1 mg/kg, i.p.) significantly inhibited the DOI-induced HTR [$F(3,41)=8.717$, $p<0.001$ by one-way ANOVA; DOI 5 mg/kg + riluzole 0.3 mg/kg: $p<0.05$, DOI 5 mg/kg + riluzole 1 mg/kg: $p<0.01$ by Tukey–Kramer's post-hoc test; Fig. 2A]. Similarly, the natural glutamate analog theanine (1 and 3 mg/kg, p.o.) significantly inhibited the DOI-induced HTR [$F(3,48)=5.672$, $p<0.01$ by one-way ANOVA; DOI 5 mg/kg + theanine 1 mg/kg: $p<0.05$, DOI 5 mg/kg + theanine 3 mg/kg: $p<0.01$ by Tukey–Kramer's post-hoc test; Fig. 2B].



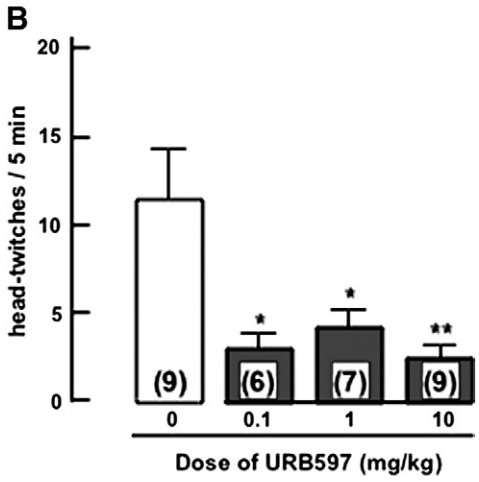
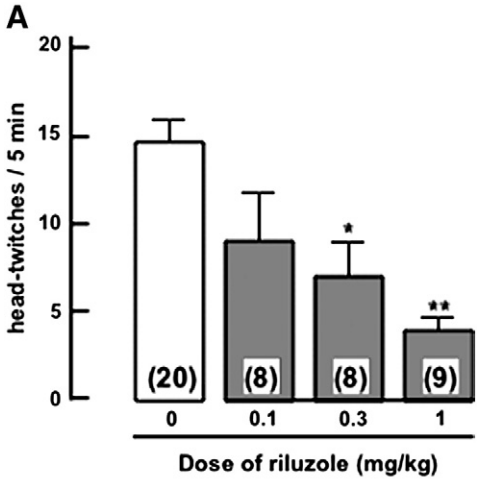


Fig. 1. Effects of DOI (5 mg/kg, i.p.) alone and in combination with AM404 (A) and URB597 (B) on DOI-induced HTR in mice. The number of head-twitches was counted for 5 min, beginning 5 min after treatment with DOI. AM404 and URB597 were injected i.p. 60 min before the number of head-twitches was counted. Values are expressed as the mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$ compared with vehicle. The number of mice is shown at the bottom of each column.



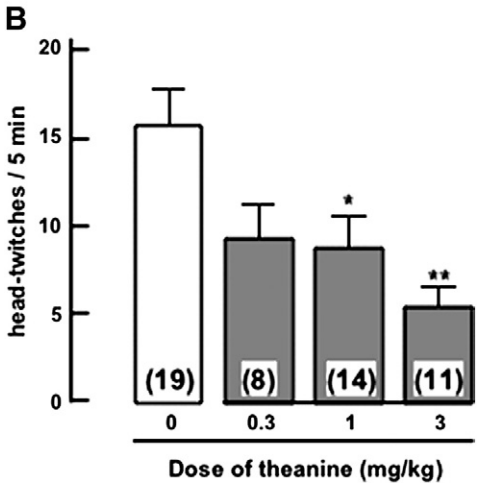


Fig. 2. Effects of DOI (5 mg/kg, i.p.) alone and in combination with riluzole (A) and theanine (B) on DOI-induced HTR in mice. The number of head-twitches was counted for 5 min, beginning 5 min after treatment with DOI. Riluzole was injected i.p. 30 min before the number of head-twitches was counted. Theanine was injected orally 60 min before the number of head-twitches was counted. Values are expressed as the mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$ compared with vehicle. The number of mice is shown at the bottom of each column.

3.3. Effects of MK-801 and NBQX on DOI-induced HTR

As shown in Fig. 3A, the noncompetitive NMDA receptor antagonist MK-801 (0.1 and 1 mg/kg, i.p.) did not affect the DOI-induced HTR. On the other hand, the AMPA receptor antagonist NBQX (0.01–0.3 mg/kg, i.p.) significantly inhibited the DOI-induced HTR [$F(4,46)=10.709$, $p<0.001$ by one-way ANOVA; DOI 5 mg/kg + NBQX 0.01–0.3 mg/kg: $p<0.01$ by Tukey–Kramer's post-hoc test; Fig. 3B].

3.4. Effects of aniracetam on inhibition of HTR by NBQX and URB597

The AMPA receptor positive modulator aniracetam (30 and 100 mg/kg, p.o.) significantly reversed inhibition of HTR by NBQX (0.3 mg/kg, i.p.) [$F(3,34)=13.855$, $p<0.001$ by one-way ANOVA; DOI 5 mg/kg + NBQX 0.3 mg/kg + aniracetam 30 mg/kg: $p<0.05$, DOI 5 mg/kg + NBQX 0.3 mg/kg + aniracetam 100 mg/kg: $p<0.01$ by Tukey–Kramer's post-hoc test; Fig. 4A]. Similarly. aniracetam (100 mg/kg, p.o.) significantly reversed the inhibition of HTR by URB597 (1 mg/kg, i.p.) [$F(3,30)=4.244$, $p<0.05$ by one-way ANOVA; DOI 5 mg/kg + URB597 1 mg/kg + aniracetam 100 mg/kg: $p<0.05$ by Tukey–Kramer's post-hoc test; Fig. 4B]. In addition, aniracetam (30 and 100 mg/kg, p.o.) alone did not induce HTR.

3.5. Effect of AM281 on inhibition of HTR by URB597

The CB_1 receptor antagonist AM281 (1 and 3 mg/kg, i.p.) had no effect on the inhibition of HTR by URB597 (1 mg/kg, i.p.) (Fig. 5).

4. Discussion

The results of the present study revealed that the glutamate release inhibitor riluzole and the natural glutamate analog theanine inhibited DOI-induced HTR in mice. Theanine, a component of Japanese green tea (*Camellia sinensis*), competitively antagonizes glutamate receptors (Maruyama and Takeda, 1994). Kakuda et al. (2002) reported that theanine binds the glutamate receptor subtypes AMPA and NMDA receptors in rat cortical neurons. The inhibitory effect of theanine on the AMPA receptor is 10-fold higher than its inhibitory effect on the NMDA receptor. Theanine also inhibits glutamate transport in neurons and astroglia in rat brain (Kakuda et al., 2008). Moreover, intra-striatum administration of theanine increases the release of dopamine and glycine in conscious rats, and the AMPA receptor antagonist NBQX inhibits these effects of theanine (Yamada et al., 2009). Recently, activation of adenosine A_1 heteroceptors on glutamatergic terminals, which decreases glutamate release in the prefrontal cortex like mGlu2 autoreceptors (Marek et al., 2000), has been reported to suppress the

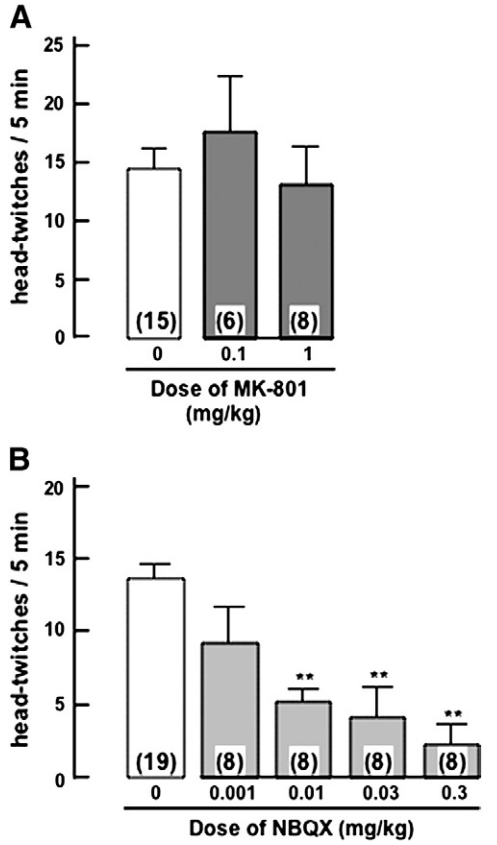


Fig. 3. Effects of DOI (5 mg/kg, i.p.) alone and in combination with MK-801 (A) and NBQX (B) on DOI-induced HTR in mice. The number of head-twitches was counted for 5 min, beginning 5 min after treatment with DOI. MK-801 and NBQX were injected i.p. 30 min before the number of head-twitches was counted. Values are expressed as the mean ± SEM. $^{**}p<0.01$ compared with vehicle. The number of mice is shown at the bottom of each column.

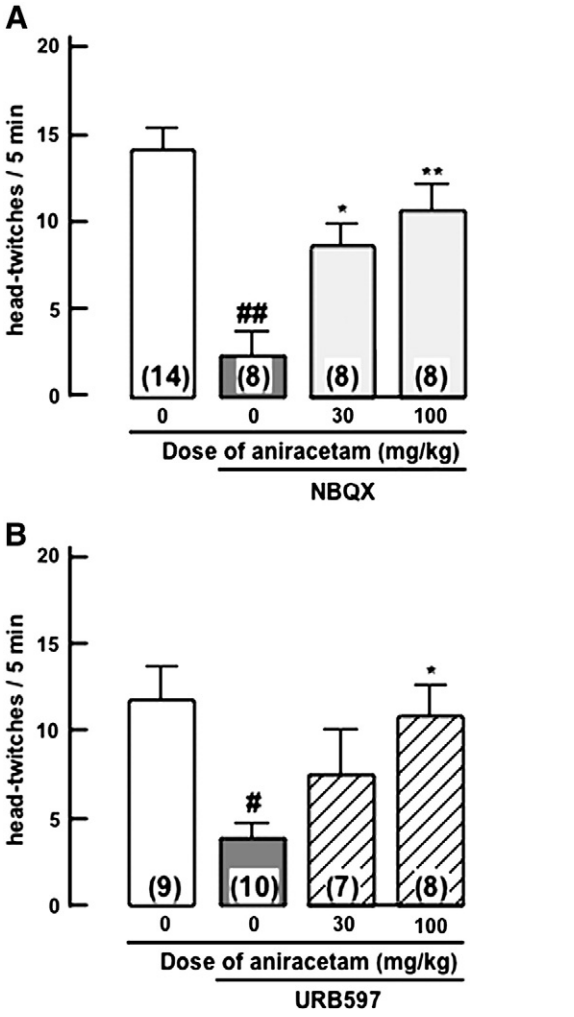


Fig. 4. Effects of DOI (5 mg/kg, i.p.) alone and in combination with aniracetam, NBQX (0.3 mg/kg) (A) and URB597 (1 mg/kg) (B) on DOI-induced HTR in mice. The number of head-twitches was counted for 5 min, beginning 5 min after treatment with DOI. Aniracetam was injected orally 60 min before the number of head-twitches was counted. NBQX and URB597 were injected i.p. 30 and 60 min before testing, respectively. Values are expressed as the mean ± SEM. $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with DOI alone. $^{*}p<0.05$, $^{**}p<0.01$ compared with DOI + NBQX or DOI + URB597. The number of mice is shown at the bottom of each column.

DOI-induced head shakes in rats (Marek, 2009). Similarly, the selective mGlu2/3 receptor agonists LY354740 and LY379268 inhibit the DOI-induced HTR and head-shakes in mice and rats (Gewirtz and Marek, 2000; Kłodzinska et al., 2002). Moreover, LY379268 attenuates the DOI-induced c-*fos* expression in the prefrontal cortex (Zhai et al., 2003). In addition, the mGlu2 receptor-selective positive allosteric modulator biphenyl-indanone (BINA) reduces HTR and Fos expression in the prefrontal cortex induced by the 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-bromoamphetamine (DOB) in mice (Benneyworth et al., 2007). Taken together with these findings, the present results suggest that the inhibition of the glutamatergic system blocks the ability of 5-HT_{2A} receptors to modulate HTR. Also, antipsychotic drugs such as clozapine and haloperidol inhibit the DOI-induced HTR in mice (Hayslett and Tizabi, 2005; Rojas-Corrales et al., 2007). Our data suggest that riluzole and theanine might counteract the effects of hallucinogenic drugs.

Human brain imaging demonstrates a common pattern of increased activity in the frontal cortex caused by psychotomimetic drugs and acute schizophrenia (Cleghorn et al., 1989; Kaplan et al., 1993; Vollenweider et al., 1997), suggesting a potential role of the excitatory neurotransmitter glutamate in psychotic behaviors. Moreover, the bilateral microinjection of DOI elicits dose-dependent head shakes in rats when injected into the prefrontal cortex (Willins and Meltzer, 1997), which suggests that the prefrontal cortex is a part of the circuitry mediating the HTR. Systemic or intracortical DOI administration has also been reported to increase cortical extracellular glutamate levels in rats (Scruggs et al., 2003). The increase in glutamate levels elicited by intracortical DOI injection was blocked by treatment with the selective 5-HT_{2A} receptor antagonist MDL100907, indicating a 5-HT_{2A} receptor-mediated effect. These data support the hypothesis that 5-HT_{2A} receptor-mediated regulation of glutamate release is the mechanism through which hallucinogens activate the cerebral cortex.

The present finding also showed that the AMPA receptor antagonist NBQX inhibited DOI-induced HTR, whilst the noncompetitive NMDA receptor antagonist MK-801 did not affect this response. Moreover, the AMPA receptor positive modulator aniracetam reversed inhibition of HTR by NBQX. Our data suggest that the activation of AMPA receptors modulates the action of DOI to induce HTR. This idea agrees with previous data indicating that DOI-induced expression of Fos protein in the cerebral cortex is depended on the AMPA receptor-mediated mechanism (Scruggs et al., 2000). Our data also agree with a previous report indicating that DOI-induced increase of 5-HT release in the medial prefrontal cortex is reversed by NBQX and mGlu2/3 receptor agonist but not by MK-801 (Martín-Ruiz et al., 2001). Furthermore, our data are essentially consistent with the report that the expression of DOI-induced wet dog shakes or head-shakes is reduced by pretreatment with the AMPA/kainate receptor antagonists DNQX and LY293558, or AMPA receptor antagonist GYKI52466 in rats (Gorzalka et al., 2005; Zhang and Marek, 2008).

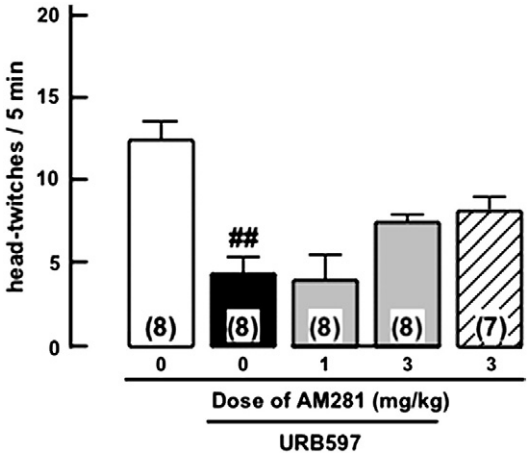


Fig. 5. Effects of DOI (5 mg/kg, i.p.) alone and in combination with AM281 and URB597 (1 mg/kg) on DOI-induced HTR in mice. The number of head-twitches was counted for 5 min, beginning 5 min after treatment with DOI. AM281 and URB597 were injected i.p. 60 min before the number of head-twitches was counted. White, black, gray and striped bars represent DOI alone, DOI + URB597, DOI + URB597 + AM281, DOI + AM281, respectively. Values are expressed as the mean ± SEM. $^{\#\#}p<0.01$ compared with DOI alone. The number of mice is shown at the bottom of each column.

Thus, our data support that AMPA receptors play a role in the DOI-induced behavioral responses.

Zhang and Marek (2008) reported that MK-801 (0.2 mg/kg, i.p.) enhances the head shakes induced by DOI at low and medium doses (0.313 and 0.625 mg/kg) but not a high dose (1.25 mg/kg) in rats. However, we did not observe that MK-801 (0.1 and 1 mg/kg, i.p.) could enhance HTR induced by DOI (5 mg/kg, i.p.) in mice. This discrepancy may be due to the difference of experimental methods such as dose of DOI and behavioral evaluation. Also, the competitive NMDA receptor antagonist CGP43487 increases the head shakes induced by DOI (Dall'Olio et al., 1999). Moreover, the noncompetitive NMDA receptor antagonists such as MK-801, ketamine and dextrophan enhance the HTR induced by intracerebroventricular 5-HT administration in mice (Kim et al., 1998). These findings suggest that the blockade of NMDA receptors enhances the 5-HT_{2A} receptor-mediated behavioral responses. Several studies have demonstrated that systemically administered NMDA receptor antagonists such as PCP, ketamine and MK-801 increase 5-HT and glutamate release in the medial prefrontal cortex (Adams and Moghaddam, 2001; Amargós-Bosch et al., 2006; López-Gil et al., 2007; Lorrain et al., 2003; Martin et al., 1998; Moghaddam et al., 1997). Therefore, the increase of 5-HT and glutamate release by the blockade of NMDA receptors might be involved in the enhancement of the DOI-induced behavioral responses.

In the present study, we demonstrated that the anandamide transporter inhibitor AM404 (0.3–3 mg/kg, i.p.) inhibited DOI-induced HTR in mice. Furthermore, we found that the FAAH inhibitor URB597 (0.1–10 mg/kg, i.p.) inhibited the DOI-induced HTR. We previously reported that exogenous administration of the endocannabinoid anandamide inhibited this response, which leads to the hypothesis that the endocannabinoid system may participate in the mechanism of action of DOI to induce HTR. Gorzalka et al. (2005) reported that AM404 (10 mg/kg, i.p.) inhibited the DOI-induced wet dog shakes in rats, but lower doses were ineffective. These data suggest that the endocannabinoid system may be involved in the DOI-induced HTR. Also, the discrepancies of effective doses of AM404 between two studies may be due to the differences of species or administration time. In addition, none of the AM404 and URB597 showed a dose–response effect on the HTR. That is because the lowest dose was not low enough to show a dose–response. Taken together, the present results strongly indicate that endocannabinoid signaling counteracts the DOI-induced behavioral responses.

Interestingly, the AMPA receptor positive modulator aniracetam completely reversed inhibition of HTR by URB597, indicating the interaction of endocannabinoid and glutamatergic systems. Endocannabinoids regulate the excitability of dorsal raphe 5-HT neurons by modulating glutamatergic synaptic transmission to these neurons (Haj-Dahmane and Shen, 2009). Therefore, endocannabinoids might inhibit the DOI-induced HTR through an interaction with glutamatergic neurotransmission. Two cannabinoid receptors have been cloned: the CB_1 receptor in the central nervous system (Matsuda et al., 1990) and the CB_2 receptor on immune cells and peripheral tissues (Munro et al., 1993). Initially, CB_2 receptors were thought to be localized exclusively in immune cells, but recent work has suggested that low levels of these receptors are also present in the brainstem (Van Sickle et al., 2005) and possibly in other brain regions (Gong et al., 2006). Anandamide binds CB_1 and CB_2 receptors (Pertwee and Ross, 2002). Anandamide also activates to the transient receptor potential vanilloid 1 (TRPV1) receptors (Ross, 2003). The inhibition of FAAH activity impairs anandamide hydrolysis, resulting in elevated central nervous system levels of this transmitter (Cravatt et al., 2001; Fegley et al., 2005). We previously reported that the inhibitory effect of anandamide on the DOI-induced HTR was not blocked by rimonabant or a TRPV1 receptor antagonist capsazepine (Egashira et al., 2004b). In the present study, the CB_1 receptor antagonist AM281 had no effect on the inhibition of HTR by URB597. Therefore, the endogenous anandamide might inhibit the HTR via CB_2 receptor but not CB_1 receptor or TRPV1 receptor.

We previously reported that Δ^9-tetrahydrocannabinol (THC) inhibited DOI-induced HTR in mice (Egashira et al., 2004b). Darmani (2001) also reported that THC and synthetic cannabinoid receptor agonists inhibited the DOI-induced HTR. In contrast, a CB_1 receptor antagonist rimonabant (2.5–20 mg/kg, i.p.) alone has been reported to evoke HTR in mice (Darmani and Pandya, 2000). We also reported that rimonabant (3 and 10 mg/kg, i.p.) alone weakly caused the HTR in mice (Egashira et al., 2004b). Thus, the effect of THC on the HTR is completely different from that of other hallucinogenic drugs such as LSD. Therefore, the DOI-induced HTR is thought to be a model used for investigating the 5-HT_{2A} receptors rather than a model of hallucinogenic or psychotic symptoms. Also, the psychosis related to CB_1 receptor activation may be unrelated mechanistically to the psychosis-like response of hallucinogenic drugs that stimulate the 5-HT_{2A} receptors.

In conclusion, the present results suggest that the endocannabinoid system modulates DOI-induced HTR via the interaction with glutamatergic neurotransmission. Moreover, the present data suggest that the activation of AMPA receptors modulates the DOI-induced HTR. These findings confirm and extend previous findings concerning the key role of endocannabinoid and glutamatergic systems in the effects of hallucinogen.

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