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

Nobuaki Egashira [a,b,*], Atsunori Shirakawa [a], Ryoko Okuno [a], Kenichi Mishima [a], Katsunori Iwasaki [a], Ryozo Oishi [b], Michihiro Fujiwara [a]

[a] Department of Neuropharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814–0180, Japan
[b] Department of Pharmacy, Kyushu University Hospital, Fukuoka 812–8582, Japan

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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.

* Corresponding author at: Department of Pharmacy, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan. Tel.: +81 92 642 5920; fax: +81 92 642 5937.
E-mail address: n-egashi@pharm.med.kyushu-u.ac.jp (N. Egashira).

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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].