Full Paper

Correlation of Receptor Occupancy of Metabotropic Glutamate Receptor Subtype 1 (mGluR1) in Mouse Brain With In Vivo Activity of Allosteric mGluR1 Antagonists

Gentaroh Suzuki^{1,2,*}, Hiroko Kawagoe-Takaki¹, Takao Inoue¹, Toshifumi Kimura¹, Hirohiko Hikichi¹, Takashi Murai¹, Akio Satow¹, Mikiko Hata¹, Shunsuke Maehara¹, Satoru Ito¹, Hiroshi Kawamoto¹, Satoshi Ozaki¹, and Hisashi Ohta¹

¹Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba, Ibaraki 300-2611, Japan

²Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan

Received January 14, 2009; Accepted May 13, 2009

Abstract. The aim of this study was to clarify the relationship between receptor occupancy and in vivo pharmacological activity of mGluR1 antagonists. The tritiated mGluR1-selective allosteric antagonist [³H]FTIDC (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) was identified as a radioligand having high affinity for mGluR1-expressing CHO cells ($K_D = 2.1$ nM) and mouse cerebellum ($K_D = 3.7$ nM). [³H]FTIDC bound to mGluR1 was displaced by structurally unrelated allosteric antagonists, suggesting there is a mutual binding pocket shared with different allosteric antagonists. The binding specificity of [³H]FTIDC for mGluR1 in brain sections was demonstrated by the lack of significant binding to brain sections prepared from mGluR1-knockout mice. Ex vivo receptor occupancy with [³H]FTIDC revealed that the receptor occupancy level by FTIDC correlated well with FTIDC dosage and plasma concentration. Intracerebroventricular administration of (*S*)-3,5-dihydroxyphenylglycine is known to elicit face washing behavior that is mainly mediated by mGluR1. Inhibition of this behavioral change by FTIDC correlated with the receptor occupancy level of mGluR1 in the brain. A linear relationship between the receptor occupancy and in vivo activity was also demonstrated using structurally diverse mGluR1 antagonists. The receptor occupancy assays could help provide guidelines for selecting appropriate doses of allosteric mGluR1 antagonist for examining the function of mGluR1 in vivo.

Keywords: metabotropic glutamate receptor, receptor occupancy, radioligand binding, allosteric antagonist, central nervous system

Introduction

Metabotropic glutamate receptors (mGluRs) belong to a family of G-protein-coupled receptors that are activated by L-glutamate. Eight subtypes of the receptor (mGluR1–mGluR8) have been cloned to date and are classified into groups I, II, and III. mGluR1 and mGluR5 are classified into group I, and they are coupled to phospholipase C via Gq protein and subsequently

mobilize intracellular calcium (1).

Application of functional assays such as Ca²⁺ mobilization has led to the identification of allosteric antagonists that interact with sites different from the L-glutamate binding site of mGluR1. These allosteric mGluR1 antagonists exhibit high selectivity to mGluR1, in contrast to orthosteric antagonists, which lack sufficient selectivity between mGluR1 and mGluR5 (2–9). An increasing body of animal studies using these mGluR1-selective allosteric antagonists indicate that blockade of mGluR1 could ameliorate CNS disorders such as neuropathic pain (10–12), stroke (13, 14), epilepsy (15, 16), and psychiatric diseases (17–20). On the

*Corresponding author (affiliation #1). gentaroh_suzuki@merck.com
Published online in J-STAGE on June 19, 2009 (in advance)
doi: 10.1254/jphs.09011FP

other hand, behavioral studies using mGluR1 knockout mice or some mGluR1 antagonists suggest that inhibition of mGluR1 might elicit adverse effects such as deficits in motor functions (9, 18, 21, 22). Therefore, it is important to determine the level of receptor occupancy of mGluR1 necessary to cause these beneficial and adverse effects and whether they are indeed due to the blockade of mGluR1. A representative mGluR1-specific allosteric radioligand, [^3H]1-(3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-2-phenyl-1-ethanone (R214127) was used to measure receptor occupancy of mGluR1 in rat brain (7, 23). These studies indicated that ex vivo receptor occupancy assays using [^3H]R214127 are useful for evaluating brain penetration of systemically administered allosteric mGluR1 antagonists. However, there have been no reports of an apparent relationship between in vivo pharmacological effects with these compounds and receptor occupancy under the same experimental conditions.

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) is a recently identified allosteric mGluR1 antagonist with a novel chemical structure (24). The compound has high selectivity and equal inhibitory activity toward recombinant human, rat, and mouse mGluR1. We recently reported that central administration of (*S*)-3,5-dihydroxyphenylglycine [(*S*)-3,5-DHPG, a mGluR1/mGluR5 dual agonist] causes face washing behavior and that this behavior is mainly mediated by the activation of mGluR1 (25). Systemic administration of FTIDC inhibits the behavioral change. These findings indicate that FTIDC is a novel and excellent allosteric mGluR1 antagonist with respect to antagonist potency, selectivity, species difference, and brain penetrability (24). Therefore, radiolabelled FTIDC could be useful for elucidating the function of mGluR1.

The aim of the present study was to clarify the relationship between receptor occupancy and in vivo antagonistic activity of mGluR1 antagonists using [^3H]FTIDC. Tritiated FTIDC was pharmacologically characterized using membranes prepared from recombinant mGluR1-expressing cells and from mouse brain. [^3H]FTIDC exhibited high affinity and high selectivity toward mGluR1. An ex vivo receptor occupancy assay using [^3H]FTIDC in mouse brain revealed a clear correlation between mGluR1 occupancy and the dose or plasma concentration of the peripherally administered mGluR1 antagonist. The current study demonstrates that inhibition of (*S*)-3,5-DHPG-induced face-washing behavior by allosteric mGluR1 antagonists is proportional to receptor occupancy level in the brain, confirming this inhibition is due to blockade of mGluR1 in the brain. In addition, these studies with [^3H]FTIDC clarify

Fig. 1. Chemical structure of [^3H]FTIDC.

the receptor occupancy level necessary for the behavioral change elicited by allosteric mGluR1 antagonists.

Materials and Methods

Reagents and chemicals

FTIDC and [^3H]FTIDC (85 Ci/mmol) were synthesized in-house (Fig. 1). L-Glutamate was purchased from Sigma-Aldrich (St. Louis, MO, USA). (+)-2-Methyl-4-carboxyphenylglycine (LY367385), (*S*)-3,5-DHPG, L-quisqualate, and 2-methyl-6-(phenylethynyl)pyridine (MPEP) were purchased from Tocris Bioscience (Bristol, UK). 2-[4-(Indan-2-ylamino)-5,6,7,8-tetrahydroquinazolin-2-ylsulfanyl]-ethanol, HCl (LY456066), R214127, 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo [3,2-*a*]benzimidazole-2-carboxamide (YM-298198), (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM), 6-[1-(2-fluoro-3-pyridinyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-quinoline (Compound A), 1,4-piperazinedicarboxylic acid, 1-(2,2-dimethylpropyl) 4-(1-methylethyl) ester (Compound B), and 3-cyclohexyl-7-cyanopropoxy-4*H*-pyrano[2,3-*b*]pyridin-4-one (Compound C) were synthesized in-house. L-proline was purchased from Wako Pure Chemical Industries (Osaka). Dialyzed fetal bovine serum, culture media, and other reagents used for cell culture were purchased from Invitrogen (Carlsbad, CA, USA). All other reagents used were of molecular or analytical grade.

Animals

Male CD-1 (ICR) mice (5–8-week-old) were obtained from Japan SLC (Shizuoka). mGluR1 knockout mice were generated by Deltagen (San Carlos, CA, USA). In brief, the *Grm1* gene was inactivated by homologous recombination in ES cells (derived from the 129/OlaHsd mouse substrain) by deletion of nucleotides 433 to 471 of the coding sequence of the *Grm1* gene, followed by introduction of a LacZ-Neo cassette. F1 mice were generated and were repeatedly backcrossed with C57BL/6 females to produce heterozygous offspring (N5). These mice were introduced from Deltagen (identification number, T801)

and additionally backcrossed twice to a C57BL/6N background (N7) in house. Homozygous knockout and wild-type littermates used in the present study were generated by intercrosses of N7 mice.

All mice were group-housed (5–6 mice per cage) in a controlled animal room (room temperature: $23 \pm 2^\circ\text{C}$, humidity: $55 \pm 15\%$) on a 12-h light/dark cycle (lights on between 7:00 AM and 7:00 PM), with food and water available ad libitum. Experiments were carried out between 9:00 AM and 5:00 PM. All animal studies were approved by Banyu Institutional Animal Care and Use Committee.

Stable cell lines

CHO (Chinese hamster ovary)–dhfr (dihydrofolate reductase)[−] cells stably expressing human mGluR1a (CHO-hmGluR1a) were obtained as described previously (26). The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% dialyzed fetal bovine serum, 100 units/ml penicillin, 100 units/ml streptomycin, and 1% proline at 37°C with 5% CO_2 in a humidified atmosphere.

Intracellular Ca^{2+} mobilization

Functional assays based on intracellular Ca^{2+} mobilization were conducted according to previously described methods (24). In brief, CHO-hmGluR1a was seeded at 5×10^4 cells/well in a 96-well plate and cultured overnight. The cells were then incubated with $4 \mu\text{M}$ Fluo-3 for 1 h, and then the extracellular dye was removed by washing the cells. Ca^{2+} flux was measured using a fluorometric imaging plate reader (FLIPR; Molecular Devices, Sunnyvale, CA, USA). Test compounds were applied 5 min before the application of L-glutamate. The final concentration of L-glutamate was $10 \mu\text{M}$.

Membrane preparation

CHO-hmGluR1a was cultured in the medium described above. Confluent cells were washed with ice-cold phosphate-buffered saline and stored at -80°C until membrane preparation. After thawing, the cells were suspended in 3 volumes of ice-cold buffer A (10 mM MOPS, 154 mM NaCl, 10 mM KCl, 0.8 mM CaCl_2 , pH 7.4) containing 20% sucrose and homogenized using a Polytron homogenizer (Kinematica, Basel, Switzerland). The homogenate was centrifuged at $10,000 \times g$ for 20 min at 4°C . The supernatant was collected and centrifuged at $100,000 \times g$ for 60 min at 4°C . The resultant pellet was suspended in buffer B (20 mM HEPES, pH 7.4 and 0.1 mM EDTA) supplemented with protease inhibitor cocktail (Complete EDTA-free; Roche Diagnostics, Mannheim, Germany)

and centrifuged at $100,000 \times g$ for 60 min at 4°C . The pellet was resuspended in the same buffer and stored in aliquots at -80°C until use. Protein content was measured by the BCA method (Sigma-Aldrich) with bovine serum albumin as a standard.

Brains were removed from male CD-1 mice. The cerebellum was dissected on ice and homogenized in ice-cold 20 mM HEPES, pH 7.4 containing 10 mM EDTA using a Polytron homogenizer. The homogenate was centrifuged at $1,000 \times g$ for 15 min at 4°C and then the supernatant was centrifuged at $40,000 \times g$ for 15 min at 4°C . The resultant pellet was suspended in ice-cold 50 mM Tris, pH 7.4 supplemented with protease inhibitor cocktail and centrifuged at $40,000 \times g$ for 15 min at 4°C . The pellet was resuspended in the same buffer and stored in aliquots at -80°C until use.

[^3H]FTIDC binding to membrane preparations

The membranes prepared from CHO-hmGluR1a and mouse cerebellum were incubated with 1 nM [^3H]FTIDC in 0.2 ml of 50 mM Tris containing 1.2 mM MgCl_2 and 2 mM CaCl_2 (pH 7.4) at room temperature for 2 h in the presence or absence of different concentrations of the test compounds. Bound and free radioligand were separated by rapid filtration using UniFilter-96 GF/C filter plates (PerkinElmer Life and Analytical Sciences, Boston, MA, USA) presoaked in 0.5% polyethyleneimine, and a Filtermate 196 harvester (PerkinElmer Life and Analytical Sciences). Filter-bound radioactivity was counted using TopCount after the addition of microscint-0 (PerkinElmer Life and Analytical Sciences). Nonspecific binding was defined as binding occurring in the presence of $10 \mu\text{M}$ LY456066, which was comparable to that measured using $10 \mu\text{M}$ unlabelled FTIDC. In saturation binding experiments, membranes from CHO-hmGluR1a or mouse cerebellum were incubated with various concentrations (0.078–10 nM for CHO-hmGluR1a and 0.31–20 nM for cerebellum) of [^3H]FTIDC for 2 h.

Ex vivo receptor occupancy with [^3H]FTIDC

Mice received an intraperitoneal injection of vehicle (0.5% methylcellulose); FTIDC (1–100 mg/kg); EMQMCM (1–10 mg/kg); Compounds A, B, and C (10–30 mg/kg). Blood samples were collected into plastic tubes containing heparin by cardiac puncture under ketamine/xylazine anesthesia 30 min after administration of the compounds. The brains were rapidly removed from the skulls and were immediately frozen in dry ice-cooled 2-methylbutane (-40°C) for determination of receptor occupancy and then stored at -80°C until sectioning. Twenty- μm -thick sections were cut from the frozen brains using a cryostat microtome

(Microm HM 500 O; Microm, Walldorf, Germany) and thaw-mounted on SuperFrost Plus microscope slides (Thermo Fisher Scientific, Waltham, MA, USA). The sections were stored at -20°C until use.

Brain sections were thawed and incubated with 4 nM [^3H]FTIDC in 50 mM Tris containing 1.2 mM MgCl_2 , 2 mM CaCl_2 (pH 7.4), and 0.1% BSA for 3 min at room temperature. Incubation was limited to 3 min to minimize dissociation of the administered drug from the receptor. After incubation, the sections were washed (3×4 min) with ice-cold 50 mM Tris containing 1.2 mM MgCl_2 and 2 mM CaCl_2 (pH 7.4). The sections were then dried under a stream of air and radioligand binding was evaluated using a β -imager 2000 (BioSpace, Paris, France). The levels of bound radioactivity in the brain areas were directly determined by counting the number of β -particles emerging from the delineated area (region of interest) by using the Beta vision + program (version 2.0, BioSpace). Consequently, the radioligand-binding signal was expressed in counts per minute per square millimeter.

Quantitative autoradiography of brain sections from wild-type and mGluR1 knockout mice using [^3H]FTIDC was performed under the same experimental conditions as the ex vivo receptor occupancy assay. In order to obtain higher resolution images, the sections were exposed to imaging plates (BAS-TR2025; Fuji Photo Film, Tokyo). Imaging plates were scanned using a BAS-5000 Bio-Imaging Analyzer (Fuji Photo Film).

Total binding of [^3H]FTIDC in each mouse was obtained by calculating the mean value of radioactivity from six brain sections. Nonspecific binding was defined as the radioactivity measured in the presence of 10 μM unlabelled FTIDC and was obtained by calculating the mean value of nine brain sections from three mice treated with vehicle (three brain sections/mouse). Specific binding of [^3H]FTIDC in each mouse was calculated by subtracting the nonspecific binding from the total binding. Specific binding of [^3H]FTIDC in drug-treated mice was expressed as the percentage of specific binding in vehicle-treated animals. Receptor occupancy by the drug was expressed as 100% minus the percentage of specific binding in the treated animals.

Compound concentrations in blood samples were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Compounds in plasma samples were extracted with ethanol containing the internal standard. Quantitative analyses were performed by the relative calibration curve method using both an authentic standard and an internal standard.

(S)-3,5-DHPG-induced face washing behavior in mice

(S)-3,5-DHPG-induced face washing behavior studies

in mice were performed according to the previously described method (25). In brief, (S)-3,5-DHPG (10 nmol) was dissolved in 0.9% NaCl, and 10 μl was intracerebroventricularly administered using a Hamilton syringe. Test compounds suspended in 0.5% methylcellulose solution were given intraperitoneally 30 min prior to administration of (S)-3,5-DHPG. Animals were placed in an acrylic box ($10 \times 15 \times 13$ cm) immediately after the administration of (S)-3,5-DHPG, and their behavior was monitored between 5 and 10 min after the (S)-3,5-DHPG injection. (S)-3,5-DHPG caused characteristic behaviors including face-washing behavior. 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 the previous report (27). Duration of face-washing behavior was recorded by the time-recorder with keypads (Neuroscience, Tokyo). Effects of various drugs on face-washing behavior were recorded 5 min after administration of (S)-3,5-DHPG for 5 min. The numbers of animals tested in each group (n) were 5 – 11, 6, 6, 10, and 5 – 10 for evaluations of FTIDC, Compound A, Compound B, Compound C, and EMQMCM, respectively.

Percent inhibition of (S)-3,5-DHPG-induced face-washing time was defined as follows:

$$\text{Percent inhibition (\%)} = 100 - \{[(A - B) / (C - B)] \times 100\}$$

, where A is the face-washing time in mice treated with both (S)-3,5-DHPG and a test compound, B is the mean face-washing time in each mouse treated with vehicle alone, and C is the mean face-washing time in mice treated with (S)-3,5-DHPG alone.

Data analyses and statistics

Unless stated otherwise, all data analyses were performed by using Prism (version 4.03) from GraphPad Software (La Jolla, CA, USA). In saturation binding and competition binding experiments using membranes prepared from CHO-hmGluR1a, the dissociation constant (K_D) and the maximal number of binding sites (B_{max}) for [^3H]FTIDC and affinities (K_i) for the other compounds were determined by fitting to a saturation binding model and competition binding models that take into account ligand depletion (28). K_D and B_{max} values of [^3H]FTIDC binding to membranes prepared from mouse cerebellum were determined by nonlinear regression and fitting to a one-site binding model.

Each percentage of receptor occupancy was plotted against the dose or plasma concentration of the compound and analyzed by nonlinear regression. Williams' test was performed with SAS (Preclinical Package version 5.0; SAS Institute, Tokyo) to analyze

Fig. 2. Saturation binding experiments with $[^3\text{H}]\text{FTIDC}$ to membranes prepared from CHO-hmGluR1a (A) and mouse cerebellum (B). Membranes were incubated with various concentrations of $[^3\text{H}]\text{FTIDC}$ for 2 h. Non-specific binding was defined as binding occurring in the presence of $10 \mu\text{M}$ LY456066. Values for total binding (closed squares), specific binding (open squares), and nonspecific binding (closed triangles) were expressed in dpm. Data values are each the mean $\pm$ S.D. (A) or mean (B) of the representative experiment in three independent experiments performed in triplicate (A) or in duplicate (B). Saturation analysis of $[^3\text{H}]\text{FTIDC}$ provided a K_D value of 2.1 ± 0.40 nM and a B_{max} of 26 ± 2.2 pmol/mg (mean $\pm$ S.E.M., $n = 3$) for membranes prepared from CHO-hmGluR1a. K_D and B_{max} values for mouse cerebellar membranes were 3.7 ± 0.26 nM and 0.99 ± 0.11 pmol/mg protein (mean $\pm$ S.E.M., $n = 3$), respectively.

test compound dose-dependent inhibition of (*S*)-3,5-DHPG-induced face-washing behavior. A probability level of <0.05 was considered statistically significant. The relationship between receptor occupancy level and degree of inhibition of (*S*)-3,5-DHPG-induced face washing behavior was analyzed by linear regression.

Results

$[^3\text{H}]\text{FTIDC}$ binding to recombinant and native mGluR1 using membrane preparations

Saturation studies of $[^3\text{H}]\text{FTIDC}$ binding were performed at the apparent binding equilibrium (2-h incubation, data not shown) using membranes prepared from CHO-hmGluR1a or mouse cerebellum. In CHO-hmGluR1a, $[^3\text{H}]\text{FTIDC}$ binding to recombinant mGluR1a was saturable and of high affinity (Fig. 2A), with a K_D value of 2.1 ± 0.40 nM and a B_{max} value of 26 ± 2.2 pmol/mg protein (mean $\pm$ S.E.M., $n = 3$). Saturation binding curves using mouse cerebellar membranes indicated a K_D value of 3.7 ± 0.26 nM and a B_{max} value of 0.99 ± 0.11 pmol/mg protein (mean $\pm$ S.E.M., $n = 3$) (Fig. 2B).

Representative mGluR1 ligands were tested for inhibition of $[^3\text{H}]\text{FTIDC}$ binding to membranes prepared from CHO-hmGluR1a (Fig. 3). FTIDC itself displaced $[^3\text{H}]\text{FTIDC}$ bound to hmGluR1a with a K_i value of

Fig. 3. Displacement by group I mGluR ligands of $[^3\text{H}]\text{FTIDC}$ bound to membranes prepared from CHO-hmGluR1a. Results are expressed as a percentage of the total binding of $[^3\text{H}]\text{FTIDC}$ (1 nM) and each value is the mean $\pm$ S.E.M. from more than three individual experiments performed in duplicate. The total binding of $[^3\text{H}]\text{FTIDC}$ was 7000 ± 700 dpm (mean $\pm$ S.E.M., $n = 4$) in the presence of 1% dimethyl sulfoxide (DMSO).

2.0 ± 0.34 and a Hill coefficient of 0.91 ± 0.050 (mean $\pm$ S.E.M., $n = 4$). In contrast, not even 1 mM of the orthosteric amino acid-like ligands, LY367385 and L-quisqualate, could displace $[^3\text{H}]\text{FTIDC}$. Other structurally unrelated allosteric mGluR1 antagonists (LY456066, R214127, and YM-298198) displaced bound $[^3\text{H}]\text{FTIDC}$ in a concentration-dependent manner, but an mGluR5 antagonist MPEP did not. In addition, $[^3\text{H}]\text{FTIDC}$ binding was also inhibited by Compound A, a novel allosteric mGluR1 antagonist. K_i values and Hill coefficients of LY456066, R214127, YM-298198, and Compound A are summarized, together with their functional activities, in Table 1.

$[^3\text{H}]\text{FTIDC}$ binding to mGluR1 in mouse brain sections

The binding of $[^3\text{H}]\text{FTIDC}$ to native mGluR1 was examined using brain sections prepared from wild-type and mGluR1-knockout mice. Strong $[^3\text{H}]\text{FTIDC}$ binding was found in the cerebellum from wild-type mice, with the highest binding occurring in molecular layers of the cerebellum (Fig. 4A). In forebrain sections at the striatal level, a high degree of $[^3\text{H}]\text{FTIDC}$ binding was observed in the dorsolateral septal nucleus. The striatum showed lower but significant $[^3\text{H}]\text{FTIDC}$ binding (Fig. 4B). Unlabelled $10 \mu\text{M}$ FTIDC displaced $[^3\text{H}]\text{FTIDC}$ bound in the cerebellar and the striatal sections, as shown in Fig. 4, C and D, respectively. No significant $[^3\text{H}]\text{FTIDC}$ binding was observed in either the cerebellum or the striatum from mGluR1 knockout mice (Fig. 4: E and F). Quantification analyses revealed that $[^3\text{H}]\text{FTIDC}$ binding in the striatal region of wild-type mice was $37 \pm 1.0\%$ (mean $\pm$ S.E.M., $n = 8$) of that in the cerebellum (Fig. 4G).

Table 1. Potencies of various mGluR1 antagonists in inhibiting [3 H]FTIDC binding, and functional (intracellular Ca^{2+} mobilization) assay in CHO-hmGluR1a

Compound	Structure	Binding K_i (nM)	Hill coefficient	Functional IC_{50} (nM) [#]
Compound A		14 ± 1.8	1.0 ± 0.022	3.6 ± 0.49
LY456066		21 ± 2.9	1.0 ± 0.11	15 ± 1.6
R214127		29 ± 2.8	1.2 ± 0.097	26 ± 7.1
YM-298198		120 ± 23	1.0 ± 0.039	110 ± 31

[#]Except for Compound A, IC_{50} values for the functional assay (Ca^{2+} mobilization) are taken from a previous study (24). Data are expressed as means $\pm$ S.E.M. from more than three individual experiments.

Fig. 4. [3 H]FTIDC binding to brain sections from mGluR1 wild-type and knockout mice. Coronal brain sections at the cerebellar (A, C, and E) and the striatal (B, D, and F) levels were prepared from wild type (A–D) and mGluR1 knockout (E and F) mice. The sections were incubated with 4 nM [3 H]FTIDC in the absence (A, B, E, and F) or presence (C and D) of unlabelled 10 μM FTIDC. The autoradiograms were acquired using a BAS-5000. Radioactivities were quantified using a β -imager and are shown as % of radioactivity in the cerebellum from wild-type mice (G). Data in G are indicated as the mean $\pm$ S.E.M. ($n = 8/\text{group}$). Note that no significant [3 H]FTIDC binding was observed in brain sections from mGluR1-knockout mice (E and F).

Ex vivo receptor occupancy for mGluR1

Ex vivo receptor occupancy assays for mGluR1 were conducted in order to examine the relationship between

receptor occupancy level in the brain and either the dose of systemically administered allosteric mGluR1 antagonist or its plasma concentration. [3 H]FTIDC binding was dose-dependently inhibited in cerebellar and striatal sections prepared from mice that had received FTIDC systemically. Receptor occupancy of mGluR1 was detectable at more than 3 mg/kg, and maximal occupancy was obtained at 100 mg/kg. ED_{50} values (dose of the compound required to occupy 50% of mGluR1) were 25 mg/kg (19–32, 95% confidence limits) and 13 mg/kg (11–15, 95% confidence limits) in the cerebellum and the striatum, respectively (Fig. 5A). The plasma concentrations of FTIDC were determined in the same mice. As shown in Fig. 5B, the receptor occupancy level by FTIDC was clearly correlated with the plasma concentration of the compound. Plasma EC_{50} values (plasma concentration of the compound required to occupy 50% of mGluR1) in the cerebellum and the striatum were 430 nM (290–640, 95% confidence limits) and 150 nM (110–200, 95% confidence limits), respectively.

Correlation between receptor occupancy and in vivo activities by allosteric mGluR1 antagonists

We examined the relationship between the level of mGluR1-receptor occupancy by allosteric mGluR1 antagonists and the degree of inhibition in face-washing

Fig. 5. Receptor occupancy of mGluR1 in mouse brain after intraperitoneal dosing with FTIDC. Brain sections were cut at the level of the cerebellum and the striatum from mice treated with FTIDC. The sections were incubated with 4 nM [3 H]FTIDC for 3 min at room temperature. Radioligand binding was evaluated using a β -imager. The percentage of receptor occupancy was determined by designating [3 H]FTIDC binding in the control (vehicle-treated) animals in the absence and presence of 10 μ M unlabelled FTIDC as 0% and 100%, respectively. The dose–receptor occupancy curve (A) and the plasma concentration–receptor occupancy curve (B) were generated by means of log doses and log plasma concentrations of FTIDC, respectively. Data are expressed as individual values and mean curves.

Table 2. Inhibition of (*S*)-3,5-DHPG–induced face washing behavior by FTIDC

(<i>S</i>)-3,5-DHPG (nmol/mouse)	FTIDC (mg/kg)	Face-washing time (s)	Inhibition (%)
Vehicle	Vehicle	12 $\pm$ 4.1	
10	Vehicle	78 $\pm$ 8.8	0 $\pm$ 13
10	1.0	88 $\pm$ 11	–15 $\pm$ 16
10	3.0	48 $\pm$ 14*	45 $\pm$ 21*
10	10	32 $\pm$ 9.2***	69 $\pm$ 14***
10	30	15 $\pm$ 6.7***	96 $\pm$ 10***

* $P < 0.05$ and *** $P < 0.001$ vs. animals treated with (*S*)-3,5-DHPG alone. Data are expressed as means $\pm$ S.E.M. ($n = 5 - 11$).

behavior elicited by the group I mGluR agonist (*S*)-3,5-DHPG. FTIDC inhibited (*S*)-3,5-DHPG–induced face washing behavior in a dose-dependent manner (Table 2). The percent inhibition of (*S*)-3,5-DHPG–induced face washing behavior by FTIDC was plotted against the receptor occupancy level at the corresponding dose of FTIDC (Fig. 6A). There was good linear correlation between the receptor occupancy level and the degree of inhibition of (*S*)-3,5-DHPG–induced face washing behavior by FTIDC ($r^2 = 0.83$ and 0.88 in the cerebellum and the striatum, respectively). The receptor occupancy levels of FTIDC required to achieve 50% inhibition of (*S*)-3,5-DHPG–induced face washing behavior were calculated to be 24% and 35% in the cerebellum and striatum, respectively.

Fig. 6. Relationship between the degree of receptor occupancy on mGluR1 and inhibition of (*S*)-3,5-DHPG–induced face washing behavior by allosteric mGluR1 antagonists. The percent inhibition of (*S*)-3,5-DHPG–induced face washing behavior was plotted against the receptor occupancy level in the cerebellum (closed triangles) and the striatum (inverted open triangles) at the corresponding dose of the allosteric mGluR1 antagonists, FTIDC (A) and Compound A (B).

Table 3. Dose, plasma concentration, and receptor occupancy level of Compound A

Dose (mg/kg)	Plasma concentration (μ M)	Receptor occupancy (%)	
		cerebellum	striatum
0	—	0 $\pm$ 11	0 $\pm$ 3.3
10	0.17 $\pm$ 0.079	57 $\pm$ 13	60 $\pm$ 13
30	1.26 $\pm$ 0.046	89 $\pm$ 1.7	94 $\pm$ 0.44

Data are expressed as means $\pm$ S.E.M. ($n = 3$).

To further validate the correlation between receptor occupancy of mGluR1 and inhibition of the behavioral change, Compound A was used. This compound also exhibited dose-dependent and plasma concentration–dependent receptor occupancy in the cerebellum and striatum (Table 3). Like FTIDC, Compound A inhibited (*S*)-3,5-DHPG–induced face-washing behavior by 46 $\pm$ 14% ($P < 0.05$) and 97 $\pm$ 6.6% ($P < 0.001$) (mean $\pm$ S.E.M., $n = 6$) at 10 and 30 mg/kg, respectively. Compound A produced a linear correlation between the receptor-occupancy level and the degree of inhibition of (*S*)-3,5-DHPG–induced face washing behavior ($r^2 = 0.96$ and 0.96 in the cerebellum and the striatum, respectively; Fig. 6B).

Other mGluR1 antagonists with diverse structures (Fig. 7A) were used to confirm the correlation between mGluR1-receptor occupancy and inhibition of (*S*)-3,5-DHPG–induced face-washing behavior. Compounds B and C were novel mGluR1 antagonists with IC_{50} values (nM) of 26 $\pm$ 5.7 ($n = 3$) and 110 $\pm$ 38 ($n = 3$) for L-glutamate-induced Ca^{2+} mobilization in CHO-hmGluR1a. EMQMCM is a known mGluR1 antagonist (29) with the functional IC_{50} value of 8.1 $\pm$ 0.73 nM ($n = 25$). The

Fig. 7. Relationship between the degree of receptor occupancy on mGluR1 and in vivo effects by allosteric mGluR1 antagonists with diverse chemical structures. Chemical structures of diverse allosteric mGluR1 antagonists, Compounds B and C and EMQMCM (A). Correlation of receptor occupancy with inhibition of (*S*)-3,5-DHPG-induced face washing behavior by various mGluR1 antagonists. The percent inhibition of (*S*)-3,5-DHPG-induced face washing behavior was plotted against the receptor occupancy level in the striatum at the corresponding dose of Compound B (closed triangles), Compound C (open triangles), and EMQMCM (inverted closed triangles) in addition to FTIDC (closed squares) and Compound A (open squares) (B).

percent inhibition of the face-washing behavior by these compounds in addition to FTIDC and Compound A was collectively plotted against the receptor occupancy level in the striatum at the corresponding dose of the compounds (Fig. 7B), indicating a good linear correlation ($r^2 = 0.85$).

Discussion

[3 H]FTIDC bound with high affinity to membranes prepared from CHO-hmGluR1a and mouse cerebellum. [3 H]FTIDC bound to mGluR1 was not displaced by the orthosteric amino acid-like ligands L-quisqualate and LY367385. This result is in accordance with our earlier observation that FTIDC does not displace [3 H]L-quisqualate (an orthosteric radioligand) bound to mGluR1a (24), thus confirming the allosteric property of FTIDC. In contrast to orthosteric ligands, allosteric mGluR1 antagonists with diverse chemical structures inhibit [3 H]FTIDC binding to membranes prepared from CHO-hmGluR1a. This result suggests that these allosteric mGluR1 antagonists share a mutual binding region, consistent with the results obtained from studies using other allosteric mGluR1 radioligands, [3 H]LY456066 (4, 30), [3 H]R214127 (6, 30), and [3 H]YM-298198 (8, 30). The rank order of K_i of the allosteric antagonists to mGluR1 in the present study is similar to that of their inhibitory potencies (IC_{50}) obtained from functional assays using the same cell line, as shown in Table 1. These results show that structurally unrelated allosteric mGluR1 antagonists can be pharmacologically characterized by a single allosteric mGluR1 radioligand such as [3 H]FTIDC.

Receptor occupancy levels in the cerebellum and

the striatum were determined in the present study. The molecular layer of the cerebellum is the brain region most abundantly expressing mGluR1, but it required careful measurement of the region of interest (ROI) because the molecular layer is not uniform in the cerebellum. The striatum is the forebrain region with a moderate level of mGluR1 expression and relatively homogenous structure, where receptor occupancy levels can be more easily and precisely determined compared with other brain regions. Therefore, those two brain regions were chosen for determination of receptor occupancy. High specificity of [3 H]FTIDC binding to mGluR1 in the cerebellum and the striatum was clearly demonstrated by comparison of [3 H]FTIDC binding to brain sections prepared from wild-type and mGluR1-knockout mice. These results indicate that [3 H]FTIDC is an excellent radioligand for ex vivo receptor occupancy. The receptor occupancy of mGluR1 in mice following systemic administration of FTIDC was correlated to dosage levels and concentrations of FTIDC in the plasma. ED_{50} and plasma EC_{50} values (25 mg/kg and 430 nM, respectively) in the cerebellum were somewhat higher than those in the striatum (13 mg/kg and 150 nM, respectively). Consistent with these results, the ED_{50} value of (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl) (*cis*-4-methoxycyclohexyl) methanone (JNJ16259685) in the cerebellum was higher than that in the thalamus in an ex vivo receptor occupancy study in rats using [3 H]R214127. The difference in ED_{50} s from different brain regions might be due to a difference in distribution of the compound among the brain regions (7). Further studies will be necessary to determine if the observed regional difference in receptor occupancy by FTIDC is biologically significant.

The pharmacological effects of allosteric mGluR1 antagonists indicate the involvement of mGluR1 in CNS disorders, but to date, the amount of receptor occupancy by mGluR1 antagonists required to obtain these pharmacological results has not been demonstrated. The first *ex vivo* receptor occupancy study using [³H]R214127 indicated a relationship between receptor occupancy level and a given dose for two allosteric mGluR1 antagonists ([[(3a*S*,6a*S*)-6a-naphthalen-2-ylmethyl-5-methylidenehexahydro-cyclopent[*c*]furan-1-on] (BAY 36-7620) and 2-quinoxaline-carboxamide-*N*-adamantan-1-yl (NPS 2390)) 1 h after subcutaneous administration to rats (23). However, *in vivo* pharmacological studies with BAY 36-7620 have not been performed under conditions corresponding to the receptor occupancy study (13, 15), and no *in vivo* pharmacological study has been published for NPS2390. Receptor occupancy by another allosteric mGluR1 antagonist, JNJ16259685, was evaluated at various time points after subcutaneous dosing to rats (7), and *in vivo* rat efficacy studies have been carried out by intraperitoneal dosing (18, 31). Alternatively, the *in vivo* pharmacological effects of JNJ16259685 in mice have been discussed based on the results of receptor occupancy determined using rats (32). As stated above, there have been no directly comparable data between *in vivo* pharmacological studies and receptor occupancy experiments for mGluR1; therefore, the relationship between receptor occupancy level of mGluR1 and *in vivo* activity of allosteric mGluR1 antagonists has been unclear. To evaluate the precise relationship between receptor occupancy and *in vivo* antagonistic effects, studies should be conducted under the same experimental conditions including the same animal species, compound dose, routes of administration, and timing of evaluation after dosing, for both receptor occupancy experiments and *in vivo* pharmacological tests.

In the present study, we examined the relationship between the degree of receptor occupancy on mGluR1 and (*S*)-3,5-DHPG-induced face washing behavior as an *in vivo* pharmacological test under comparable experimental conditions. (*S*)-3,5-DHPG is a dual agonist for mGluR1 and mGluR5 (33). Some behavioral changes, including facial grooming, are elicited when (*S*)-3,5-DHPG is intracerebroventricularly administered to mice (27). We also observed similar behavior following intracerebroventricular administration of (*S*)-3,5-DHPG and referred to it as 'face-washing behavior' (25). This behavioral change was inhibited by specific mGluR1 antagonists such as [(4-methoxy-phenyl)-(6-methoxyquinazolin-4-yl)-amine, HCl] (LY456236), and FTDC, but not by an mGluR5 antagonist, MPEP (25, 27). The dose of MPEP used in our previous study was 30 mg/kg

(i.p.) that was threefold higher than dose occupying about 70% of mGluR5 in the receptor occupancy assay (34). Furthermore, a group II/III mGluR antagonist, LY341495, did not inhibit the behavioral change (25). These behavioral studies indicated that (*S*)-3,5-DHPG-induced face-washing behavior is mediated via activation of mGluR1. Therefore, (*S*)-3,5-DHPG-induced face-washing behavior is a useful assay to evaluate *in vivo* antagonism of mGluR1 function. Experiments with mGluR1-knockout mice may be useful for further validation of the involvement of mGluR1 in (*S*)-3,5-DHPG-induced face washing behavior. However it is difficult to evaluate face-washing behavior due to severe cerebellar ataxia observed in the mice (21, 22). The physiological or pathophysiological relevance of (*S*)-3,5-DHPG-induced face washing behavior is not clear, but it might be related to ritualistic behavior (such as hand-washing) observed in obsessive-compulsive disorder (OCD) in humans. Fronto-striatal abnormalities have been hypothesized to represent the core pathology in human OCD (35), and glutamate levels in CSF have been reported to be significantly higher in OCD patients compared to control subjects (36). Therefore, (*S*)-3,5-DHPG-induced face-washing behavior might be a potential animal model for obsessive compulsive behavior. To validate this hypothesis, further studies will be necessary. Especially, it will be important to evaluate effects of some drugs like selective serotonin reuptake inhibitors on (*S*)-3,5-DHPG-induced face-washing behavior.

In the current study, FTDC was intraperitoneally administered to mice 30 min before intracerebroventricular injection of (*S*)-3,5-DHPG, and then the amount of time spent in face-washing behavior was measured for 5 min starting at 5 min after injection of (*S*)-3,5-DHPG. We determined mGluR1-receptor occupancy levels 30 min after intraperitoneal injection of mGluR1 antagonists at the corresponding dose. These matched experimental conditions allowed us to accurately assess the relationship between receptor occupancy and *in vivo* activity of allosteric mGluR1 antagonists. Correlation analysis revealed a linear relationship between the receptor occupancy level of mGluR1 and the degree of inhibition of (*S*)-3,5-DHPG-induced face washing behavior by FTDC. The correlation obtained by the results with only FTDC may also be interpreted by off-target (mGluR1-unrelated) activities derived from its chemical structure. Thus, correlation of receptor occupancy with inhibition of the behavioral change was further validated using various known and novel allosteric mGluR1 antagonists with diverse chemical structures (Compounds A – C and EMQMCM). The correlation analysis revealed that there is an

excellent correlation between receptor occupancy and inhibition of the behavioral change by various allosteric mGluR1 antagonists regardless of their chemical structures. These results provided further proof that the correlation was due to functional blockade of mGluR1 by the compounds, but not due to off-target activities of FTIDC. This is the first demonstration of a correlation between mGluR1-receptor occupancy and in vivo activity by allosteric mGluR1 antagonists under the same experimental conditions. It is difficult to directly compare in vitro antagonistic potency of tested compounds on intracellular Ca^{2+} mobilization with in vivo antagonistic potency on (*S*)-3,5-DHPG-induced face-washing behavior since the in vivo antagonistic activity does not simply reflect the in vitro antagonistic activity and is affected by the pharmacokinetic properties of compounds. Thus, potency in receptor occupancy could be used as a surrogate of in vivo antagonistic potency.

mGluR1-knockout mice exhibited decreased spontaneous locomotor activity and impaired rotorod performance (21, 22) whereas systemic administration of allosteric mGluR1 antagonists, A-841720 and JNJ16259685, to rats decreased locomotor activity and/or impaired motor coordination (9, 18), suggesting that blockade of mGluR1 may elicit a deficit in motor functions. In the present study, the doses of FTIDC were correlated with receptor occupancy in the brain, and a dose of 30 mg/kg almost fully inhibited (*S*)-3,5-DHPG-induced face-washing behavior. In contrast, the doses of FTIDC in the present study neither decreased spontaneous locomotor activity nor impaired rotorod performance (20). These results suggested that motor deficit was not elicited in mice at least at doses of FTIDC that significantly occupied mGluR1 in the brain and antagonized *S*-(3,5)-DHPG-induced face washing behavior.

In conclusion, [^3H]FTIDC has been identified as a novel allosteric mGluR1 radioligand. [^3H]FTIDC exhibits potent affinity and high selectivity toward mGluR1. The binding region of [^3H]FTIDC on mGluR1 is shared with other structurally unrelated allosteric mGluR1 antagonists. Specificity of [^3H]FTIDC binding to mGluR1 in brain sections has been demonstrated by the lack of significant binding to brain sections prepared from mGluR1-knockout mice. Ex vivo receptor occupancy assays using [^3H]FTIDC show a clear correlation of the degree of mGluR1 occupancy with both the dose of FTIDC and the plasma concentration of FTIDC. Furthermore, we have shown that inhibition of (*S*)-3,5-DHPG-induced face washing behavior by FTIDC is dependent on the receptor occupancy level by the compound. Finally, the correlation between the receptor occupancy and inhibition of the face washing behavior

was validated using structurally diverse mGluR1 antagonists. From a broader perspective, the receptor occupancy assay using [^3H]FTIDC may be a useful tool for determining direct relationships between given doses of allosteric mGluR1 antagonists and various in vivo pharmacological effects and help provide guidelines for selecting appropriate doses of allosteric mGluR1 antagonists for examining the function of mGluR1 in vivo.

Acknowledgments

The authors thank Professor Kazuhide Inoue (Kyushu University) for supporting the preparation of this article and thank Satomi Yoshinaga for technical assistance.

References

- 1 Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol.* 1997;37: 205–237.
- 2 Litschig S, Gasparini F, Rueegg D, Stoeckli N, Flor PJ, Vranesic I, et al. CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol.* 1999;55:453–461.
- 3 Carroll FY, Stolle A, Beart PM, Voerste A, Brabet I, Mauler F, et al. BAY36-7620: a potent non-competitive mGlu1 receptor antagonist with inverse agonist activity. *Mol Pharmacol.* 2001; 59:965–973.
- 4 Li L, Tomlinson R, Wang Y, Tsui HCT, Chamberlain MJ, Johnson MP, et al. A novel series of potent and selective noncompetitive antagonists of metabotropic glutamate receptor 1. *Neuropharmacology.* 2002;43:295.
- 5 Malherbe P, Kratochwil N, Knoflach F, Zenner MT, Kew JN, Kratzenstein C, et al. Mutational analysis and molecular modeling of the allosteric binding site of a novel, selective, noncompetitive antagonist of the metabotropic glutamate 1 receptor. *J Biol Chem.* 2003;278:8340–8347.
- 6 Lavreysen H, Janssen C, Bischoff F, Langlois X, Leysen JE, Lesage AS. [^3H]R214127: a novel high-affinity radioligand for the mGlu1 receptor reveals a common binding site shared by multiple allosteric antagonists. *Mol Pharmacol.* 2003;63:1082–1093.
- 7 Lavreysen H, Wouters R, Bischoff F, Nóbrega Pereira S, Langlois X, Blokland S, et al. JNJ16259685, a highly potent, selective and systemically active mGlu1 receptor antagonist. *Neuropharmacology.* 2004;47:961–972.
- 8 Kohara A, Taya T, Tamura S, Watabiki T, Nagakura Y, Shitaka Y, et al. Radioligand binding properties and pharmacological characterization of 6-amino-N-cyclohexyl-N,3-dimethylthiazolo [3,2-a]benzimidazole-2-carboxamide (YM-298198), a high-affinity, selective, and noncompetitive antagonist of metabotropic glutamate receptor type 1. *J Pharmacol Exp Ther.* 2005;315:163–169.
- 9 El-Kouhen O, Lehto SG, Pan JB, Chang R, Baker SJ, Zhong C, et al. Blockade of mGluR1 receptor results in analgesia and disruption of motor and cognitive performances: effects of A-841720, a novel non-competitive mGluR1 receptor antagonist. *Br J Pharmacol.* 2006;149:761–774.

- 10 Varty GB, Grilli M, Forlani A, Fredduzzi S, Grzelak ME, Guthrie DH, et al. The antinociceptive and anxiolytic-like effects of the metabotropic glutamate receptor 5 (mGluR5) antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in rodents: a comparison of efficacy and side-effect profiles. *Psychopharmacology (Berl)*. 2005;179:207–217.
- 11 Kohara A, Nagakura Y, Kiso T, Toya T, Watabiki T, Tamura S, et al. Antinociceptive profile of a selective metabotropic glutamate receptor 1 antagonist YM-230888 in chronic pain rodent models. *Eur J Pharmacol*. 2007;571:8–16.
- 12 Zhu CZ, Baker S, El-Kouhen O, Lehto SG, Hollingsworth PR, Gauvin DM, et al. Analgesic activity of metabotropic glutamate receptor 1 antagonists on spontaneous post-operative pain in rats. *Eur J Pharmacol*. 2008;580:314–321.
- 13 De Vry J, Horváth E, Schreiber R. Neuroprotective and behavioral effects of the selective metabotropic glutamate mGlu(1) receptor antagonist BAY 36-7620. *Eur J Pharmacol*. 2001;428:203–214.
- 14 Kohara A, Takahashi M, Yatsugi S, Tamura S, Shitaka Y, Hayashibe S, et al. Neuroprotective effects of the selective type 1 metabotropic glutamate receptor antagonist YM-202074 in rat stroke models. *Brain Res*. 2008;1191:168–179.
- 15 Chapman A, Nanan K, Meldrum BS, Schreiber R, De Vry J, Müller T. Anticonvulsant activity in mice of BAY 36-7620, a novel, selective mGluR1 antagonist. *Soc Neurosci Abstracts*. 2000;618.11.
- 16 Shannon HE, Peters SC, Kingston AE. Anticonvulsant effects of LY456236, a selective mGlu1 receptor antagonist. *Neuropharmacology*. 2005;49 Suppl 1:188–195.
- 17 Pietraszek M, Sukhanov I, Maciejak P, Szyndler J, Gravius A, Wisłowska A, et al. Anxiolytic-like effects of mGlu1 and mGlu5 receptor antagonists in rats. *Eur J Pharmacol*. 2005;514:25–34.
- 18 Steckler T, Lavreysen H, Oliveira AM, Aerts N, Van Craenendonck H, Prickaerts J, et al. Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. *Psychopharmacology (Berl)*. 2005;179:198–206.
- 19 Belozertseva IV, Kos T, Popik P, Danyś W, Besspalov AY. Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. *Eur Neuropsychopharmacol*. 2007;17:172–179.
- 20 Satow A, Maehara S, Ise S, Hikichi H, Fukushima M, Suzuki G, et al. Pharmacological effects of the metabotropic glutamate receptor 1 antagonist compared with those of the metabotropic glutamate receptor 5 antagonist and metabotropic glutamate receptor 2/3 agonist in rodents: detailed investigations with a selective allosteric mGluR1 antagonist, FTIDC, (4-[1-(2-fluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide). *J Pharmacol Exp Ther*. 2008;326:577–586.
- 21 Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, et al. Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell*. 1994;79:377–388.
- 22 Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, et al. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature*. 1994;372:237–243.
- 23 Lavreysen H, Pereira SN, Leysen JE, Langlois X, Lesage AS. Metabotropic glutamate 1 receptor distribution and occupancy in the rat brain: a quantitative autoradiographic study using [³H]R214127. *Neuropharmacology*. 2004;46:609–619.
- 24 Suzuki G, Kimura T, Satow A, Kaneko N, Fukuda J, Hikichi H, et al. Pharmacological characterization of a new, orally active and potent allosteric metabotropic glutamate receptor 1 antagonist, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide (FTIDC). *J Pharmacol Exp Ther*. 2007;321:1144–1153.
- 25 Hikichi H, Iwahori Y, Murai T, Maehara S, Satow A, Ohta H. Face-washing behavior induced by the group I metabotropic glutamate receptor agonist (S)-3,5-DHPG in mice is mediated by mGlu1 receptor. *Eur J Pharmacol*. 2008;586:212–216.
- 26 Ohashi H, Maruyama T, Higashi-Matsumoto H, Nomoto T, Nishimura S, Takeuchi Y. A novel binding assay for metabotropic glutamate receptors using [³H] L-quisqualic acid and recombinant receptors. *Z Naturforsch [C]*. 2002;57:348–355.
- 27 Barton ME, Shannon HE. Behavioral and convulsant effects of the (S) enantiomer of the group I metabotropic glutamate receptor agonist 3,5-DHPG in mice. *Neuropharmacology*. 2005;48:779–787.
- 28 Motulsky HJ, Christopoulos A. Fitting radioligand and enzyme kinetics data. In: *Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting*. San Diego: GraphPad Software Inc.; 2003. p. 187–255.
- 29 Mabire D, Coupa S, Adelinet C, Poncelet A, Simonnet Y, Venet M, et al. Synthesis, structure-activity relationship, and receptor pharmacology of a new series of quinoline derivatives acting as selective, noncompetitive mGlu1 antagonists. *J Med Chem*. 2005;48:2134–2153.
- 30 Johnson MP, Nisenbaum ES, Large TH, Emkey R, Baez M, Kingston AE. Allosteric modulators of metabotropic glutamate receptors: lessons learnt from mGlu1, mGlu2 and mGlu5 potentiators and antagonists. *Biochem Soc Trans*. 2004;32:881–887.
- 31 Besheer J, Faccidomo S, Grondin JJ, Hodge CW. Effects of mGlu1-receptor blockade on ethanol self-administration in inbred alcohol-preferring rats. *Alcohol*. 2008;42:13–20.
- 32 Steckler T, Oliveira AF, Van Dyck C, Van Craenendonck H, Mateus AM, Langlois X, et al. Metabotropic glutamate receptor 1 blockade impairs acquisition and retention in a spatial Water maze task. *Behav Brain Res*. 2005;164:52–60.
- 33 Pin JP, De Colle C, Bessis AS, Acher F. New perspectives for the development of selective metabotropic glutamate receptor ligands. *Eur J Pharmacol*. 1999;375:277–294.
- 34 Anderson JJ, Bradbury MJ, Giracello DR, Chapman DF, Holtz G, Roppe J, et al. In vivo receptor occupancy of mGlu5 receptor antagonists using the novel radioligand [³H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine. *Eur J Pharmacol*. 2003;473:35–40.
- 35 Rosenberg DR, Macmillan SN. Imaging and neurocircuitry of OCD. In: Davis KL, Charney D, Coyle JT, Nemeroff C, editors. *Neuropsychopharmacology. The fifth generation of progress*. Philadelphia: Lippincott Williams and Wilkins; 2002. p. 1621–1645.
- 36 Chakrabarty K, Bhattacharyya S, Christopher R, Khanna S. Glutamatergic dysfunction in OCD. *Neuropsychopharmacology*. 2005;30:1735–1740.