European Journal of Pharmacology 586 (2008) 212–216

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Face-washing behavior induced by the group I metabotropic glutamate receptor agonist (*S*)-3,5-DHPG in mice is mediated by $mGlu_1$ receptor

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ARTICLE INFO

Article history:
Received 31 August 2007
Received in revised form 13 February 2008
Accepted 20 February 2008
Available online 29 February 2008

Keywords:
$mGlu_1$ receptor
$mGlu_5$ receptor
$mGlu_{2/3}$ receptor
DHPG
Face-washing behavior
Convulsion

ABSTRACT

It is known for the non-selective group I metabotropic glutamate (mGlu) receptors agonist (*S*)-3,5-dihydroxyphenylglycine (*S*-3,5-DHPG) to cause convulsions, which are mediated by $mGlu_1$ receptor. However, the behavioral changes other than convulsions caused by (*S*)-3,5-DHPG have not been well studied. The purpose of the present study was to explore the behavioral changes elicited by activation of group I mGlu receptors with (*S*)-3,5-DHPG and to clarify which, $mGlu_1$ receptor or $mGlu_5$ receptor, is responsible for such behavior. (*S*)-3,5-DHPG at doses of 3–30 nmol caused characteristic face-washing behavior. This behavioral change was inhibited by both the competitive $mGlu_1$ receptor antagonists (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA) and (*S*)-4-carboxyphenylglycine (*S*-4CPG) and the non-competitive $mGlu_1$ receptor antagonist, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide (FTIDC), but not by the $mGlu_5$ receptor antagonist 2-Methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP), the $mGlu_{2/3}$ receptor agonist (−)-2-oxa-4-aminobicyclo [3.1.0]hexane-4,6-dicarboxylate (LY379268), the $mGlu_{2/3}$ receptor antagonist (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495), the *N*-methyl-D-asparate (NMDA) receptor antagonist 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801), or the competitive non-NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*] quinoxaline-7-sulfonamide (NBQX). These findings indicate that face-washing behavior is due to selective activation of $mGlu_1$ receptor by (*S*)-3,5-DHPG, and that the face-washing behavior induced by (*S*)-3,5-DHPG in mice can be used for *in vivo* testing of the antagonistic potency of both competitive and non-competitive $mGlu_1$ receptor antagonists.

1. Introduction

The metabotropic glutamate (mGlu) receptors have been classified into eight subtypes ($mGlu_{1-8}$) by sequence homology and second-messenger coupling. Group I subtype receptors consist of $mGlu_1$ receptor and $mGlu_5$ receptor, which couple with Gq, activate phospholipase C, and produce diacylglycerol and inositol triphosphate while group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) subtype receptors couple with Gi and inhibit production of cyclic AMP. Group I receptors are primarily located in the post-synaptic membrane and mediate neuronal excitation, whereas group II and group III receptors are primarily located in the presynaptic membrane and are believed to be autoreceptors regulating excessive release of glutamate (Conn and Pin, 1997; Pin and Duvoisin, 1995; Nakanishi and Masu, 1994).

$mGlu_1$ receptor and $mGlu_5$ receptor are broadly distributed in the central nervous system, including the hippocampus, cerebellum, cortex, and thalamus. Perturbations of the glutamatergic system are thought to play a major role in many diseases, such as epilepsy, anxiety, depression, cerebral ischemia, schizophrenia, and neurodegenerative diseases (Bordi and Ugolini, 1999; Meldrum, 2000; Spooren et al., 2001). However, the roles of $mGlu_1$ receptor and $mGlu_5$ receptor in these abnormalities are not yet clearly understood. Efforts to discover and develop mGlu receptors ligands including allosteric modulators have continued to reveal the roles of mGlu receptors and their potential therapeutic usages. However, the behavioral outcomes of activation of these receptors *in vivo* have not been well characterized. It is thus difficult to conclude at present whether the pharmacological effects of agents that have been developed are actually due to activation/inhibition of these receptors *in vivo* or whether the behavioral changes caused by such agents are actually specific to effects on certain types of receptors. Previously, Laudrup and Klitgaard (1993) and Tizzano et al. (1993) reported that central administration of the non-selective groups I and II mGlu receptor agonist, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) caused face-washing behavior/scratching and seizures in mice. Later, Tizzano et al. (1995), Chapman et al. (2000) and Moldrich et al. (2001) reported that selective group I agonist, (*R*,*S*)-3,5-

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doi:10.1016/j.ejphar.2008.02.061

dihydroxyphenylglycine (*RS*-3,5-DHPG) also caused face-washing behavior/scratching and seizures. Barton and Shannon (2005) reported that lower doses of the non-selective $mGlu_{1/5}$ receptor agonist (*S*)-3,5-dihydroxyphenylglycine (*S*-3,5-DHPG) (≤30 nmol) mainly produced scratching behavior, whereas higher doses of (*S*)-3,5-DHPG (≥100 nmol) caused dose-related increases in seizure-like behaviors such as slow forelimb clonus in CD-1 mice. The seizures produced by (*S*)-3,5-DHPG have been well studied by various laboratories. However, less attention was paid to which subtype of mGlu receptors is involved in the face-washing behavior/scratching induced by (*S*)-3,5-DHPG. That is, the seizure produced by (*RS*)-3,5-DHPG or (*S*)-3,5-DHPG were well studied with various mGlu receptors-related compounds (Barton and Shannon, 2005; Kingston et al., 2002; Tizzano et al., 1995, Moldrich et al., 2001; Chapman et al., 2000). The results of these studies indicated that activation of $mGlu_1$ receptor by (*RS*)-3,5-DHPG or (*S*)-3,5-DHPG was primarily responsible to elicit seizures, however, modulation of other mGlu receptors also affected the seizure caused by administration of (*RS*)-3,5-DHPG and (*S*)-3,5-DHPG. On the other hand, the scratching syndrome caused by (*S*)-3,5-DHPG were only characterized by Barton and Shannon (2005). They found that (4-methoxy-phenyl)-(6-methoxy-quinazolin-4-yl)-amine hydrochloride (LY456236), the $mGlu_1$ receptor antagonist and 1-4-aminophenyl-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466), the non-*N*-methyl-D-asparate (NMDA) receptor antagonist suppressed the scratching syndrome.

We have observed face-washing behavior, mainly caused by lower doses of (*S*)-3,5-DHPG (3 to 30 nmol), as well as convulsions caused by higher doses of (*S*)-3,5-DHPG (100 to 400 nmol), findings similar to those noted in a previous report (Barton and Shannon, 2005). Face-washing behavior is thought to be the first behavioral changes caused by the $mGlu_{1/5}$ receptor agonist (*S*)-3,5-DHPG prior to convulsions, and might be more relevant than convulsions to study of agonist–antagonist interaction *in vivo*. The purpose of this study was to clarify which subtype of mGlu receptors is involved in the behavioral changes produced by (*S*)-3,5-DHPG at low doses in mice. In the present studies, we evaluated not only the amino acid-derived $mGlu_1$ receptor antagonist AIDA and *S*-4CPG, but also the negative allosteric modulator 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide (FTIDC) that we recently identified (Suzuki et al., 2007) to investigate the pharmacology of (*S*)-3,5-DHPG-inuced face-washing behavior. In addition, we evaluated the $mGlu_{2/3}$ receptor agonist LY379268, the $mGlu_{2/3}$ receptor antagonist LY341495 and the ionotropic receptor antagonist such as the NMDA receptor antagonist 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801) and the competitive non-NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX) against this face-washing behavior. We demonstrated that the face-washing behavior elicited by (*S*)-3,5-DHPG was mainly due to activation of $mGlu_1$ receptor but not $mGlu_5$ receptor, which supported the original results reported by Barton and Shannon (2005). In addition to the competitive $mGlu_1$ receptor antagonist, the non-competitive negative allosteric modulator of $mGlu_1$ receptor was also able to attenuate face-washing behavior elicited by (*S*)-3,5-DHPG.

2. Materials and methods

2.1. Animals

Male CD-1 (ICR) mice (5–8-weeks old, Japan SLC, Inc., Shizuoka, Japan) were housed in a controlled animal room (room temperature; 23±2 °C, humidity 55±15%) with a 12 h light–dark cycle (light on: 07:00–19:00). Mice were maintained in groups of 5–6 mice per cage. Food and water were available *ad libitum*. All animal studies were approved by Banyu Institutional Animal Care and Use Committee.

2.2. Behavioral observation after administration of (*S*)-3,5-DHPG in mice

(*S*)-3,5-DHPG (3 to 400 nmol) was intracerebroventricularly administered to mice at a volume of 10 μl per head using a Hamilton syringe. Animals were placed in an acrylic box (10×15×13 cm) immediately after the administration of (*S*)-3,5-DHPG, and their behavior was monitored for 60 min. (*S*)-3,5-DHPG caused characteristic behaviors included face-washing behavior and convulsions. The face-washing behavior was defined as wiping of the face from the ear to the mouth with forelimbs, which is likely similar to facial grooming of the scratching syndrome described by Barton and Shannon (2005). Duration of face-washing behavior was recorded by the time-recorder with keypads (Neuroscience, Tokyo, Japan). The recorder was activated by pressing a key and measured duration while holding pressed key. Measurements were conducted blind fashion. In the case of face-washing behavior, the duration was recorded for the first 30 min immediately after administration of (*S*)-3,5-DHPG and expressed total duration every 5 min, while convulsive activity was monitored to measure the presence or absence of clonic convulsions (CC), tonic convulsions (TC), and mortality for 60 min for each mouse. Effects of various drugs on face-washing behavior were recorded 5 min after administration of (*S*)-3,5-DHPG for 5 min. For administration into the lateral ventricle, in the case of central administration, (*S*)-3,5-DHPG and the test compounds were given as a mixture of both. In the case of systemic administration, the test compounds were given 15 min or 30 min prior to administration of (*S*)-3,5-DHPG.

2.3. Drugs

(−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268) and 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide (FTIDC) were synthesized at Banyu Tsukuba Laboratories. (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495), (*S*)-3,5-dihydroxyphenylglycine (*S*-3,5-DHPG), (*RS*)-1-Aminoindan-1,5-dicarboxylic acid (AIDA), (*S*)-4-carboxyphenylglycine (*S*-4CPG), 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP), and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX) were purchased from Tocris Bioscience (Bristol, UK). 5*R*,10*S*-(+)-5-Methyl-10, 11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine hydrogen maleate (MK-801) was purchased from Sigma-Aldrich (St. Louis, MO). In the case of intracerebroventricular administration, all compounds were dissolved in physiological saline. For systemic administration, LY341495 and MK-801 were dissolved in physiological saline, and FTIDC and LY379268 were suspended in a 0.5% methylcellulose solution. NBQX was dissolved a small quantity of 1 N NaOH and this solution was diluted in distillated water to obtain a target concentration.

2.4. Data analysis

Results are expressed as the mean±S.E.M. In dose–response testing of (*S*)-DHPG-induced face-washing behavior, one-way analysis of variance (ANOVA) followed by Dunnett's test was used to examine the duration of face-washing behavior. The χ^2 test was used to examine the incidence of convulsions. In the study of antagonism of face-washing, ANOVA followed by multiple comparison test (Dunnett's test) was used for examination of the effects of drugs on groups administered (*S*)-3,5-DHPG. Probability levels <0.05 were considered significant.

3. Results

3.1. (*S*)-3,5-DHPG-induced behavioral changes in mice

When (*S*)-3,5-DHPG (3–400 nmol) was intracerebroventricularly administered, significant face-washing behavior was observed from