Journal of Pharmacological Sciences 127 (2015) 352–361

HOSTED BY

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full paper

Antipsychotic profiles of TASP0443294, a novel and orally active positive allosteric modulator of metabotropic glutamate 2 receptor

Hirohiko Hikichi [a, *], Tetsuaki Hiyoshi [a], Toshiyuki Marumo [a], Yasumitsu Tomishima [a], Ayaka Kaku [a], Izumi Iida [b], Hiroki Urabe [c], Tomoko Tamita [c], Akito Yasuhara [c], Jun-ichi Karasawa [a], Shigeyuki Chaki [a]

[a] Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan
[b] Drug Safety and Pharmacokinetics Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan
[c] Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan

ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form
30 January 2015
Accepted 2 February 2015
Available online 9 February 2015

Keywords:
TASP0443294
mGlu2 receptor positive allosteric modulator
Hyperlocomotion
Social memory
EEG

ABSTRACT

Glutamatergic dysfunction has been implicated in psychiatric disorders such as schizophrenia. The stimulation of metabotropic glutamate (mGlu) 2 receptor has been shown to be effective in a number of animal models of schizophrenia. In this study, we investigated the antipsychotic profiles of (2*S*)-5-methyl-2-{[4-(1,1,1-trifluoro-2-methylpropan-2-yl)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole-6-carboxamide (TASP0443294), a newly synthesized positive allosteric modulator of the mGlu2 receptor. TASP0443294 potentiated the response of human mGlu2 and rat mGlu2 receptors to glutamate with EC_{50} values of 277 and 149 nM, respectively, without affecting the glutamate response of human mGlu3 receptor. TASP0443294 was distributed in the brain and cerebrospinal fluid after peroral administration in rats. The peroral administration of TASP0443294 inhibited methamphetamine-induced hyperlocomotion in rats, which was attenuated by an mGlu2/3 receptor antagonist, and improved social memory impairment induced by 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801) in rats. Furthermore, TASP0443294 reduced the ketamine-induced basal gamma hyperactivity in the prefrontal cortex and suppressed rapid eye movement (REM) sleep in rats. These findings indicate that TASP0443294 is an mGlu2 receptor positive allosteric modulator with antipsychotic activity, and that the suppression of aberrant gamma oscillations and REM sleep could be considered as neurophysiological biomarkers for TASP0443294.

1. Introduction

In addition to the dopamine hypothesis of schizophrenia, abnormalities in glutamatergic transmission have also been suggested to be involved in the pathophysiology of schizophrenia. Among glutamate receptors, metabotropic glutamate (mGlu) receptors, which consist of 8 subtypes (mGlu1-8), have emerged as attractive therapeutic targets for the development of novel interventions for psychiatric disorders.

Of the mGlu receptor subtypes, group II mGlu receptors, consisting of mGlu2 and mGlu3 receptors, are primarily localized presynaptically and are negatively coupled via Gi proteins to adenylate cyclase, leading to the inhibition of glutamate release upon neuronal activation (1). In addition, mGlu2 and mGlu3 receptors are expressed postsynaptically and glial cells, respectively, where they regulate glutamate transmission (2,3). These receptors have been proposed to play important roles in the pathophysiology of schizophrenia (4,5). Indeed, several potent dual mGlu2/3 receptor agonists, which have structurally constrained analogs of glutamate, have been identified, and a growing body of evidence has shown that several mGlu2/3 receptor agonists exhibit antipsychotic activity in numerous experimental animal models of schizophrenia (4). Among mGlu2/3 receptors, the stimulation of the mGlu2

* Corresponding author. Tel.: +81 48 669 3069; fax: +81 48 652 7254.
E-mail address: h-hikichi@so.taisho.co.jp (H. Hikichi).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.02.004
1347-8613/

receptor has been proposed to mediate the antipsychotic effects of mGlu2/3 receptor agonists in animal models for schizophrenia, since the antipsychotic effects of mGlu2/3 receptor agonists are no longer observed in mice lacking the mGlu2 receptor but not the mGlu3 receptor (6,7). These mGlu2/3 receptor agonists bind at an orthosteric binding site to which endogenous glutamate ligands bind. On the other hand, positive allosteric modulators do not activate the receptor directly, but act at an allosteric site on the receptor to potentiate glutamate-induced activation and produce their effects only in the presence of an endogenous ligand (glutamate) (8), thereby reducing the risk of side effects and tolerance related to continuous receptor stimulation with orthosteric agonists. Therefore, allosteric modulators may offer advantages over orthosteric agonists as therapeutic agents.

In this sense, selective positive allosteric modulators of mGlu2 receptor should be of interest and may provide an advantage over orthosteric mGlu2/3 receptor agonists. Indeed, positive allosteric modulators preferentially acting on the mGlu2 receptor over the mGlu3 receptor reportedly exert antipsychotic effects in animal models (9–12). Therefore, the selective stimulation of the mGlu2 receptor may be a useful approach for the treatment of schizophrenia. However, some important differences exist in the effects of mGlu2 receptor positive allosteric modulators in animal models (10), with these differences possibly being ascribed to the differential actions of each compound on the mGlu2 receptor. Thus, an investigation of the pharmacological profiles with structurally distinct mGlu2 receptor positive allosteric modulators should provide additional information regarding the usefulness of mGlu2 receptor positive allosteric modulators as antipsychotics. Recently, we synthesized a structurally novel and orally active mGlu2 receptor positive allosteric modulator, (2*S*)-5-methyl-2-{[4-(1,1,1-trifluoro-2-methylpropan-2-yl)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole-6-carboxamide (TASP0443294) (Fig. 1).

The aim of this study was to further support the assumptions that the stimulation of mGlu2 receptors results in antipsychotic effects using TASP0443294. Moreover, to evaluate the neurophysiological mechanisms of mGlu2 positive allosteric modulators, we investigated the effects of TASP0443294 on ketamine-induced basal gamma hyperactivity on electroencephalograms (EEG) showing activity in the prefrontal cortex and on rapid eye movement (REM) sleep on polysomnograms in rats.

2. Methods

2.1. Animals

Male Sprague–Dawley and Wistar rats (purchased from Charles River, Yokohama, Japan) were used for this study. The rats were housed in a controlled animal room (room temperature: 23 ± 3 °C, humidity: 50 ± 20%) with a 12-h light–dark cycle (lights on: 07:00–19:00). Rats were maintained in groups of 2 or 4 rats per cage. Food and water were available *ad libitum*. All the studies were conducted in accordance with the criteria of the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987).

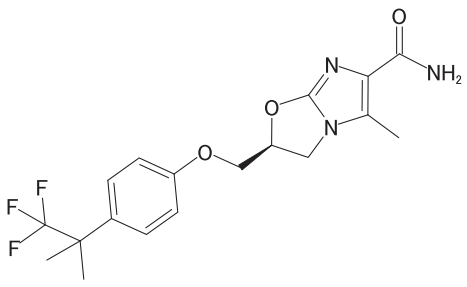

Fig. 1. Chemical structure of TASP0443294.

2.2. In vitro studies

2.2.1. Cell culture and membrane preparation

Chinese hamster ovary (CHO) cell lines stably expressing rat mGlu2 receptor were kindly donated by Dr. Nakanishi (Kyoto University, Kyoto, Japan). CHO cell lines stably expressing human mGlu receptors (mGlu2, mGlu3 and mGlu8 receptor) were established in house. The cells expressing mGlu2 or mGlu3 receptor were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% dialyzed fetal bovine serum (FBS), 2 mM L-glutamine, 1% proline, penicillin (50 units/mL for rat mGlu2 receptor, 100 units/mL for human mGlu2 and mGlu3 receptor expressed cells) and streptomycin (50 μg/mL for rat mGlu2 receptor, 100 μg/mL for human mGlu2 and mGlu3 receptor expressed cells), 1 mM sodium pyruvate, 1 mM succinic acid, 1 mM succinic acid disodium salt and hygromycin B (400 μg/mL for human mGlu2, or 300 μg/mL for human mGlu3 receptor expressed cells). The cells expressing human mGlu8 receptor were cultured in Ham's F-12 Nutrient Mixture (F-12) containing 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 500 μg/mL G418. The cells were in an incubator maintained at 37 °C in a humidified atmosphere of 5% CO_2. Confluent cells expressing each mGlu receptor were washed in phosphate-buffered saline, scraped, and centrifuged at 190 × *g* for 5 min at 4 °C. The pellet was homogenized with 20 mM HEPES buffer (pH 7.4) for mGlu2 receptor expressed cells or 20 mM HEPES buffer containing 1 mM EDTA (pH 7.4) for mGlu3 or mGlu8 receptor expressed cells, then centrifuged at 48,000 × *g* for 20 min at 4 °C. The pellet was washed twice and suspended with 20 mM HEPES buffer or 20 mM HEPES buffer containing 1 mM EDTA (pH 7.4) to obtain the crude membrane fraction, which was stored at −80 °C.

2.2.2. [^{35}S]GTPγS binding assay

The above mentioned membranes were diluted in 20 mM HEPES buffer (pH 7.4), containing 1 mM EDTA (for mGlu3 and mGlu8 receptor), 100 mM NaCl, 10 mM $MgCl_2$, 8.4 μM GDP, 10 μg/mL saponin, and 0.1% BSA to yield a protein concentration of 10 (for mGlu2 and mGlu8 receptor) or 15 (for mGlu3 receptor) μg/assay. The membranes were pre-incubated with various concentrations of TASP0443294 for 20 min at 30 °C; subsequently, various concentrations of glutamate and [^{35}S]GTPγS (0.15 nM) were added and the membranes were further incubated for 60 min at 30 °C. The reaction was terminated by rapid filtration under a vacuum through a UniFilter GF/C microplate (PerkinElmer Life Science, Boston, MA, USA), after which the filters were washed with 1 mL of ice-cold 20 mM HEPES buffer or 20 mM HEPES buffer containing 1 mM EDTA (pH 7.4) using a UniFilter-96 harvester (PerkinElmer Life Science, Boston, MA, USA). After drying the filters, 20 μL of Microscint-O (PerkinElmer Life Science, Boston, MA, USA) was added, and the membrane-bound radioactivity was counted with a TopCount NXT™ (PerkinElmer Life Science, Boston, MA, USA). The specific binding of [^{35}S]GTPγS was calculated by subtracting the nonspecific binding in the absence of glutamate. The amount of [^{35}S]GTPγS binding was normalized with the response to 1 mM glutamate. The EC_{50} values of TASP0443294 for mGlu2 positive allosteric modulator activity were determined in the presence of an EC_{20}-equivalent concentration of glutamate (1 μM for human mGlu2 receptor or 3 μM for rat mGlu2 receptor). The effect of TASP0443294 on human mGlu3 receptor was examined in

the presence of an EC_{40}-equivalent concentration of glutamate at 100 nM.

2.2.3. Pharmacokinetics, and brain and plasma levels in rats

For the pharmacokinetic study, TASP0443294 was intravenously administered at 0.25 mg/kg to fasted male Sprague–Dawley rats (7 weeks old). Then blood samples were repeatedly collected at several time points (5, 15, and 30 min and 1, 2, 4, 8, and 24 h) after the administration of TASP0443294 from the tail vein using EDTA as the anticoagulant. To evaluate the time course of the plasma levels of TASP0443294 prior to the behavioral studies, TASP0443294 was perorally administered at a dose of 3 mg/kg to male Wistar rats (7 weeks old) and blood samples were then collected at 0.5, 1, 2, and 3 h after the administration of TASP0443294 from the tail vein using EDTA. To evaluate the brain, plasma and cerebrospinal fluid (CSF) levels after the systemic administration of TASP0443294, TASP0443294 was administered at doses of 3, 10, and 30 mg/kg perorally to male Wistar rats. Then, the rats were sacrificed after collecting a blood sample at 2.5 h after TASP0443294 administration, and the CSF and brain were rapidly collected and removed, respectively. Plasma was obtained following centrifugation, was subjected to protein precipitation, and was subsequently analyzed for TASP0443294 using a liquid chromatography/tandem mass spectrometry qualified research method on an API4000 instrument (Applied Sciex, Foster City, CA, USA). The pharmacokinetic parameters were calculated using WinNonlin software (Pharsight Corporation, Mountain View, CA, USA).

2.3. Behavioral studies

2.3.1. Locomotor activity

The locomotor activity of male Wistar rats (7 weeks old) was measured using a SCANET apparatus (Melquest Ltd., Toyama, Japan) according to previously reported methods (13). Rats were individually placed into the test chamber (47 cm width × 28 cm length × 30 cm height), which in turn was placed in a soundproof box, at 120 min after TASP0443294 administration. Spontaneous locomotor activity was then measured for 60 min. In the drug interaction study, the rats were individually habituated to the test chamber placed in a soundproof box for 60 min, and methamphetamine was then subcutaneously administered at a dose of 1 mg/kg; the locomotor activities were immediately recorded for 120 min. TASP0443294 was administered perorally 120 min prior to the methamphetamine administration. LY341495 was administered intraperitoneally 60 min prior to the methamphetamine administration. Under the same condition, peroral administration of clozapine at doses of 30 and 100 mg/kg significantly inhibited methamphetamine-induced locomotor and rearing hyperactivities in rats.

2.3.2. Social recognition test

The social recognition test was performed as described previously (14). The behavior tests were performed in an open-topped box (47 cm width × 28 cm length × 30 cm height) containing sawdust. Adult male Sprague–Dawley rats (9 weeks old) were used in this test because Wistar rats exhibited shorter interaction time than Sprague–Dawley rats. A rat was placed in the test cage and was allowed to habituate for 30 min. Then, an unfamiliar juvenile rat (4 weeks old) was placed in the test cage with the adult rat for 5 min (the first test session). The length of time during which the adult rat exhibited exploratory behavior (sniffing, grooming, and close following) toward the juvenile rat during the test session was recorded and was defined as the first investigation duration. The juvenile was then removed from the test cage and returned to its home cage, while the adult was left in the test cage. Thirty minutes later, the same juvenile (familiar) was then placed in the test cage once again for a 5-min test session (the second test session); the length of time spent by the adult in exploring the juvenile during this test session was defined as the second investigation duration. Observers who were blinded to the treatment group scored the investigation duration (the first and the second test sessions). The social memory for each adult rat was defined by determining the ratio of the second investigation duration to the first investigation duration. MK-801 was administered intraperitoneally to the adult rats 30 min prior to the first exposure to the juvenile. TASP0443294 was administered perorally to the rats at 120 min prior to the MK-801 administration. Under the same condition, intraperitoneal administration of clozapine at a dose of 0.3 mg/kg but not haloperidol significantly improved MK-801-induced social memory deficits in rats (14).

2.4. Quantitative electroencephalogram and polysomnogram studies

2.4.1. Implantation of electrodes

To implant the electrodes for the quantitative electroencephalogram (qEEG) or polysomnogram recording, male Sprague–Dawley rats (9–10 weeks old) were anaesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) and fixed in a stereotaxic frame. After drilling holes in the skull, stainless steel screw electrodes (E363/20; PlasticsOne, Roanoke, VA, USA) were placed on the cerebral dura mater at positions according to a rat brain atlas (15) as follows: the recording electrode was placed over the frontal cortex at 1.5 mm anterior and 2.0 mm lateral from the bregma, the reference electrode was placed over the cerebellum at 11.5 mm posterior and 0 mm lateral from the bregma, and the ground electrode was placed over the contralateral occipital cortex at 6.0 mm posterior and 2.0 mm lateral from the bregma. For the polysomnogram recording, two stainless steel subcutaneous electrodes (E363/76; PlasticsOne, Roanoke, VA, USA) with needles were bipolarly implanted into the dorsal neck region to record the electromyogram (EMG). All the electrodes were socketed into an electrode pedestal (MS363; PlasticsOne, Roanoke, VA, USA), which was then fixed to the skull using a combination of dental acrylic resin and alpha-cyanoacrylate adhesive. The electrode-implanted rats were then singly housed and allowed to recover for more than 5 days before the qEEG or polysomnogram recordings.

2.4.2. EEG recording for measurement of gamma band oscillation power

The qEEG recording was performed as described previously (16). Cortical EEG recordings and drug administrations were performed under freely moving and unrestrained conditions. Rats were individually transferred to an acrylic chamber (30 cm width × 30 cm length × 35 cm height) placed within an electrically shielded sound-proof box and were tethered to a lead wire connected to a slip-ring commutator (SPM-15-6P; Hikari Denshi, Tokyo, Japan). The EEG signals were amplified (20,000 times) and bandpass-filtered (0.5–1000 Hz) using a biophysical amplifier (AB-611J; Nihon Kohden, Tokyo, Japan), digitized at a sampling rate of 2.5 kHz with an analog-to-digital converter (AD16-16U(PCI)EH; Contec, Osaka, Japan), and recorded using the data acquisition program VitalRecorder® (Ver. 1.3; Kissei Comtec, Matsumoto, Japan). After a baseline EEG recording for 30 min in parallel with the acclimation of the animal to the measurement environment, TASP0443294 or the vehicle was orally administered, followed 120 min later by the subcutaneous administration of ketamine at a dose of 5 mg/kg. The EEG recording was continued for 120 min after the administration of ketamine. The animals were repeatedly used for the EEG

recordings a maximum of three times after a more than 7-day interval to ensure drug washout.

The EEG data were individually analyzed off-line with the data analysis program SleepSign® (Ver. 3.0; Kissei Comtec, Matsumoto, Japan). After digital filtering (0.5–200 Hz bandpass), a fast Fourier transformation was performed for each 4-s epoch for a power spectrum analysis. The total power in the gamma band frequency (30–80 Hz) was averaged for 1-min bins and was normalized with the mean value of the 30-min baseline recordings to plot the time course. The area under the curve (AUC) for the gamma power change for 120 min after the administration of TASP0443294 and for 60 min after the ketamine injection were calculated to evaluate the effects of TASP0443294 on the baseline gamma power and the ketamine-increased gamma power, respectively. Under the same condition, subcutaneous administration of LY379268 at a dose of 1 mg/kg significantly inhibited ketamine-induced gamma hyperactivities in rats (16).

2.4.3. Polysomnogram recordings for the determination of sleep stage

The polysomnogram recordings were performed in the same recording environment as the above qEEG recordings. Rats were acclimated to the measurement environment under freely moving and unrestrained conditions (lighting period: 07:00 to 19:00, free access to food and water) for 16–19 h before drug administration. The polysomnogram (cortical EEG and EMG) was recorded from 06:00 to 15:00 on the administration day. TASP0443294 was perorally administered between 10:50 and 11:00. The EEG and EMG signals were amplified (20,000 times) and band-passed (EEG, 0.5–30 Hz; EMG, 15–1000 Hz) using a biophysical amplifier (AB-611J; Nihon Kohden, Tokyo, Japan), digitized at a sampling rate of 1024 Hz with an analog-to-digital converter (AD16-16U(PCI)EH; Contec, Osaka, Japan), and recorded using the data acquisition program VitalRecorder® (Ver. 1.3; Kissei Comtec, Matsumoto, Japan). The animals were repeatedly used for the polysomnogram recording a maximum of three times after a more than 7-day interval to ensure drug washout.

The polysomnogram was analyzed off-line using the data analysis program SleepSign® (Ver. 3.0; Kissei Comtec, Matsumoto, Japan). The sleep–wake states were classified in each 8-s epoch as wakefulness, non-rapid eye movement (NREM) sleep, and REM sleep by a visual verification based on the EEG and EMG patterns according to a previous report (17). Each state was characterized as follows: wakefulness, high EMG amplitude and low EEG amplitude; NREM sleep, low EMG amplitude and high EEG amplitude with a high-power density in the delta band (0.5–4.0 Hz); REM sleep, very low EMG amplitude and low EEG amplitude with high values in the theta band (4.0–8.0 Hz). The cumulative period of REM sleep during 2 h after the drug administration was calculated and was presented as a percentage of all the states. Under the same condition, peroral administration of paroxetine at a dose of 3 mg/kg significantly decreased the duration of REM sleep in rats.

2.4.4. Drugs

TASP0443294 was synthesized at Taisho Chemistry Laboratories. Methamphetamine hydrochloride (methamphetamine) was purchased from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). [^{35}S]GTPγS (specific radioactivity: 46.25 TBq/mmol) was purchased from PerkinElmer Life Science (Boston, MA, USA). MK-801 hydrogen maleate (MK-801) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ketamine was purchased as veterinary Ketalar® 50 from Sankyo Yell Pharmaceutical Co., Ltd. (Tokyo, Japan). LY341495 was purchased from Tocris Bioscience (Bristol, UK). TASP0443294 was suspended in 0.5% methylcellulose for peroral administration for the in vivo studies and was dissolved in polyethylene glycol 400 for intravenous administration in the pharmacokinetic study. Methamphetamine and MK-801 were dissolved in saline for subcutaneous and intraperitoneal administration, respectively. Ketamine was diluted in saline for subcutaneous administration. LY341495 was dissolved in 66.6 mM phosphate buffer (pH 8.0) for intraperitoneal administration. The volume of administration was 2 mL/kg. The dose selections for all the drugs were based on previous reports (14,16) and our preliminary studies.

2.4.5. Data analysis

For the in vitro experiments, data analyses were performed using GraphPad Prism 5 from GraphPad Software Inc. (San Diego, CA, USA). The concentration–effect curves for [^{35}S]GTPγS binding were fitted using nonlinear regression analysis. All the statistical analyses were performed using SAS software (SAS Institute Japan, Tokyo, Japan). The effects of TASP0443294 on the time course plot were analyzed using a two-way repeated-measures analysis of variance (ANOVA). Data were analyzed using the Student's *t*-test, or a one-way ANOVA followed by Dunnett's test and Tukey–Kramer test. A value of $P < 0.05$ was regarded as significant.

3. Results

3.1. In vitro profiles of TASP0443294

Glutamate produced an increase in [^{35}S]GTPγS binding in CHO cells expressing the human or rat mGlu2 receptor in a concentration dependent fashion, and the glutamate EC_{50} values for human and rat mGlu2 receptors were calculated to be 5.4 and 8.2 μM, respectively. The EC_{50} values for potentiation by TASP0443294 on glutamate-increased [^{35}S]GTPγS binding at the human and rat mGlu2 receptor in the presence of an EC_{20}-equivalent concentration of glutamate (1 μM) were estimated to be 277 and 149 nM, respectively (Fig. 2A, B and Table 1). The maximal responses in human and rat mGlu2 receptors, compared with the response of 1 mM glutamate, were increased to 102% and 132% upon the addition of TASP0443294, respectively (Table 1). Of note, TASP0443294 enhanced the response induced by glutamate, shifting the glutamate concentration response curve both upward and leftward (Fig. 2C). In contrast, TASP0443294 did not alter basal [^{35}S]GTPγS binding at the rat mGlu2 receptor at a concentration of up to 10 μM (data not shown). Moreover, TASP0443294 did not affect the response to glutamate (100 nM) in [^{35}S]GTPgS binding to membranes expressing human mGlu3 receptor (Table 1), whereas glutamate increased [^{35}S]GTPgS binding in a concentration dependent fashion, with an EC_{50} value of 286 nM. In addition, TASP0443294 did not potentiate glutamate-increased [^{35}S]GTPγS binding in CHO cells expressing the human mGlu8 receptor at a concentration of up to 10 μM (data not shown).

3.2. Pharmacokinetic study in rats

The results of the pharmacokinetic study, including the plasma concentrations after the intravenous administration of TASP0443294 in rats, are shown in Fig. 3. TASP0443294 had a 1.3 ± 0.1 h half-life, with a volume of distribution of 1.18 ± 0.04 L/kg and a plasma clearance of 0.64 ± 0.05 L/h/kg. The plasma concentrations of TASP0443294 at 0.5, 1, 2, and 3 h after the peroral administration of TASP0443294 are shown in Table 2A. The T_{max} was observed at 2 h after the administration of TASP0443294. Therefore, for the in vivo study, TASP0443294 was perorally administered 2 h prior to the test. The plasma, brain and CSF concentrations of TASP0443294 at 2.5 h after the peroral administration of TASP0443294 at doses of 3, 10, and 30 mg/kg in rats are shown in Table 2B. In rats, the plasma, brain and CSF concentrations of TASP0443294 increased in a dose-dependent fashion.

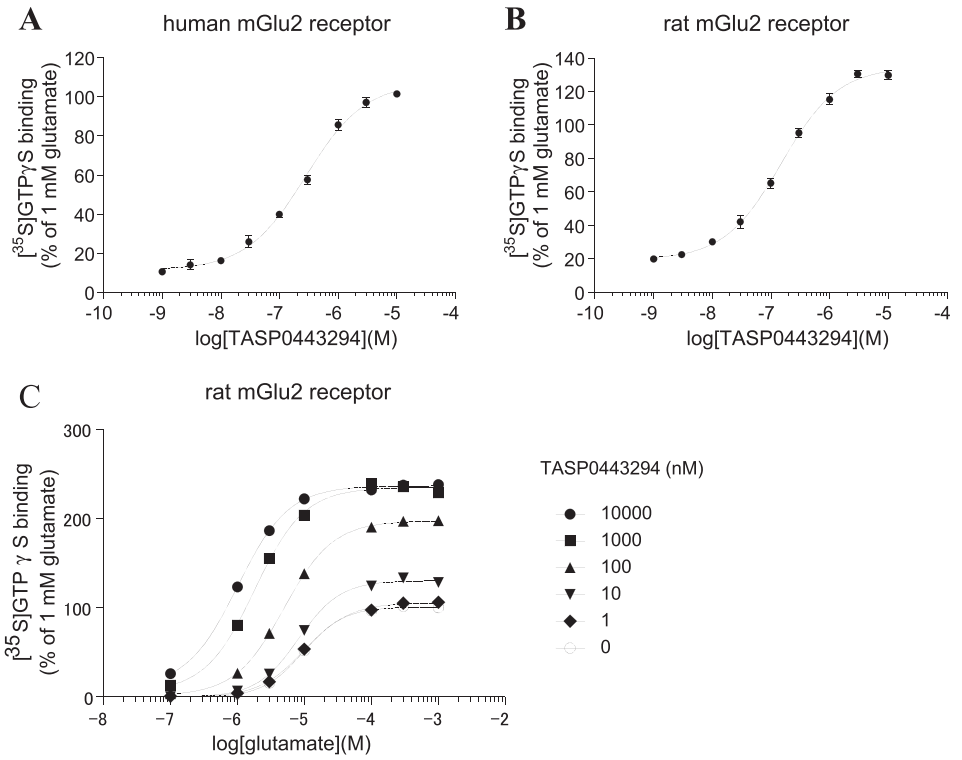

Fig. 2. The concentration–effect curves for TASP0443294 on the binding of [^{35}S]GTPγS at an EC_{20}-equivalent concentration of glutamate in the human mGlu2 (A) and rat mGlu2 receptors (B). The concentration–effect curves for TASP0443294 on the concentration–response curve of the [^{35}S]GTPγS binding by glutamate (C). Data are expressed as percentage of the maximal response to glutamate and are mean ± S.E.M. in the case of 3 experiments, each done in duplicate.

3.3. Spontaneous and methamphetamine-induced locomotor and rearing activities in rats

Subcutaneous administration of methamphetamine at a dose of 1 mg/kg significantly increased locomotor (all $P < 0.01$) (Figs. 4A, 5A and 5B) and rearing (all $P < 0.01$) (Figs. 4C, 5C and 5D) activities. TASP0443294 at doses of 30 and 100 mg/kg significantly inhibited the locomotor [$F(3,28) = 3.97$, $P < 0.05$] ($P < 0.01$) (Fig. 4A), [$F(2,27) = 5.49$, $P < 0.01$] ($P < 0.05$) (Fig. 5A) and rearing [$F(3,28) = 3.94$, $P < 0.05$] ($P < 0.05$) (Fig. 4C), [$F(2,27) = 10.25$, $P < 0.01$] ($P < 0.01$) (Fig. 5C) activities elicited by the subcutaneous administration of methamphetamine. In contrast, TASP0443294 did not affect spontaneous locomotor and rearing activities up to a dose of 100 mg/kg [$F(3,28) = 1.7$, $P > 0.05$] (Fig. 4B) and [$F(3,28) = 0.67$, $P > 0.05$] (Fig. 4D). In addition, pretreatment with the mGlu2/3 receptor antagonist LY341495 at a dose of 3 mg/kg significantly blocked the effect of TASP0443294 on methamphetamine-induced locomotor [$F(2,27) = 5.49$, $P < 0.01$] ($P < 0.05$) (Fig. 5A) and rearing [$F(2,27) = 10.25$, $P < 0.01$] ($P < 0.05$) (Fig. 5C) hyperactivities, indicating that the stimulation of the mGlu2 receptor mediates the effect of TASP0443294 on locomotor and rearing activities. Of note, LY341495 did not affect methamphetamine-induced locomotor and rearing hyperactivities at the doses used in the present study [$F(2,21) = 0.08$, $P > 0.05$] (Fig. 5B) and [$F(2,21) = 0.12$, $P > 0.05$] (Fig. 5D).

Table 1
Functional activity of TASP0443294 at human/rat recombinant mGlu2 receptors and human recombinant mGlu3 receptor in the presence of glutamate.

	Activity in presence of glutamate	
	EC_{50} (nM)	E_{max} (%)
Human mGlu2	277 (182–425)	102 (101–102)
Rat mGlu2	149 (120–185)	132 (123–140)
Human mGlu3	>10,000	

Glutamate is used at its EC_{20} and EC_{40} concentrations for mGlu2 and mGlu3 receptor, respectively. Values are an average of 3 experiments. Numbers in parentheses indicated 95% confidence intervals.

3.4. MK-801-induced social memory deficits in rats

The intraperitoneal administration of MK-801 at a dose of 0.1 mg/kg significantly increased the ratio of investigation duration ($P < 0.01$) (Fig. 6), indicating that MK-801 impaired short-term social memory. TASP0443294 at a dose of 30 mg/kg significantly

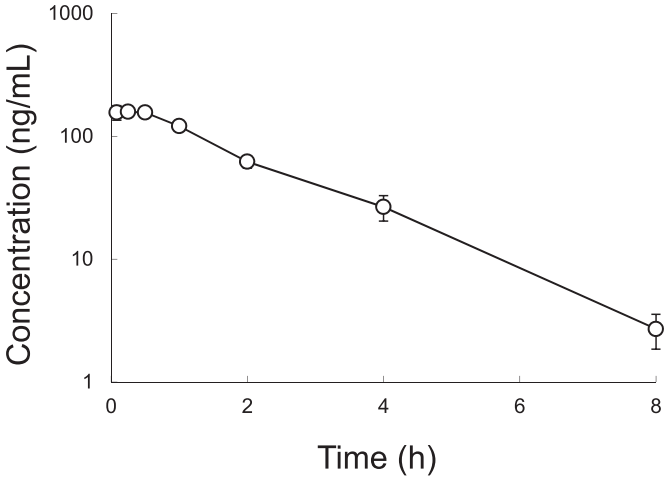

Fig. 3. Pharmacokinetic profiles of TASP0443294 after intravenous administration in rats. Plasma concentrations of TASP0443294 were measured at 5, 15 and 30 min and 1, 2, 4 and 8 h after administration. Three animals were used for this study. TASP0443294 at a dose of 0.25 mg/kg was administered intravenously in rats. Values are mean ± S.D.

Table 2
Brain and plasma concentrations of TASP0443294 in rats after peroral administration. A: Pharmacokinetic profiles of TASP0443294 after peroral administration. B: Brain, plasma and CSF concentrations 2.5 h after administration of TASP0443294.

A				
3 mg/kg, p.o.	Time			
	0.5 h	1 h	2 h	3 h
Plasma (ng/mL)	384 ± 65	531 ± 122	593 ± 100	458 ± 157
B				
Dose (mg/kg, p.o.)	Brain (ng/g)	Plasma (ng/mL)	CSF (ng/mL)	
3	835 ± 320	441 ± 200	26 ± 11	
10	3830 ± 1010	1650 ± 343	94 ± 29	
30	8400 ± 3700	3720 ± 1900	213 ± 80	

A: Plasma concentrations of TASP0443294 were measured at 0.5, 1, 2, and 3 h after administration. TASP0443294 at a dose of 3 mg/kg was administered perorally in rats. B: TASP0443294 at doses of 3, 10, and 30 mg/kg was administered perorally in rats. Brain, plasma and CSF samples were collected 2.5 h after administration. Values are mean ± S.D., n = 3 animals per each group. Brain: brain concentration, Plasma: plasma concentration, CSF: CSF concentration.

inhibited the increase in ratio of investigation duration elicited by MK-801 [$F(2,52) = 4.44$, $P < 0.05$) ($P < 0.01$) (Fig. 6).

3.5. Ketamine-induced gamma hyperactivities in the prefrontal cortex of rats

In the time course analyses, a two-way repeated measures ANOVA demonstrated that TASP0443294 (10–30 mg/kg) showed significant dose–time interactions [$F(478,5019) = 1.24$, $P < 0.01$] on the ketamine-increased gamma power (Fig. 7A). TASP0443294 at a dose of 30 mg/kg significantly inhibited the ketamine-increased gamma power ($P < 0.05$) (Fig. 7B).

3.6. REM sleep in rats

TASP0443294 decreased the duration of REM sleep during 2-h period, compared with the vehicle, in a dose-dependent fashion [$F(2,30) = 6.46$, $P < 0.05$]. The effect of TASP0443294 was statistically significant at a dose of 10 mg/kg ($P < 0.01$) (Fig. 8).

4. Discussion

In this study, we demonstrated that an orally active mGlu2 receptor positive allosteric modulator, TASP0443294, improved methamphetamine-induced hyperlocomotion and MK-801-induced social memory deficits. Moreover, TASP0443294 attenuated ketamine-induced basal gamma hyperactivity in the prefrontal cortex and decreased the duration of REM sleep. The present results not only confirm the previous findings that mGlu2 receptor positive allosteric modulators exert antipsychotic effects in animal models, but show that mGlu2 receptor positive allosteric modulators can improve the same neural circuit deficits in the same manner as orthosteric mGlu2/3 receptor agonists.

Regarding the glutamate-induced increase in [^{35}S]GTPγS binding, TASP0443294 exerted similar potentiated activities and a maximal response for human and rat mGlu2 receptors, with EC_{50}

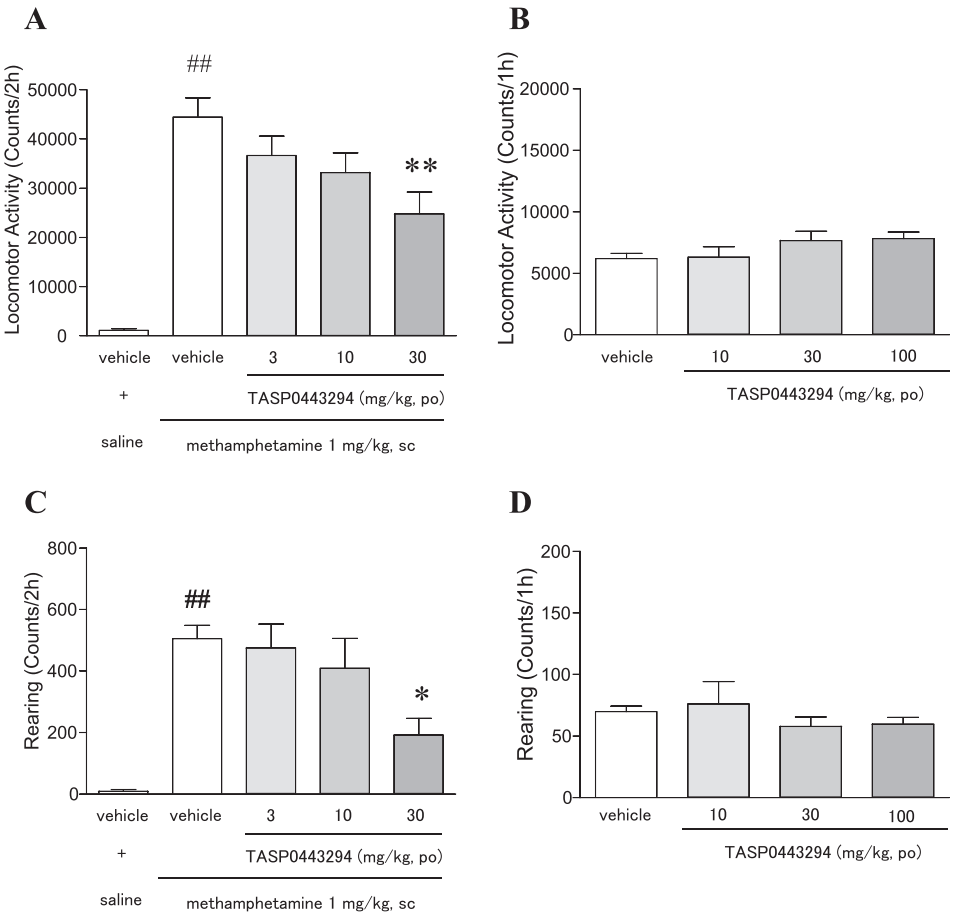

Fig. 4. Effects of TASP0443294 on methamphetamine-induced locomotor (A) and rearing (C) hyperactivities and on spontaneous locomotor (B) and rearing (D) activities in rats. Data represent the mean ± S.E.M., n = 8 animals per each group. $^{\#\#}P < 0.01$, compared with response to vehicle + saline (Student's t-test). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the response to vehicle + methamphetamine (Dunnett's test).

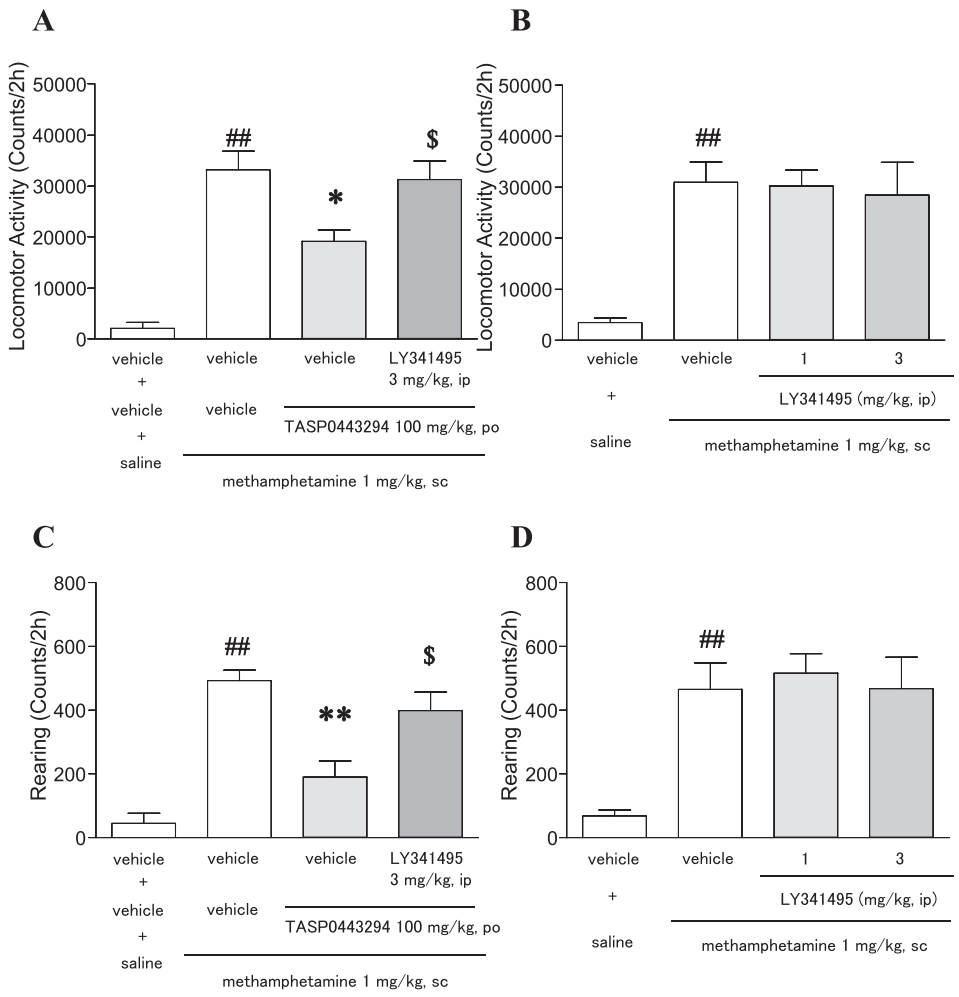

Fig. 5. Effects of LY341495 on TASP0443294 inhibition on methamphetamine-induced locomotor (A) and rearing (C) hyperactivities and effects of LY341495 on methamphetamine-induced locomotor (B) and rearing (D) hyperactivities in rats. Data represent the mean ± S.E.M., n = 8–10 animals per each group. $^{\#\#}P < 0.01$, compared with response to vehicle + vehicle + saline or vehicle + saline (Student's t-test) or. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the response to vehicle + vehicle + methamphetamine (Tukey–Kramer test). $^{\$}P < 0.05$, compared with the response to vehicle + TASP0443294 + methamphetamine (Tukey–Kramer test).

values of 277 and 149 nM and Emax values of 102 and 132%, respectively, without affecting the basal [^{35}S]GTPγS binding. On the other hand, TASP0443294 did not affect the glutamate-induced increase in [^{35}S]GTPγS binding for the human mGlu3 receptor. These findings suggested that TASP0443294 is a selective positive allosteric modulator at the mGlu2 receptor among group II mGlu receptors. Moreover, pharmacokinetic studies revealed that TASP0443294 penetrated into the brain and CSF in quantities sufficient to exert pharmacological effects after peroral administration. Therefore, TASP0443294 might be a useful pharmacological tool for studying the function of mGlu2 receptors in vivo.

The antipsychotic effects of TASP0443294 were evaluated using two animal models of schizophrenia, methamphetamine-induced locomotor hyperactivity and MK-801-impaired social memory. Psychostimulant-induced hyperlocomotion is considered to be an animal model for the positive symptoms of schizophrenia (18), while the induction of social memory impairment by a non-competitive NMDA receptor antagonist, MK-801, in rats is considered to be useful as an animal model for evaluating improvements in cognitive dysfunction in schizophrenia (14). In the present study, TASP0443294 reversed methamphetamine-induced locomotor hyperactivity and improved MK-801-induced social memory impairment in rats. These results further support our previous findings and those of others that both mGlu2/3 receptor agonists and mGlu2 receptor positive allosteric modulators are effective in these models (9,11,19). However, some discrepancies exist among mGlu2 receptor positive allosteric modulators. For example, an mGlu2 receptor positive allosteric modulator, LY487379, attenuated amphetamine-induced locomotor hyperactivity as well as spontaneous locomotor activity in mice (9), while TASP0443294 did not affect spontaneous locomotor activity. Moreover, another mGlu2 receptor positive allosteric modulator, 3'-[[(2-cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1*H*-inden-5-yl)oxy]methyl]-[1,1'-biphenyl]-4-carboxylic acid (BINA), reportedly did not reverse amphetamine-induced hyperlocomotion (10). Although these differences may be ascribed to differences in the mode of potentiation and modest intrinsic agonist activity at the mGlu2 receptor, the reason for these differences among mGlu2 receptor allosteric modulators remains unclear. Different classes of positive allosteric modulators may have different in vivo effects as a result of differential actions on receptors (10,20). Thus, TASP0443294, which is structurally distinct from LY487379 and BINA, may be useful for delineating the molecular mechanisms of the above-mentioned differences among mGlu2 receptor positive allosteric modulators. Of note, the effect of TASP0443294 on methamphetamine-induced locomotor hyperactivity is not an off-target effect, but rather

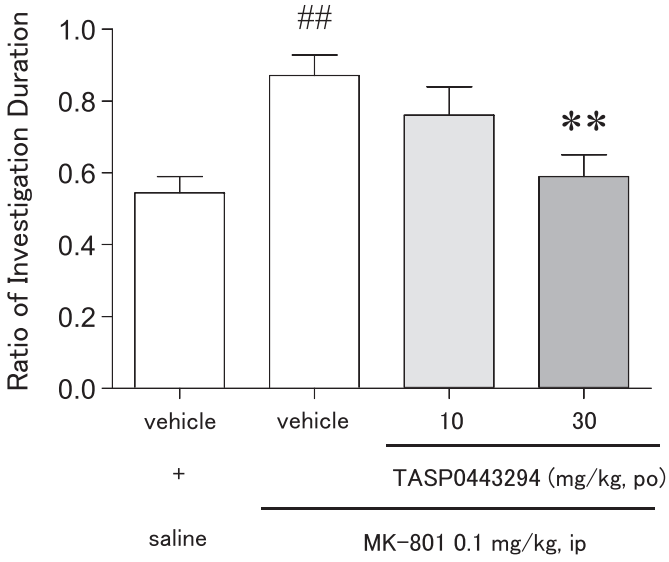

Fig. 6. Effects of TASP0443294 on MK-801-induced social memory deficits in rats. Data represent mean ± S.E.M. n = 18–19 animals per each group. $^{\#\#}P < 0.01$ compared with response to vehicle + saline (Student's *t*-test). $^{**}P < 0.01$ compared with response to vehicle + MK-801 (Dunnett's test).

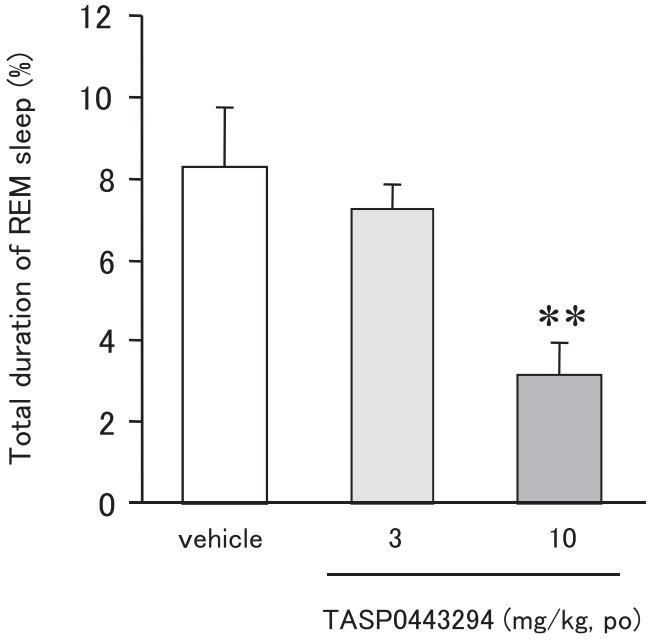

Fig. 8. Effects of TASP0443294 on duration of REM sleep in rats for 2 h after oral administration. Data represent the mean ± S.E.M. n = 11–12 animals per group. $^{**}P < 0.01$, compared with the response to vehicle treatments (Dunnett's test).

mediates the stimulation of the mGlu2 receptor, since the effect of TASP0443294 was completely blocked by an mGlu2/3 receptor antagonist, LY341495, at a dose that did not affect methamphetamine-induced locomotor hyperactivity by itself. However, in addition to the mGlu2/3 receptor, LY341495 also has antagonistic activities at the mGlu7 and mGlu8 receptors (21). Because we have not yet tested the activity of TASP0443294 at the mGlu7 receptor, a possible role of the mGlu7 receptor in the in vivo effect of TASP0443294 cannot be fully ruled out.

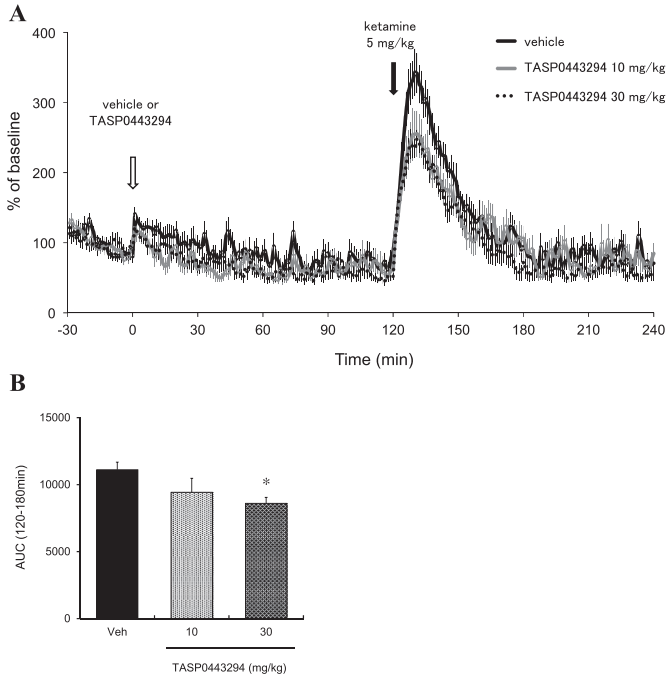

Fig. 7. Effects of TASP0443294 on ketamine-induced elevations in gamma power in rats. (A) Time course changes in gamma power in the frontal cortex of rats. Values are a percentage of the baseline value, which corresponds to the mean value before drug administration. The open arrow indicates the timing of TASP0443294 administration, and the closed arrow indicates ketamine administration. (B) AUC values (120–180 min) for the gamma power in the rat frontal cortex after the administration of ketamine. Data represent the mean ± S.E.M. n = 8 animals per group. $^{*}P < 0.05$, compared with the response to vehicle treatments (Dunnett's test).

In the present study, the neurophysiological effects of TASP0443294 were evaluated using two EEG recordings: REM sleep and ketamine-induced gamma oscillation. EEG is considered to be a translational biomarker for targets and compounds, since EEG can be recorded in both humans and rodents. The present result that TASP0443294 reduced the amount of REM sleep in rats is consistent with previous findings that orthosteric mGlu2/3 receptor agonists and other mGlu2 receptor positive allosteric modulators, such as BINA, THIIC and JNJ-40068782, reduced the amount of REM sleep in rats (12,22–24). Thus, REM sleep as observed on a polysomnogram may be useful as a sensitive biomarker for mGlu2 receptor activation. Glutamate levels reportedly increase during REM sleep in the orbitofrontal and cerebral cortex of rats (25,26). Therefore, the inhibition of glutamate release elicited by the stimulation of mGlu2 receptors may result in a decrease in the duration of REM sleep, and mGlu2 receptor positive allosteric modulators may share the same neuronal mechanisms for their effects on the reduction in REM sleep and their antipsychotic actions.

Aberrant gamma oscillations have been reported to be associated with the symptoms of schizophrenia (27,28). The administration of NMDA-receptor antagonists reportedly increase basal gamma oscillation on EEG recording of activity in the prefrontal cortex, presumably as a result of the disinhibition of pyramidal neurons through a reduction in γ-aminobutyric acid (GABA) interneurons activities (16), which may represent the neuronal mechanism for the glutamate hypothesis explaining schizophrenia (29). In the present study, TASP0443294 inhibited the cortical spontaneous gamma hyperactivity elicited by ketamine in freely moving rats. In a preliminary study, TASP0443294 slightly but dose-dependently reduced the baseline of gamma oscillation power with the same effective doses (data not shown). However, TASP0443294 inhibited the trough-to-peak response of ketamine, although it was not statistically significant (data not shown). Given

that the mGlu2/3 receptor agonist LY379268 inhibits both ketamine-induced increases in gamma oscillations and reduces spontaneous gamma oscillations in the cortex of rats (16,30), the activation of the mGlu2 receptor may play a role in the modulation of gamma oscillations. Of note, this is the first result to show that the attenuation of NMDA receptor antagonist-induced aberrant gamma oscillations by an mGlu2/3 receptor agonist is mediated through the stimulation of the mGlu2 receptor, and that mGlu2 receptor positive allosteric modulators may improve the same neuronal circuit deficits possibly observed in schizophrenia as do orthosteric mGlu2/3 receptor agonists. The hippocampus (31), amygdala (32), and prefrontal cortex (33) are thought to be involved in social memory in rodents. Therefore, the improvement in social memory impairment induced by an mGlu2 receptor positive allosteric modulator may be partially attributable to the suppression of the aberrant excitability of pyramidal neurons through GABAergic disinhibition, at least in the prefrontal cortex. It would be interesting to compare the effect of mGlu2 receptor positive allosteric modulators on ketamine-increased gamma oscillation and locomotor activity in future studies.

In summary, the orally active mGlu2 receptor positive allosteric modulator TASP0443294 exhibits antipsychotic-like activities in animal models. TASP0443294 inhibited not only dopamine-related, but also NMDA receptor-related abnormal behaviors that are considered to be animal models for the positive symptoms and cognitive dysfunction of schizophrenia, respectively. Moreover, because TASP0443294 improved aberrant gamma oscillations, gamma oscillations, in addition to REM sleep, may be useful as translatable biomarkers for mGlu2 receptor positive allosteric modulators. In addition, TASP0443294 may provide a useful tool for examining the usefulness of mGlu2 receptor positive allosteric modulators for the treatment of schizophrenia as well as the neuronal mechanisms underlying the antipsychotic effects of mGlu2 receptor positive allosteric modulators.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

We thank Dr. Daiji Kambe for his valuable assistance in analysis of EEG data. We thank Mr. Katsuya Iwata and Dr. Takeshi Aoki for assistance in the pharmacokinetics studies and the in vitro assays, respectively. We thank Mr. Shinsuke Kurosu for consultations of statistical analysis. We also thank Dr. Shigetada Nakanishi of Osaka Bioscience Institute for providing us with CHO cell lines expressing rat mGlu2 receptor.

References

(1) Cartmell J, Schoepp DD. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem. 2000;75:889–907.
(2) Ohishi H, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study. J Comp Neurol. 1993;335:252–266.
(3) Shigemoto R, Kinoshita A, Wada E, Nimura S, Ohishi H, Takada M, et al. Differential presynaptic localization of metabotropic glutamate receptor subtypes in rat hippocampus. J Neurosci. 1997;17:7503–7522.
(4) Chaki S. Group II metabotropic glutamate receptor agonists as a potential drug for schizophrenia. Eur J Pharmacol. 2010;639:59–66.
(5) Marek GJ. Metabotropic glutamate2/3 (mGlu2/3) receptors, schizophrenia and cognition. Eur J Pharmacol. 2010;639:81–90.
(6) Fell MJ, Svensson KA, Johnson BG, Schoepp DD. Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist (−)-(1R, 4S, 5S, 6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4, 6-dicarboxylic acid (LY404039). J Pharmacol Exp Ther. 2008;326:209–217.
(7) Woolley ML, Pemberton DJ, Bate S, Corti C, Jones DN. The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. Psychopharmacology (Berl). 2008;196:431–440.
(8) Schaffhauser H, Rowe BA, Morales S, Chavez-Noriega LE, Yin R, Jachec C, et al. Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. Mol Pharmacol. 2003;64:798–810.
(9) Galici R, Echemendia NG, Rodriguez AL, Conn PJ. A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. J Pharmacol Exp Ther. 2005;315:1181–1187.
(10) Galici R, Jones CK, Hemstapat K, Nong Y, Echemendia NG, Williams LC, et al. Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. J Pharmacol Exp Ther. 2006;318:173–185.
(11) Hikichi H, Kaku A, Karasawa J, Chaki S. Stimulation of metabotropic glutamate (mGlu) 2 receptor and blockade of mGlu1 receptor improve social memory impairment elicited by MK-801 in rats. J Pharmacol Sci. 2013;122:10–16.
(12) Lavreysen H, Langlois X, Ahnaou A, Drinkenburg W, te Riele P, Biesmans I, et al. Pharmacological characterization of JNJ-40068782, a new potent, selective, and systemically active positive allosteric modulator of the mGlu2 receptor and its radioligand [3H]JNJ-40068782. J Pharmacol Exp Ther. 2013;346:514–527.
(13) Funakoshi T, Chaki S, Kawashima N, Suzuki Y, Yoshikawa R, Kumagai T, et al. In vitro and in vivo pharmacological profile of 5-[2-[4-(6-fluoro-1H-indole-3-yl)piperidin-1-yl]ethyl]-4-(4-fluorophenyl)thiazole-2-carboxylic acid amide (NRA0562), a novel and putative atypical antipsychotic. Life Sci. 2002;71:1371–1384.
(14) Shimazaki T, Kaku A, Chaki S. D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. Psychopharmacology (Berl). 2010;209:263–270.
(15) Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. New York: Elsevier/Academic Press; 1998.
(16) Hiyoshi T, Hikichi H, Karasawa J, Chaki S. Metabotropic glutamate receptors regulate cortical gamma hyperactivities elicited by ketamine in rats. Neurosci Lett. 2014;567:30–34.
(17) Akanmu MA, Honda K. Selective stimulation of orexin receptor type 2 promotes wakefulness in freely behaving rats. Brain Res. 2005;1048:138–145.
(18) Bubeníková-Valesová V, Horácek J, Vrajová M, Höschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. Neurosci Biobehav Rev. 2008;32:1014–1023.
(19) Rorick-Kehn LM, Johnson BG, Knitowski KM, Salhoff CR, Witkin JM, Perry KW, et al. In vivo pharmacological characterization of the structurally novel, potent, selective mGlu2/3 receptor agonist LY404039 in animal models of psychiatric disorders. Psychopharmacology (Berl). 2007;193:121–136.
(20) Zhang Y, Rodriguez AL, Conn PJ. Allosteric potentiators of metabotropic glutamate receptor subtype 5 have differential effects on different signaling pathways in cortical astrocytes. J Pharmacol Exp Ther. 2005;315:1212–1219.
(21) Kingston AE, Ornstein PL, Wright RA, Johnson BG, Mayne NG, Burnett JP, et al. LY341495 is a nanomolar potent and selective antagonist of group II metabotropic glutamate receptors. Neuropharmacology. 1998;37:1–12.
(22) Ahnaou A, Dautzenberg FM, Geys H, Imogai H, Gibelin A, Moechars D, et al. Modulation of group II metabotropic glutamate receptor (mGlu2) elicits common changes in rat and mice sleep-wake architecture. Eur J Pharmacol. 2009;603:62–72.
(23) Feinberg I, Campbell IG, Schoepp DD, Anderson K. The selective group mGlu2/3 receptor agonist LY379268 suppresses REM sleep and fast EEG in the rat. Pharmacol Biochem Behav. 2002;73:467–474.
(24) Fell MJ, Witkin JM, Falcone JF, Katner JS, Perry KW, Hart J, et al. N-(4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyryl)phenoxy)methyl)benzyl)-1-methyl-1Himidazole-4-carboxamide (THIIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant properties: in vivo profiling suggests a link between behavioral and central nervous system neurochemical changes. J Pharmacol Exp Ther. 2011;336:165–177.
(25) Lopez-Rodriguez F, Medina-Ceja L, Wilson CL, Jhung D, Morales-Villagran A. Changes in extracellular glutamate levels in rat orbitofrontal cortex during sleep and wakefulness. Arch Med Res. 2007;38:52–55.
(26) Dash MB, Douglas CL, Vyazovskiy VV, Cirelli C, Tononi G. Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. J Neurosci. 2009;29:620–629.
(27) Spencer KM, Nestor PG, Perlmutter R, Niznikiewicz MA, Klump MC, Frumin M, et al. Neural synchrony indexes disordered perception and cognition in schizophrenia. Proc Natl Acad Sci U S A. 2004;101:17288–17293.
(28) Suazo V, Díez Á, Martín C, Ballesteros A, Casado P, Martín-Loeches M, et al. Elevated noise power in gamma band related to negative symptoms and memory deficit in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2012;38:270–275.
(29) Krystal JH, D'Souza DC, Mathalon D, Perry E, Belger A, Hoffman R. NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. Psychopharmacology (Berl). 2003;169:215–233.
(30) Jones NC, Reddy M, Anderson P, Salzberg MR, O'Brien TJ, Pinault D. Acute administration of typical and atypical antipsychotics reduces EEG γ power,

but only the preclinical compound LY379268 reduces the ketamine-induced rise in γ power. Int J Neuropsychopharmacol. 2012;15:657–668.
(31) Bannerman DM, Lemaire M, Yee BK, Iversen SD, Oswald CJ, Good MA, et al. Selective cytotoxic lesions of the retrohippocampal region produce a mild deficit in social recognition memory. Exp Brain Res. 2002;142:395–401.
(32) Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci. 2001;21:8278–8285.
(33) Dantzer R, Tazi A, Bluthe RM. Cerebral lateralization of olfactorymediated affective processes in rats. Behav Brain Res. 1990;40:53–60.