Neuroscience Letters 567 (2014) 30–34

Contents lists available at ScienceDirect

Neuroscience Letters

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

# Metabotropic glutamate receptors regulate cortical gamma hyperactivities elicited by ketamine in rats

Tetsuaki Hiyoshi*, Hirohiko Hikichi, Jun-ichi Karasawa, Shigeyuki Chaki

*Discovery Pharmacology I, Molecular Function and Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan*

## HIGHLIGHTS

- Ketamine increases basal gamma oscillation in the prefrontal cortex of rats.
- An mGluR2/3 agonist reduces baseline and ketamine-increased gamma oscillation power.
- An mGluR1 antagonist reduces ketamine-increased gamma oscillation power.

## ARTICLE INFO

*Article history:*
Received 28 January 2014
Received in revised form 12 March 2014
Accepted 15 March 2014

*Keywords:*
EEG
Gamma
Oscillations
Ketamine
mGlu2/3 receptor agonist
mGlu1 receptor antagonist

## ABSTRACT

Abnormalities in electroencephalogram gamma oscillations have been implicated in schizophrenic symptoms. N-methyl-D-aspartate (NMDA) receptor antagonists produce behavioral abnormalities that are similar to the symptoms of schizophrenia, including social and cognitive impairment, and also increase the power of spontaneous gamma oscillations in the frontal cortex in rodents. Both mGlu2/3 receptor agonists and mGlu1 receptor antagonists reportedly improve behavioral abnormalities elicited by NMDA receptor antagonists in rodents. The present study evaluated the effects of an mGlu2/3 receptor agonist and an mGlu1 receptor antagonist on aberrant basal gamma oscillations elicited by an NMDA receptor antagonist, ketamine, in the rat frontal cortex. Ketamine increased spontaneous cortical gamma oscillations. Pretreatment with an mGlu2/3 receptor agonist, (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268), or an mGlu1 receptor antagonist, (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxycyclohexyl)-methanone (JNJ16259685), reduced the ketamine-induced basal gamma hyperactivity. These findings indicate that the stimulation of mGlu2/3 receptors and the inhibition of mGlu1 receptors reverse aberrant gamma oscillations, and these effects may partially explain the antipsychotic-like properties of mGlu2/3 receptor agonists and mGlu1 receptor antagonists.



## 1. Introduction

Glutamatergic dysfunction, particularly the hypofunction of N-methyl-D-aspartate (NMDA) receptors, has been suggested to be involved in the pathophysiology of schizophrenia [1–4]. Consistent with this hypothesis, non-competitive NMDA receptor antagonists, such as ketamine and phencyclidine, can induce positive and negative symptoms and cognitive dysfunction reminiscent of those seen in schizophrenia in healthy volunteers and exacerbate the symptoms in schizophrenic patients [5–8]. Moreover, they produce behavioral abnormalities in rodents that are similar to the symptoms observed in schizophrenia [9,10].

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 these, group I (mGlu1 and mGlu5) and group II (mGlu2 and mGlu3) receptors have been proposed to have important roles in the pathophysiology of schizophrenia. The activation of group I mGlu receptor coupled to $G_{q/11}$ leads to postsynaptic excitatory signal transduction, whereas activation of group II mGlu receptor coupled to $G_{i/o}$ protein leads to presynaptic inhibitory signal transduction. Indeed, mGlu2/3 receptor agonists and mGlu1 receptor antagonists reportedly reversed NMDA receptor antagonist-induced hyperlocomotion and social deficits in rodents [9,11,12]. In addition, we previously reported

* Corresponding author. Tel.: +81 48 669 3067; fax: +81 48 654 6650.
*E-mail address:* t-hiyoshi@so.taisho.co.jp (T. Hiyoshi).

http://dx.doi.org/10.1016/j.neulet.2014.03.025
0304-3940/

that an mGlu2/3 receptor agonist and an mGlu1 receptor antagonist improved NMDA receptor antagonist-induced social memory impairment in rats [10]. Therefore, both the stimulation of the mGlu2/3 receptor and the blockade of the mGlu1 receptor may be useful approaches for the treatment of schizophrenia.

The oscillatory activity in neuronal populations is considered to be associated with the coordination of individual neurons [13]. Disturbances in cortical oscillations have been suggested as a possible neural basis for the symptoms of schizophrenia [14,15]. In particular, increased gamma-frequency oscillations have been reported in schizophrenic patients [16–18]. A higher power of gamma oscillations is correlated with the visual hallucinations score [19] and the severity of negative symptoms [18] and is inversely correlated with verbal memory performance [18] in schizophrenia, suggesting that aberrant gamma oscillations may be associated with the symptoms of schizophrenia. Previous studies in healthy humans and rodents have shown that NMDA receptor antagonists increase the power of gamma oscillations [20–23]. Of note, the aberrant gamma oscillations induced by ketamine were dissociated from the motor effects of ketamine, since the aberrant increases were generated even under the influence of anesthesia [23]. Thus, the increased gamma oscillations elicited by the NMDA receptor antagonists may reflect schizophrenic-like behaviors and symptoms.

Therefore, in the present study, we investigated the effects of an mGlu2/3 receptor agonist and an mGlu1 receptor antagonist on ketamine-induced aberrant cortical gamma oscillations in freely moving rats.

## 2. Methods

### 2.1. Animals

Male Sprague–Dawley rats (9–15-week-old, Charles River Japan, Inc., Yokohama, Japan) were used for this study. The rats were housed in a controlled animal room (room temperature; 23 ± 3 °C, humidity 55 ± 20%) with a 12 h light-dark cycle (light on; 07:00–19:00). Rats were maintained in groups of 2 rats per cage. Food and water were available *ad libitum*. All the studies were reviewed by 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).

### 2.2. EEG electrode implantation

Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus. After drilling holes in the skull, stainless steel screw electrodes (E363/20, PlasticsOne) were placed on the cerebral dura mater of the lateral frontal cortex (recording electrode: 1.0 mm anterior, 1.5 mm lateral from the bregma), cerebellum cortex (reference electrode: 10.0 mm posterior, 0.0 mm lateral from the bregma) and parietal cortex (ground electrode: 8.0 mm posterior, 1.5 mm lateral from the bregma) for the electroencephalogram (EEG) recording, with the positions determined according to a rat brain atlas [24]. The electrodes were socketed into an electrode pedestal (MS363; PlasticsOne), which was fixed to the skull with dental resins and superglue. The rats were allowed to recover and were singly housed for 5 days or more before EEG recording.

### 2.3. EEG recording environment

Rats were individually placed in a cage (30 cm wide × 30 cm deep × 35 cm high) that was placed in a soundproof box (60 cm wide × 50 cm deep × 50 cm high). The electrode pedestals were tethered to a lead wire with a slip ring (NSR-15-6P; Solton), and the animals were each placed in the cage to enable measurements to be obtained under freely moving and unrestrained conditions. After each rat was acclimated to the cage for 30 min, they were injected subcutaneously with either (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268, 0.3-1 mg/kg), (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxycyclohexyl)-methanone (JNJ16259685, 1 mg/kg) or the respective vehicles. After a further 30 min, the rats were then injected subcutaneously with ketamine (5 mg/kg). The EEG recording was started at 30 min before the drug administration (LY379268 or JNJ16259685) and ended at 120 min after the ketamine administration.

### 2.4. EEG data acquisition processing

The signals from the EEG electrodes were amplified (20,000 times) and bandpass filtered (0.5–1000 Hz) with a biophysical amplifier (AB-611 J; Nihon Kohden, Tokyo, Japan). The signals were digitized at a sampling rate of 2.5 kHz with an analog-to-digital converter (AD16-16U(PCI)EH; Contec) and recorded using the data acquisition program SleepSign® (ver.3.0, Kissei Comtec). After the digital filtering (0.5–200 Hz bandpass), the power of the gamma frequency band (30–80 Hz) was determined using fast Fourier transformations for each 4-s epoch over the entire recording period, and the average power was then calculated for each 1-min bin. Each gamma power data was expressed as a percent change to the baseline response, which was the averaged power during the 30-min pre-treatment period. The area under the curve (AUC) for the gamma power change was calculated to evaluate the drug's effects.

### 2.5. Drugs

LY379268 and JNJ16259685 were purchased from Tocris Bioscience (Bristol, UK). Ketamine was purchased as veterinary Ketalar® 50 from Sankyo Yell Pharmaceutical Co., Ltd. (Tokyo, Japan). LY379268 and JNJ16259685 were dissolved in saline and 10% hydroxypropyl-β-cyclodextrin, respectively. Ketamine was diluted with saline. The dose selection for all the drugs were based on previous reports [10,25,26]. LY379268 and JNJ16259685 did not cause overt behavioral changes when administered alone. All the drugs were injected at a volume of 2 mL/kg body weight.

### 2.6. Statistical analysis

All the statistical analyses were performed using SAS software (SAS Institute Japan, Tokyo). The time courses for the gamma oscillations power changes were analyzed using a two-way repeated measures analysis of variance (ANOVA). Data from the AUC of the gamma oscillations power change were analyzed using a one-way ANOVA followed by Dunnett's post hoc test or using the Student's *t*-test. A value of $P < 0.05$ was regarded as significant.

## 3. Results

### 3.1. Effects of the mGlu2/3 receptor agonist

A two-way repeated measures ANOVA revealed a significant dose effect ($F_{2,21} = 8.43$, $P < 0.01$), time effect ($F_{149,3129} = 51.37$, $P < 0.01$), and dose-time interactions ($F_{298,3129} = 1.32$, $P < 0.01$) of LY379268 (0.3–1 mg/kg) on the spontaneous and ketamine (5 mg/kg)-increased gamma powers (Fig. 1A). LY379268 decreased the spontaneous gamma power compared with the vehicle, in a dose-dependent fashion ($F_{2,21} = 7.305$, $P < 0.01$; Fig. 1B). The effect of LY379268 was statistically significant at a dose of 1 mg/kg. Ketamine (5 mg/kg) produced an acute increase in the gamma power compared with the baseline before the administration of