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

Hirohiko Hikichi *, Yuki Iwahori, Takeshi Murai, Shunsuke Maehara, Akio Satow, Hisashi Ohta

Pharmacology, Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan

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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-

* Corresponding author. Tel.: +81 29 877 2000; fax: +81 29 877 2024.
E-mail address: hirohiko_hikichi@merck.com (H. Hikichi).

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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

3 to 30 nmol (Fig. 1A). Face-washing behavior began immediately after injection of (*S*)-3,5-DHPG (3–400 nmol) (Fig. 1B). Face-washing behavior was maintained over the 30 min observation period after administration of 3 and 10 nmol of (*S*)-3,5-DHPG, while the face-washing behavior elicited by 30 to 400 nmol of (*S*)-3,5-DHPG gradually decreased within 30 min (Fig. 1B). We observed scratching of the head or ears with the hindlimbs other than face-washing behavior. Scratching behaviors were not as frequent as face-washing behavior. (*S*)-3,5-DHPG at doses of 100 and 400 nmol dose-dependently induced clonic convulsions without tonic convulsions (Table 1). The onset of clonic convulsions was noted at 51.3±4.3 (n=4) min and 28.7±6.4 (n=6) min for treatment with 100 and 400 nmol, respectively. The clonic convulsions occurred after diminution of face-washing behavior. (*S*)-3,5-DHPG at doses of 100 and 400 nmol caused death of one of seven and five of six animals, respectively.

3.2. Effects of intracerebroventricular administration of drugs on face-washing behavior elicited by (S)-3,5-DHPG in mice

When we tested the effects of various mGlu receptors-related compounds on the face-washing behavior elicited by (*S*)-3,5-DHPG, we used 10 nmol of (*S*)-3,5-DHPG and face-washing behavior was monitored for 5 min after administration of (*S*)-3,5-DHPG. Co-administration of competitive $mGlu_1$ receptor antagonists AIDA and *S*-4CPG with (*S*)-3,5-DHPG reduced the duration of face-washing in dose-dependent fashion (Fig. 2A, B). These inhibitory effects of AIDA and *S*-4CPG were statistically significant at 500 nmol and 50–100 nmol, respectively. On the other hand, the $mGlu_5$ receptor antagonist MPEP had no effect on face-washing behavior (Fig. 2C). All compounds did not cause any noticeable behavioral changes including face-washing behavior or sedation by themselves.

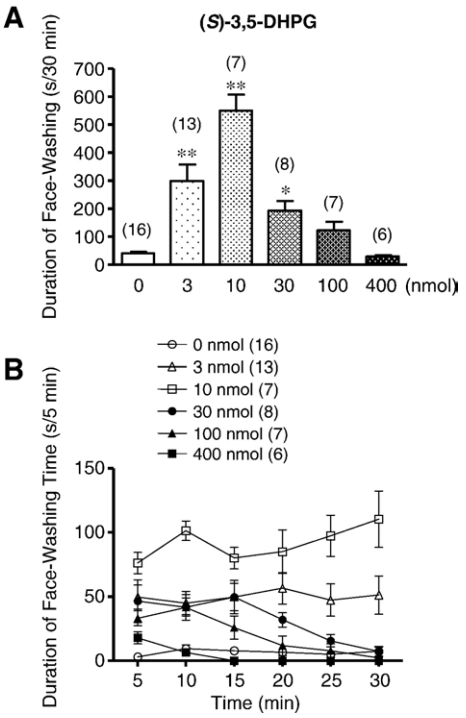

Fig. 1. Face-washing behavior caused by (*S*)-3,5-DHPG in mice. (A) Total duration of face-washing behavior during the 30-min observation period. (B) Time-course of the duration of face-washing behavior in 5-min intervals of the 30-min observation period. Duration of face-washing behavior is expressed as means±S.E.M (s). (*S*)-3,5-DHPG was intracerebroventricularly administered in conscious mice. Numbers in parenthesis indicate numbers of animals used (n=6–16 animals per dose). *P<0.05, **P<0.01 compared with the corresponding vehicle response.

Table 1
Induction of convulsions by (*S*)-3,5-DHPG in mice

Drug	CC	TC	Mortality
Vehicle (8)	0	0	0
(*S*)-3,5-DHPG 30 nmol (8)	0	0	0
(*S*)-3,5-DHPG 100 nmol (7)	4[a]	0	1
(*S*)-3,5-DHPG 400 nmol (6)	6[a]	0	5[a]

Numbers in parenthesis indicated numbers of animal used. Numbers in the table indicated numbers of animal exhibiting convulsions within 60 min after the administration of (*S*)-3,5-DHPG. CC; clonic convulsions, TC; tonic convulsions.
[a] P<0.01 compared with corresponding response to vehicle.

3.3. Effects of systemic administration of drugs on face-washing behavior elicited by (S)-3,5-DHPG in mice

The intraperitoneal administration of the non-competitive $mGlu_1$ receptor antagonist FTIDC inhibited the duration of face-washing behavior induced by (*S*)-3,5-DHPG in dose-dependent fashion (Fig. 3A). The inhibitory effect of FTIDC was statistically significant at 30 mg/kg. Intraperitoneal administration of the $mGlu_5$ receptor antagonist MPEP and the $mGlu_{2/3}$ receptor agonist LY379268 and subcutaneous administration of the $mGlu_{2/3}$ receptor antagonist

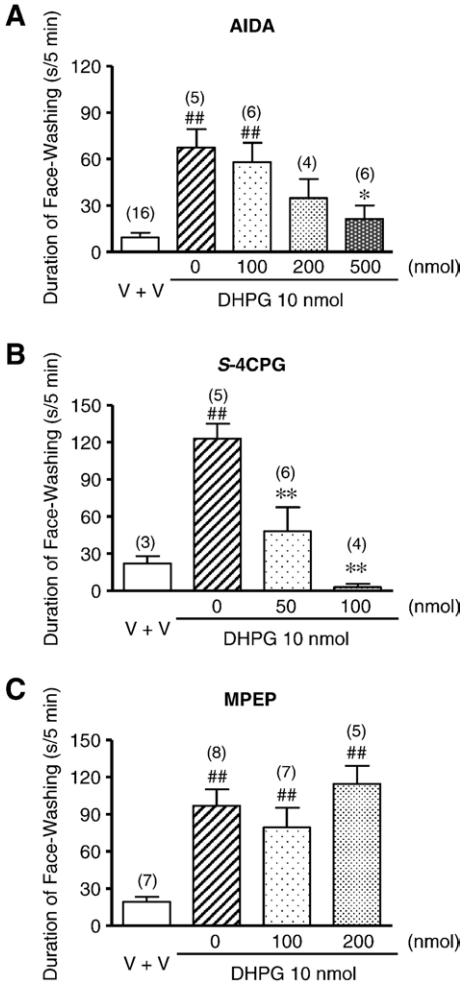

Fig. 2. Effects of intracerebroventricular co-administration of AIDA (A), (*S*)-4CPG (B), and MPEP (C) on face-washing behavior elicited by (*S*)-3,5-DHPG in mice. (*S*)-3,5-DHPG and the test compounds were given as a mixture into cerebroventricle. Drug effects on face-washing behavior were tested 5 min after administration for 5 min. Numbers in parenthesis indicated numbers of animal used (A, n=6–16 animals per each group; B, n=3–6 animals per each group; C, n=5–8 animals per each group). ##P<0.01 compared with response to vehicle. V+V: vehicle of drugs+vehicle of (*S*)-3,5-DHPG. *P<0.05, **P<0.01 compared with response to (*S*)-3,5-DHPG alone.

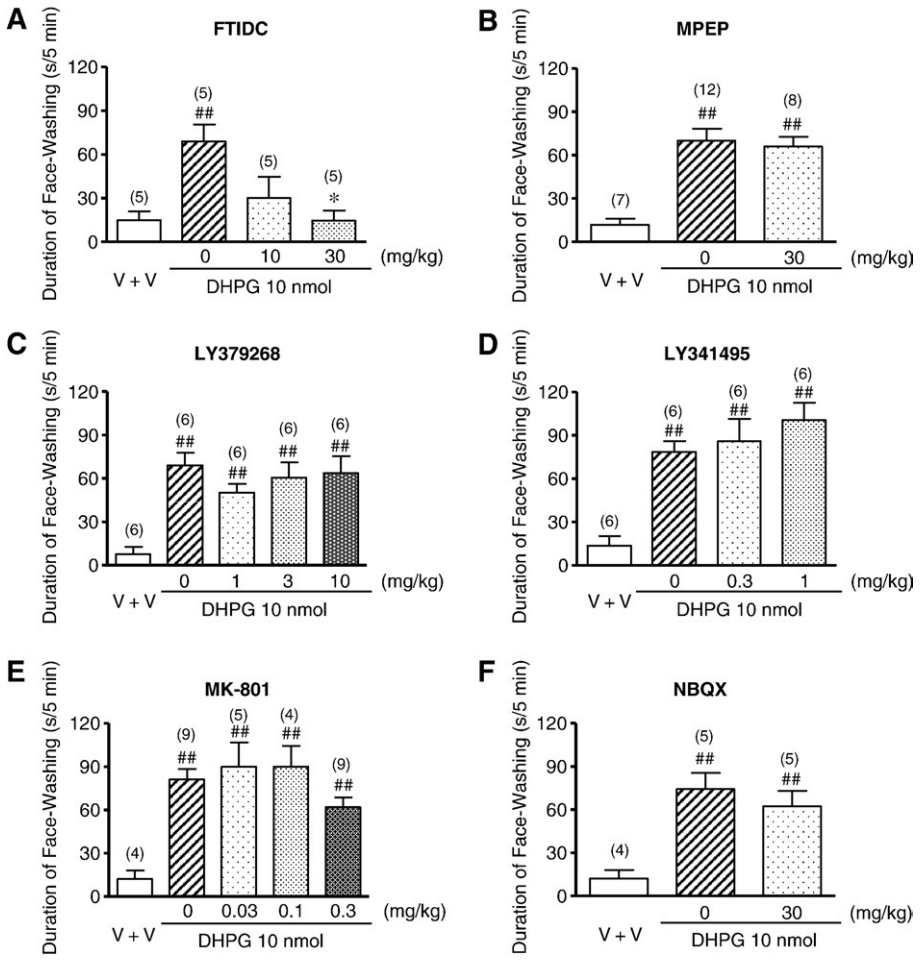

Fig. 3. Effects of FTIDC (A), MPEP (B), LY379268 (C), LY341495 (D), MK-801 (E) and NBQX (F) on face-washing behavior produced by (*S*)-3,5-DHPG in mice. Drug effects on face-washing behavior were tested 5 min after intracerebroventricular administration of (*S*)-3,5-DHPG for 5 min. FTIDC, MPEP and LY379268 or LY341495 were intraperitoneally or subcutaneously given 30 min prior to administration of (*S*)-3,5-DHPG. MK-801 and NBQX were subcutaneously gives 15 min prior to administration (*S*)-3,5-DHPG. Numbers in parenthesis indicated numbers of animal used (A, $n=5$ animals per each group; B, $n=7$–12 animals per each group; C, $n=6$ animals per each group; D, $n=6$ animals per each group; E, $n=4$–9 animals per each group; F, $n=4$–5 animals per each group). V + V: vehicle of drugs + vehicle of (*S*)-3,5-DHPG. $^{\#\#}P<0.01$ compared with response to vehicle. $^{*}P<0.05$ compared with response to (*S*)-3,5-DHPG alone.

LY341495, the NMDA receptor antagonist MK-801 and the competitive non-NMDA receptor antagonist NBQX did not affect the duration of face-washing behavior of (*S*)-3,5-DHPG (Fig. 3B, C, D, E, F). No compound caused face-washing behavior or sedation except that 0.3 mg/kg of MK-801 caused increases in locomotor activity.

4. Discussion

We have demonstrated here, with use of both competitive and non-competitive $mGlu_1$ receptor antagonists, that the face-washing behavior elicited by the non-selective $mGlu_{1/5}$ receptor agonist (*S*)-3,5-DHPG is mediated by activation of $mGlu_1$ receptor but not $mGlu_5$ receptor.

(*RS*)-3,5-DHPG has been shown to be a highly specific agonist for both $mGlu_1$ receptor and $mGlu_5$ receptor *in vitro*, with similar affinities for the two receptors (Schoepp et al., 1999; Brabet et al., 1995). (*S*)-3,5-DHPG, the (*S*) enantiomer of (*RS*)-3,5-DHPG, was about 10 times more potent than (*R*)-3,5-dihydroxyphenylglycine (*R*-3,5-DHPG) in activating phosphoinositide hydrolysis (Baker et al., 1995). We observed that intracerebroventricular administration of (*S*)-3,5-DHPG at 3-30 nmol caused significant face-washing behavior similar to the scratching behavior including facial grooming observed with (*S*)-3,5-DHPG or (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) in mice (Barton and Shannon, 2005; Thomsen and Dalby, 1998; Laudrup and Klitgaard, 1993). When doses of (*S*)-3,5-DHPG were increased to 100–400 nmol, clonic convulsions were produced in dose-dependent fashion in mice. These findings are consistent with the previous reports that (*RS*)-3,5-DHPG at doses of and above 100 nmol elicited seizures (Tizzano et al., 1995). Face-washing behavior were still observed with (*S*)-3,5-DHPG at a dose of 100 nmol. Although the etiology of face-washing behavior is unclear, intermittent convulsive behavior was observed after the disappearance of face-washing behavior at doses of and above 100 nmol, and maximum face-washing behavior was observed at a dose of 10 nmol at which no convulsive behavior was observed at all. Thus, the face-washing behavior observed in the present study did not appear to be related to convulsive behavior. These findings indicate that (*S*)-3,5-DHPG at lower doses produced scratching and at higher doses caused convulsions characterized by clonus, rearing behavior, and falling (Barton and Shannon, 2005). Since (*S*)-3,5-DHPG is a potent non-selective $mGlu_{1/5}$ receptor agonist, it is important to explore which subtypes of receptors are involved in these behaviors. We focused on characterizing face-washing behavior, since it is the first characteristic behavioral change elicited by (*S*)-3,5-DHPG and only one study has been performed to clarify the roles of $mGlu_1$ receptor and $mGlu_5$ receptor in this behavior (Barton and Shannon, 2005). On the other hand, several studies have demonstrated that the convulsive behavior caused by (*RS*)-3,5-DHPG and (*S*)-3,5-DHPG is mediated by activation of $mGlu_1$ receptor but not $mGlu_5$ receptor (Barton and Shannon, 2005; Kingston et al., 2002).

The face-washing behavior induced by (*S*)-3,5-DHPG was blocked by intracerebroventricular co-administration of the competitive $mGlu_1$ receptor antagonists AIDA and (*S*)-4CPG but not by the $mGlu_5$ receptor antagonist MPEP. AIDA at a dose of 100 nmol decreased the number of crossings in the open field test in rats (Nadlewska et al., 2003). On the other hand, another competitive $mGlu_1$ receptor antagonist, (*S*)-4CPG at doses of 25, 50 and 100 nmol did not change locomotor activity in rats (Car et al., 2001). These results suggested that attenuation of (*S*)-3,5-DHPG-induced face-washing behavior by blockade of $mGlu_1$ receptor was not due to simple reduction of motor activity. These findings indicated that the face-washing behavior was due to activation of $mGlu_1$ receptor but not $mGlu_5$ receptor by (*S*)-3,5-DHPG. We have recently identified a systemically deliverable, brain-penetrating non-competitive $mGlu_1$ receptor antagonist, FTIDC (Suzuki et al., 2007). We therefore evaluated effects of systemic administration of FTIDC as well as MPEP on the face-washing behavior elicited by (*S*)-3,5-DHPG in mice. Intraperitoneal administration of FTIDC inhibited face-washing behavior in dose-dependent fashion, while intraperitoneal administration of MPEP did not affect the duration of this behavior. FTIDC at doses of 1 to 30 mg/kg did not significantly decrease locomotor activity in mice (Suzuki et al., 2007).

Moldrich et al. (2001) reported that the seizures caused by the $mGlu_{1/5}$ receptor agonist (*RS*)-3,5-DHPG in DBA/2J mice were reduced by the $mGlu_{2/3}$ receptor agonist LY379268. Tizzano et al. (1995) have reported that the non-selective group III mGlu receptor agonists L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) and L-serine-*O*-phosphate (L-SOP) protected against the seizures induced by (*RS*)-3,5-DHPG in ICR mice. These findings suggest that the suppression of synaptic transmission by activation of group II and group III mGlu receptors, presumably located in the presynaptic membrane, is able to modulate (*RS*)-3,5-DHPG-induced seizures. Since little is known concerning the involvement of such presynaptic modulation of endogenous glutamate release on (*S*)-3,5-DHPG-induced face-washing behavior, we tested the effects of the $mGlu_{2/3}$ receptor agonist LY379268 as well as the $mGlu_{2/3}$ receptor antagonist LY341495 on the face-washing behavior elicited by (*S*)-3,5-DHPG. Neither LY379268 nor LY341495 affected the face-washing behavior caused by (*S*)-3,5-DHPG. These findings indicated that presynaptic modulation of glutaminergic tone by $mGlu_{2/3}$ receptor did not affect the face-washing behavior elicited by (*S*)-3,5-DHPG. Since there are no selective group III mGlu receptor ligands available yet, we could not rule out the possibility that presynaptic modulation of glutaminergic tone by group III mGlu receptors is able to alter the face-washing behavior elicited by (*S*)-3,5-DHPG.

We also tested the effects of the ionotropic receptor antagonist such as the NMDA receptor antagonist, MK-801 and the competitive non-NMDA receptor antagonist, NBQX on the face-washing behavior elicited by (*S*)-3,5-DHPG. MK-801 at a dose of 0.3 mg/kg increased locomotor activity, while no behavioral change was observed with NBQX at a dose of 30 mg/kg. A dose of 30 mg/kg NBQX protected lethality caused by AMPA to 50% and the effect of NBQX reached plateau (Wienrich et al., 2001). Thus MK-801 and NBQX were sufficient enough to cause pharmacological blockade of NMDA and non-NMDA receptors. Neither MK-801 nor NBQX affected the face-washing behavior caused by (*S*)-3,5-DHPG. The non-competitive non-NMDA receptor antagonist, GYKI 52466 suppressed the scratching syndrome, but only at doses that produced motor impairment, while NBQX and MK-801 did not, even at doses that produced motor impairment (Barton and Shannon, 2005). Thus it was unclear if this antagonism of GYKI 52466 on the scratching syndrome was due to mechanism based or due to motor impairment. Taken together these results, it was less likely that either NMDA or non-NMDA receptors played a critical role on face-washing behavior caused by (*S*)-3,5-DHPG.

In summary, the present study demonstrated that (*S*)-3,5-DHPG at low doses induced face-washing behavior and at high doses caused convulsions in mice, and that face-washing behavior was abolished by treatment with both competitive and non-competitive $mGlu_1$ receptor antagonists but not $mGlu_5$ receptor antagonist, $mGlu_{2/3}$ receptor agonist, $mGlu_{2/3}$ receptor antagonist, NMDA receptor antagonist or competitive non-NMDA receptor antagonist. These findings suggest that face-washing behavior is caused by activation of $mGlu_1$ receptor by (*S*)-3,5-DHPG, and that face-washing behavior is a behavioral outcome useful for evaluation of the *in vivo* potency of both competitive and non-competitive $mGlu_1$ receptor antagonists.

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