Journal of Pharmacological Sciences 127 (2015) 352–361

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).

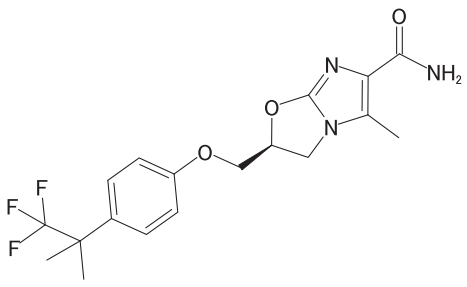

Fig. 1. Chemical structure of TASP0443294.

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