JPET Fast Forward. Published on May 22, 2008 as DOI: 10.1124/jpet.108.138107
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JPET #138107

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-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide)

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(AS, SM, SI, HH, MF, GS, TK, TT, SI, HK and HO)

Running Title: Pharmacological roles of mGluR1: Investigations with FTIDC

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Pages of text: 31

Number of tables: 3

Number of figures: 8

Number of references: 40

Words in Abstract: 172

Words in Introduction: 727

Words in Discussion: 1487

JPET Fast Forward. Published on May 16, 2008 as DOI: 10.1124/jpet.108.138107
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Nonstandard abbreviations:

CNS, central nervous system; mGluR, metabotropic glutamate receptor; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate; LY379268, (1*R*,4*R*,5*S*,6*R*)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid; MPEP, 2-methyl-6-(phenylethynyl)pyridine; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine; JNJ16259685, (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)(*cis*-4-methoxycyclohexyl)methanone; BAY36-7620, (3a*S*,6a*S*)-6a-naphtalan-2-ylmethyl-5-methyliden-hexahydro-cyclopenta[*c*]furan-1-one; YM-298198, 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide; YM-230888, *N*-cycloheptyl-6-({[(2*R*)-tetrahydrofuran-2-ylmethyl]amino}methyl)thieno[2,3-*d*]pyrimidin-4-amine; EMQMCM, (3-ethyl-2-methyl-quinolin-6-yl)(4-methoxy-cyclohexyl)methanone methansulfonate; 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; DHPG, 3,5-dihydroxyphenylglycine; i.p., intraperitoneal; s.c., subcutaneous; MAP, methamphetamine; USV, ultrasonic vocalization; SIH, stress-induced hyperthermia; PPI, prepulse inhibition; iGluR, ionotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; CHPG, 2-chloro-5-hydroxyphenylglycine; CPCCOEt, 7-(hydroxyimino)cyclopropan[*b*]chromen-1a-carboxylic acid ethyl ester; MK-801, (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine maleate

Recommended section assignment: Neuropharmacology

ABSTRACT

The functional roles of metabotropic glutamate receptor (mGluR) 1 in integrative brain functions were investigated using a potent and selective mGluR1 allosteric 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) in comparison with mGluR5 allosteric antagonist and mGluR2/3 orthosteric agonist in rodents. FTIDC reduced maternal separation-induced ultrasonic vocalization and stress-induced hyperthermia without affecting behaviors in the elevated plus maze. An mGluR5 antagonist, MPEP, and an mGluR2/3 agonist, LY379268, showed anxiolytic activities in these models, suggesting involvement of postsynaptic mGluR1 in stress-related responses comparable to mGluR5 and mGluR2/3. Analgesic effects of FTIDC were seen in the formalin test, but not in the tail immersion test. FTIDC selectively blocked methamphetamine-induced hyperlocomotion and disruption of prepulse inhibition, while MPEP and LY379268 did not alter those behaviors, suggesting that pharmacological blockade of mGluR1 could result in antipsychotic-like effects. FTIDC neither elicited catalepsy nor impaired motor functions at 10 times higher dose than doses showing antipsychotic-like action. In conclusion, blockade of mGluR1 showed antipsychotic-like effects without impairing motor functions, while blockade of mGluR5 and activation of mGluR2/3 did not display such activities.

INTRODUCTION

L-glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and acts on ionotropic and metabotropic glutamate receptors (mGluRs). The mGluR family consists of eight receptor subtypes, which are divided into three groups based on sequence homology, pharmacological profiles and signal transduction pathways (De Blasi et al., 2001; Spooren et al., 2003). Group I mGluRs comprise mGluR1 and mGluR5, which are coupled with Gq to activate phospholipase C, leading to the release of intracellular calcium. Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7 and mGluR8) mGluRs are negatively coupled *via* Gi to adenylyl cyclase, resulting in the inhibition of cyclic adenosine monophosphate production and protein kinase A activity.

The mGluRs are expressed on neuronal and glial cells, with each receptor subtype exhibiting distinct spatial and temporal expression profiles in the brain (Shigemoto et al., 1992), with the exception of mGluR6 existing in the retina (Nakajima et al., 1993). In neurons, group I mGluRs are mainly localized in somatodendritic domains and postsynaptically regulate neuronal excitability and synaptic transmission *via* several intracellular second messenger systems, whereas group II and III mGluRs are predominantly localized in axonal domains and axon terminals to presynaptically regulate neurotransmitter release (Shigemoto et al., 1992). In fact, activation of mGluR2/3 has been demonstrated using *in vivo* microdialysis techniques to reduce release of various neurotransmitters (Cartmell and Schoepp, 2000), including glutamate (Battaglia et al., 1997).

One of the most interesting and earliest breakthroughs in the development of agents targeting mGluRs was the identification of highly selective agonists for group II mGluRs, such as LY354740 and LY379268. These compounds have demonstrated anxiolytic effects in rodents (Helton et al., 1998; Kłodzińska et al., 1999) and humans (Grillon et al., 2003). In addition, activation of mGluR2/3 may lead to anticonvulsive (Kłodzińska et al.,

2000), antinociceptive (Simmons et al., 2002) and antipsychotic effects (Cartmell et al., 1999; Cartmell et al., 2000).

Since these results with activation of mGluR2/3 suggest that control of excessive excitation in glutamatergic neurotransmission is one of the underlying mechanisms, some of the pharmacological effects of mGluR2/3 activation may be mimicked by modulating postsynaptic glutamate receptors, including both ionotropic glutamate receptors (iGluRs) and mGluRs. *N*-methyl-D-aspartate (NMDA) antagonists show anxiolytic and antinociceptive effects in animal models (Fundytus, 2001; Cortese and Phan, 2005), and generally cause psychotic behaviors in animals and humans (Javitt, 2004). Non-NMDA antagonists produce antinociception in pain models, but effects on anxiety remain controversial (Cortese and Phan, 2005). These findings suggested that some effects of mGluR2/3 agonists by reducing glutamatergic neurotransmission are not fully dependent upon iGluRs pathways. Blockade of mGluR5 with its allosteric antagonists MPEP and MTEP has demonstrated amelioration of anxiety (Tatarczyńska et al., 2001; Busse et al., 2004) and nociception (Zhu et al., 2004; Varty et al., 2005). Blockade of mGluR5 and activation of mGluR2/3 may share similar outcomes to some extent. On the other hand, roles of mGluR1 in the pharmacological effects of mGluR2/3 agonists have not been well studied.

Demonstration of pharmacological effects by blockade of mGluR1 has been hampered by the lack of appropriate antagonists until the recent development of allosteric antagonists including JNJ16259685, BAY 36-7620, YM-230888 and EMQMCM (De Vry et al., 2001; Lavreysen et al., 2004; Sevostianova and Danysz, 2006; Kohara et al., 2007). Of these, only JNJ16259685 has been demonstrated to be CNS-permeable and act as a selective mGluR1 allosteric antagonist, showing full receptor occupancy in the brain at and above 0.16 mg/kg (Lavreysen et al., 2004b). JNJ16259685 demonstrated anxiolytic effects in the Vogel conflict test, reduction of spontaneous motor activities and impairment of spatial acquisition in Morris water maze (Steckler et al., 2005a; Steckler et

al., 2005b). However, the (patho)physiological roles of mGluR1 in other brain functions in which activation of mGluR2/3 altered have not been tested with JNJ16259685.

We have recently identified 4-[1-(2-fluoropyridine-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide (FTIDC) as a potent and selective mGluR1 allosteric antagonist (Suzuki et al., 2007). FTIDC blocked DHPG-induced face-washing behaviors at doses of 10 and 30 mg/kg in mice, indicating that these doses of FTIDC inhibited mGluR1-mediated brain functions *in vivo* (Suzuki et al., 2007) and that FTIDC was a useful tool for exploring the pharmacological roles of mGluR1 in CNS. The purpose of this study is to evaluate what extent of effects of FTIDC in animal models of anxiety, nociception and psychosis. Effects of FTIDC were compared with those of the mGluR5 antagonist and the mGluR2/3 agonist.

JPET Fast Forward. Published on May 16, 2008 as DOI: 10.1124/jpet.108.138107
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MATERIALS AND METHODS

Animals

All experiments were performed using adult male CD1 (ICR) mice (Japan SLC, Shizuoka, Japan), C57BL/6J mice (Clea Japan, Tokyo, Japan) and Sprague-Dawley rats (Charles River Laboratories Japan, Kanagawa, Japan). Animals were housed in an air-conditioned room with a 12:12 light/dark cycle (lights off at 19:00) and allowed to *ad libitum* access to food (CE-2; Clea Japan) and tap water. At least 6 days were allowed for acclimatization to the facility before starting experiments. All experiments were approved by our Institutional Animal Care and Use Committee.

Compounds and Administration Procedures

The structure for FTIDC was disclosed in our previous manuscript (Suzuki et al., 2007). FTIDC was used in all experiments as the free base. FTIDC was suspended into 0.5% methylcellulose for intraperitoneal (i.p.) administration. We detected 0.8 ± 0.4 μM in plasma and 0.4 ± 0.2 nmol/g in brain 30 min after administration of 30 mg/kg of FTIDC. These concentrations in plasma and brain were 26-fold and 13-fold higher concentration than the concentration required to completely suppress agonist induced increases in intracellular Ca^{2+} (0.03 μM), respectively (Suzuki et al., 2007). Thus the behavioral studies are conducted between 30 min and 60 min after administration. Specific time was noted in each behavioral assay. Dosing volume was 10 mL/kg and 1 mL/kg for mice and rats, respectively. MPEP and LY379268 (Tocris Bioscience, Ellisville, MO) were administered intraperitoneally in suspension of 0.5% methylcellulose. Methamphetamine (MAP) was purchased from Dainippon Sumitomo Pharma (Osaka, Japan) and dissolved in physiological saline for s.c. administration. The following drugs were used as positive control drugs to demonstrate sensitivity of methods in appropriate animal models. Haloperidol (Serenece®; Dainippon Sumitomo Pharma) was diluted by distilled water for subcutaneous (s.c.) administration. Morphine (Takeda Pharmaceutical,

Osaka, Japan) was dissolved in physiological saline for s.c. injection. Diazepam (Wako Junyaku Kogyo, Osaka, Japan) was administered intraperitoneally in suspension of 0.5% methylcellulose.

Elevated Plus Maze in Mice

The elevated plus maze was made from gray polyvinyl chloride and consisted of two open arms (L 30 × W 5 cm; surrounded by a 0.3-cm rim) and two enclosed arms (L 30 × W 5 cm; with 15-cm-high walls), which extended from a central platform (5 × 5 cm). The maze was elevated 70 cm above the floor and was illuminated by a dim light (60 lux) at the end of each open arm. Experiments were monitored through a CCD camera (Watec, Yamagata, Japan) placed 1.5 m above the maze then recorded and analyzed automatically using TARGET software (Neuroscience, Tokyo, Japan). A C57BL/6J mouse was brought to the vestibule of experimental room at least 30 min before start of the experiment. At 30 min after i.p. injection of test compound, mice (n = 10-26/group) were placed in the center of the maze facing an open arm. During the following 5-min period, time spent on and the numbers of entries onto each arm were recorded. An arm entry was defined when all four paws entered the arm. The maze was wiped using distilled water after each trial. Experiments were performed between 09:00 and 17:00.

Measurement of Maternal Separation-induced Ultrasonic Vocalization in Rat Pups

On the day of the study, rat pups (postnatal day 10) with their mother were moved to the experimental room and left undisturbed for at least 60 min. Pups (n = 4-12/group) received i.p. injection of test drugs, then was returned to the mother. After 30 min, each pup was placed in a stainless-steel chamber (size: ϕ 10.5 × H 16 cm) on a Cool Plate® (NCP-2215; Nisshin Rika, Tokyo, Japan), which maintained temperature of the chamber at 24ºC in a sound-proof room (AT-81; Rion, Tokyo, Japan). The number of ultrasonic vocalizations was measured for 5 min. Individual calls made by each offspring during

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this period were collected by microphone, amplified by a dedicated preamplifier (NH-05A; RION) and a main amplifier (NH-04A; Rion) with a filter (multifunction filter 3611; NF Corporation, Kanagawa, Japan; settings: high pass 1.5 kHz). Analog signals were then converted to digital signals by an A/D converter (CH-3150; Exacq Technologies, Indianapolis, IN) and stored in a personal computer. Transferred digital data were automatically counted using recording software developed by our group (Dasy Lab® 7.0, measX, Moenchengladbach, Germany). The threshold value was set at a signal amplitude of 0.1 V to exclude noise. Each pup was immediately returned to its mother in the home cage after measurement. Behavioral changes under drug treatment were observed during the experiment and body surface temperature of each pup was also monitored using an infrared thermometer (Thermo-Hunter® PT3S; Optex, Shiga, Japan) immediately before the recording sessions.

Stress-induced Hyperthermia (SIH) in Mice

The effects of FTIDC on SIH were examined using the modified classic SIH paradigm described by Olivier et al. (2003). On the experiment day, all ICR mice used in the study were transferred from group housing to individual housing before the test and were allowed to acclimatize to the experimental environment for at least 60 min. The first measurement captured basal core temperature (T_1), then 10 min later, a second measurement (T_2) captured temperature under stress. The difference between first and second temperatures ($\Delta T = T_2 - T_1$) was defined as SIH. Rectal temperature was measured with a lubricated thermistor probe (Physitemp Instruments, Clifton, NJ) inserted into the rectum of each subject. Test compounds were administered to mice (n = 7-8/group) by i.p. route 60 min before first measurement.

Formalin Test in Mice

ICR mice (n = 6-10/group) were individually placed in a clear plastic chamber (13

× 10 × 15.5 cm). Animals were habituated to the chamber for more than 60 min prior to the experimental sessions, then 20 μL of formalin (2%) was injected subcutaneously into the foot pad of the mouse hind paw using a 30-gauge needle. After injection, mice were immediately returned to the chamber and formalin-induced behaviors were recorded for a period of 40 min. All tested substances were injected 30 min before formalin administration. The duration of licking and biting of the injected paw was measured manually. For analysis of antinociceptive effects of drugs, the period from immediately to 10 min after formalin injection was defined as the first phase and the period from 15 to 25 min after formalin injection was defined as the second phase.

Spontaneous Locomotor Activity and Methamphetamine (MAP)-induced Hyperlocomotion in Mice

Locomotor activity of ICR mice was measured by an activity-monitoring system (NS-AS01; Neuroscience, Tokyo, Japan) in a test cage using previously reported methods (Narita et al., 2002). In brief, the activity monitor was composed of the infrared ray sensor placed over each test cage (L 22.5 × W 33.8 × H 14.0 cm), a signal amplification circuit and a control circuit. Motor activity was collected every 1 min and data were stored and analyzed with a computer-associated analyzing system (DAS System-24A; Neuroscience). Animals (n = 6-17/group) were administered test compounds and then immediately placed into the test cage. Spontaneous locomotor activity was then measured for 1 hour. In the drug interaction study, either mGluR1 antagonist or haloperidol was administered 30 min before MAP was subcutaneously injected at 2 mg/kg. Immediately after MAP injection, the mouse was placed into the test cage and locomotor activity was measured for 1 h.

Measurement of Prepulse Inhibition (PPI) in Rats

Sprague-Dawley rats were used to measure PPI in the Startle Response System

(SR-Lab; San Diego Instruments, San Diego, CA). The whole apparatus was housed in a ventilated chamber (L 39 × W 38 × H 58 cm). Presentation of acoustic stimuli was controlled by SR-Lab software and interface that digitized, rectified and recorded responses from the accelerometer in a personal computer. Mean startle amplitude was determined by averaging 100 one-millisecond readings taken at the pulse stimulus onset. On the day of PPI testing, rats (n = 8-13/group) were placed into the startle apparatus. After a period of 5 min, during which rats were exposed to background noise (60 dB), the session began with rats being exposed to different auditory stimuli. The session began with 15 of each of the following six trial types: a) startle stimulus (120 dB, 40 ms); b-d) startle stimulus preceded by 100 ms by prepulses at 3, 6 and 12 dB above background noise (60 dB) and lasting 20 ms; e) prepulse stimulus (72 dB, 20 ms); and f) no stimulus. These six trial types were interspersed across 15 consecutive blocks. Intertrial interval was pseudo-randomly varied between 15 and 30 s and sessions lasted approximately 35 min. Data collected in the first five blocks were discarded due to large variance of startle responses and data from the last 10 blocks were analyzed. Haloperidol was administered 15 min prior to the beginning of the session and other drugs were administered 30 min prior to the session. In the case of drug interaction studies, MAP at 3 mg/kg was subcutaneously injected 10 min prior to the session.

Catalepsy Assay in Rats

Catalepsy was determined by placing the front paws of the animal over a rod raised approximately 10 cm above the floor. The duration for which the animal remained in this forced position was measured with a "cut-off" of 60 s. The measurement procedure was repeated 3 times and the longest duration holding onto a raised rod with the front paws was taken for subsequent analysis. Test compounds were administered into rats (n = 5-15/group) 60 min before this study.

Rotarod Test in Mice

Effects of test compounds on motor coordination were evaluated using the rotarod apparatus (Model #7650; Ugo Basile, Comerio, Italy) in the ICR mouse. Mice were trained twice on the apparatus on the day of testing. In the first training session, mice were trained to walk on the rotating rod (8 revolutions/min) for 5 min. Approximately 1 h after the first training session, mice that walked for at least 3 min without falling off were selected in the second training session. Rota-rod performance prior to drug administration in selected mice was given as 120 sec. In the test session, the duration that mice (n = 9-10/group) walked on the rotating rod was measured 30, 60 and 120 min after administration of test compounds.

Grip Strength Test in Mice

Forelimb grip strength was determined using a strain gauge (GPM-100; Melquest, Toyama, Japan). The forepaws of ICR mice were allowed to grab a horizontal bar mounted on the gauge, and the tail was slowly pulled back by an experimenter. Tension was recorded from the gauge at the time the mouse released the forepaws from the horizontal bar. Measurements were repeated three times and maximum tension from the three measurements was used in analyses. Test compounds were administered into mice (n = 5-8/group) and grip strength was measured at before and 30, 60 and 120 min after dosing.

Data Analyses and Statistics

Data analyses were performed using Prism software (version 4.00; GraphPad Software, San Diego, CA). One-way analysis of variance, followed by Dunnett's test for multiple comparisons, was used when appropriate. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Elevated Plus Maze in Mice

LY379268 at 1 and 3 mg/kg did not alter behaviors in the elevated plus maze (Fig. 1A and 1E). FTIDC at both 10 and 30 mg/kg i.p. had no effect on time spent on each arm, but decreased the number of entries into closed arms at 30 mg/kg (Fig. 1B and 1F). Conversely, MPEP increased both time spent on and number of entries into open arms, with significant changes at 3 and 30 mg/kg. In addition, MPEP decreased time spent in closed arms with increases in number of entries into closed arms at 30 mg/kg (Fig. 1C and 1G). Diazepam significantly increased both time in and entry into open arms in a dose-dependent manner (Fig. 1D and 1H).

Ultrasonic Vocalization in the Rat

LY379268, FTIDC, MPEP and diazepam significantly decreased vocalizations in dose-dependent manners (Fig. 2A, 2B, 2C and 2D). LY379268 at 1 mg/kg elicited motor suppression without changes in body temperature, but body temperature was significantly decreased at 3 mg/kg ($p < 0.05$; vehicle, 36.6 ± 0.2°C; LY379268, 35.2 ± 0.5°C). FTIDC altered neither motor function nor body temperature. MPEP at 30 mg/kg significantly decreased body temperature ($p < 0.05$; vehicle, 34.6 ± 0.4°C; MPEP, 33.4 ± 0.2°C) without motor suppression. Sedation and hypothermia ($p < 0.01$; vehicle, 36.1 ± 0.3°C; diazepam, 34.2 ± 0.3°C) was seen in animals treated with diazepam at 1 mg/kg, while no changes in motor activity or body temperature were noted at 0.1 and 0.3 mg/kg.

Stress-induced Hyperthermia (SIH) in Mice

LY379268 significantly decreased SIH responses at 1 and 3 mg/kg without any effects on basal core temperature (Fig. 3A and Table 1). FTIDC suppressed SIH response at 30 mg/kg without any changes in basal body temperature (Fig. 3B and Table 1). MPEP attenuated SIH response at 30 mg/kg with significant decreases in basal body temperature

(Fig. 3C and Table 1). Diazepam at 1 mg/kg significantly decreased SIH response (Fig. 3D) without affecting basal body temperature (Table 1).

Formalin Test in Mice

Intra-foot pad injection of formalin caused biphasic licking responses. LY379268 did not show significant effects on licking behavior in either the first phase or second phase (Fig. 4A). FTIDC significantly inhibited licking or biting behavior elicited by formalin in a dose-dependent manner in both phases (Fig. 4B). MPEP significantly reduced formalin-induced licking at 30 mg/kg in both phases (Fig. 4C). Morphine significantly decreased licking latencies in a dose-dependent manner from 1 mg/kg in the first phase and 10 mg/kg of morphine was required to block licking behavior in the second phase (Fig. 4D).

Spontaneous Locomotor Activity and Methamphetamine (MAP)-induced Hyperlocomotion in Mice

LY379268 significantly inhibited hyperlocomotion induced by MAP at 3 mg/kg, with obvious reduction of spontaneous locomotor activity from 0.3 mg/kg (Fig. 5A and 5E). FTIDC significantly decreased MAP-induced hyperlocomotion in a dose-dependent manner from 1 mg/kg to 30 mg/kg (Fig. 5F) without affecting basal spontaneous locomotor activity (Fig. 5B). MPEP significantly attenuated MAP-induced hyperlocomotion only at a dose of 30 mg/kg, which caused hypolocomotion by itself (Fig. 5C and 5G). Haloperidol significantly antagonized hyperlocomotion induced by MAP in a dose-dependent manner from 0.1 mg/kg to 1 mg/kg (Fig. 5H), while haloperidol suppressed spontaneous locomotor activity at 0.3 mg/kg (Fig. 5D).

Prepulse Inhibition (PPI) in Rats

MAP (3 mg/kg s.c.) significantly disrupted PPI in rats at 6 or 12 dB prepulse

intensity (Fig. 6A-D). LY379268 affected neither impaired PPI (Fig. 6A) nor spontaneous PPI (Table 2). MAP-induced deficits of PPI were partially reversed by FTIDC at 10-30 mg/kg (Fig. 6B). FTIDC decreased spontaneous PPI at these doses (Table 2). In contrast, MPEP exaggerated MAP-induced disruption of PPI at a dose of 30 mg/kg (Fig. 6C) without affecting spontaneous PPI (Table 2). Haloperidol reversed MAP-induced deficits in PPI at doses of 0.03 and 0.3 mg/kg (Fig. 6D) without altering spontaneous PPI (Table 2).

Catalepsy Assay in Rats

FTIDC at up to 100 mg/kg did not cause any catalepsy, while haloperidol elicited catalepsy in a dose-dependent manner and 4 of 10 rats treated with 0.3 mg/kg showed a cut-off time of 60 s (Table 3).

Rotarod Test in Mice

FTIDC at doses of 3-30 mg/kg had negligible effects on motor coordination in the rotarod test (Fig. 7A). Conversely, haloperidol significantly impaired motor coordination at doses of 0.3 and 1 mg/kg (Fig. 7B).

Grip Strength Test in Mice

FTIDC at doses of 10 and 30 mg/kg did not affect muscle tone of mice in the grip test (Fig. 8A). Conversely, diazepam decreased grip strength in a dose dependent manner (Fig. 8B).

DISCUSSION

Since mGluR2/3 agonists have shown anxiolytic, antinociceptive and antipsychotic effects, modulation of glutamatergic neurotransmission has been considered as a potential therapeutic target for these CNS disorders (Kłodzińska et al., 1999; Cartmell et al., 2000; Kłodzińska et al., 2000; Simmons et al., 2002; Grillon et al., 2003). Alteration of glutamatergic neurotransmission could be achieved not only by presynaptic regulation of glutamate release with mGluR2/3, but also by postsynaptic modulation with iGluRs, mGluR1 and/or mGluR5. The studies with NMDA, non-NMDA and mGluR5 antagonists suggested that pharmacological effects of mGluR2/3 agonists *via* reduction of glutamatergic neurotransmission cannot be fully explained by postsynaptic blockade of iGluRs and mGluR5 (Fundytus, 2001; Javitt, 2004; Zhu et al., 2004; Cortese and Phan, 2005; Varty et al., 2005). In contrast to studying the pharmacological effects of iGluRs and mGluR5 antagonists, studies to explore pharmacological effects of mGluR1 antagonists have been limited. JNJ16259685 was demonstrated full mGluR1 occupancy in rat brain at and above 0.16 mg/kg (Lavreysen et al., 2004b) and showed anxiolytic effects, reduction of spontaneous locomotor activities and impairment of spatial acquisition in the Morris water maze at higher doses (Steckler et al., 2005a; Steckler et al., 2005b). Other mGluR1 allosteric antagonists demonstrated some pharmacological effects but might be insufficient to conclude that pharmacological effects were due to *in vivo* antagonism against mGluR1-mediated functions. Recently, FTIDC was identified as a potent and selective mGluR1 allosteric antagonist that reduces DHPG-induced face-washing behavior at 10 and 30 mg/kg (Suzuki et al., 2007). Since DHPG-induced face-washing behaviors are due to activation of mGluR1 (Barton and Shannon, 2005; Hikichi et al., 2008), we used these doses of FTIDC to elucidate pharmacological roles of mGluR1 *in vivo* and compared with those of mGluR2/3 agonist and mGluR5 antagonist.

New findings with FTIDC were that blockade of mGluR1 showed antipsychotic-like effects in various animal models. MAP-induced hyperlocomotion was dose-dependently attenuated by FTIDC, whereas neither LY379268 nor MPEP altered MAP-induced

hyperlocomotion. As the dose range of FTIDC effective in MAP-induced hyperlocomotion was similar to that inhibiting DHPG-induced face-washing behavior (Suzuki et al., 2007), antagonistic activities of FTIDC against MAP-induced hyperlocomotion were likely due to selective blockade of mGluR1. LY379268 dose-dependently suppressed spontaneous locomotion in mice, while MPEP only inhibited locomotion at the highest dose. FTIDC did not alter spontaneous locomotion. These results indicate that maintenance of glutamate neurotransmission *via* iGluRs is important to basal locomotor activity. We observed that mGluR1-deficient mice treated with MAP displayed smaller increases in horizontal movement than wild-type (see Supplemental data). As amphetamines are known to increase locomotion and glutamate release in the ventral tegmental area and nucleus accumbens (Xue et al., 1996; Wolf and Xue, 1999), it seems likely that increased glutamate release is participated in amphetamines-induced hyperlocomotion. Selective modulation of dopaminergic neurons *via* mGluR1 might be explained by the finding that DHPG-induced increases in intracellular calcium concentration of dopaminergic neurons are blocked by CPCCOEt, but not by MPEP (Tozzi et al., 2001).

When we investigated effects of FTIDC on MAP-induced disruption of PPI, FTIDC partially reversed MAP-induced disruption of PPI in the same dose range as in the MAP-induced hyperlocomotion test. A limited study examining the antipsychotic activities of BAY 36-7620 at 10 mg/kg reported efficacy in suppression of stereotypic behaviors induced by MK-801, but not by amphetamine or apomorphine (De Vry et al., 2001) and PPI disruption by MK-801, phencyclidine and apomorphine (Spooren et al., 2003). However, in *ex vivo* occupancy studies, BAY 36-7620 at 10 mg/kg only occupied about 30% of cerebellar and thalamic mGluR1 (Lavreysen et al., 2004a), suggesting that BAY 36-7620 at doses in these reports might be insufficient to fully block mGluR1. Although JNJ16259685 demonstrated nearly full mGluR1 occupancy at 0.16 mg/kg, JNJ16259685 has not been tested on these antipsychotic activities. The present findings are the first to demonstrate antipsychotic-like effects of mGluR1 allosteric antagonist on psychotic animal models. MPEP exaggerated

MAP-induced PPI disruption without affecting spontaneous PPI, being in the same line as findings that selective mGluR5-positive modulator antagonizes amphetamine-induced PPI deficits (Kinney et al., 2005). LY379268 affected neither spontaneous PPI nor MAP-induced PPI disruption. Although LY354740 has been reported to attenuate the disruptive effects of phencyclidine on working memory, stereotypy, locomotion and cortical glutamate efflux (Moghaddam and Adams, 1998), LY354740 and LY379268 did not reverse phencyclidine-, ketamine- or amphetamine-induced disruption of PPI (Galici et al., 2005; Imre et al., 2006), arguing against antipsychotic activities of mGluR2/3-selective agonists. Since pharmacological effects of mGluR2/3 agonist could be due to the summation of mGluR1 and mGluR5 blockade, mGluR2/3 agonist might not change MAP-induced disruption of PPI as a whole. For further support of this new possible therapeutic role of mGluR1, FTIDC should be examined in other psychotic animal models depending on non-dopaminergic mechanisms.

FTIDC neither impaired motor coordination, decreased muscle tone nor displayed catalepsy, suggesting that blockade of mGluR1 could not elicit extrapyramidal motor side effects unlike marketed antipsychotics.

We tested anxiolytic potential of mGluR ligands in three different studies. CD1 mice were used in the SIH test. We used rat pups in the USV test because we could not measure stable USVs due to low vocalization signal strength compared to those in rat pups. In the elevated plus maze test, we used C57BL/6J mice since C57BL/6J mice were more sensitive to diazepam than CD1 mice. FTIDC significantly decreased both USV in rat pups and SIH in mice. However, FTIDC did not alter behaviors in the elevated plus maze. These results may be due to different levels and/or modalities of anxiety or stress in these models. JNJ-16259685 demonstrated anxiolytic activity in the Vogel conflict model, but not in the elevated zero maze (Steckler et al., 2005a). LY379268 demonstrates anxiolytic-like effects in the USV and SIH tests, but not in the elevated plus maze test. Anxiolytic effects of LY354740 have been demonstrated in the elevated plus maze test with mice (Helton et al., 1998). In this study, NIH Swiss mice were used and they explored open arms for the same duration as closed arms in the

basal state. In our study, C57BL/6J mice preferred to explore closed arms compared to open arms. Different basal anxiety levels in these strains might explain why LY379268 could not show anxiolytic in our study. Alternatively, anxiolytic activities of LY379268 might be masked by its sedative effects. MPEP demonstrated anxiolytic activities in all three models, consistent with previous results (Tatarczyńska et al., 2001). Taking these results together, blockade of mGluR1 would exert specific anxiolytic effects rather than general anxiolytic actions shown by diazepam, and reduction of glutamate transmission *via* both mGluR1 and mGluR5 might be responsible for at least some anxiolytic effects of mGluR2/3 agonist.

Antinociceptive effects of mGluR1 antagonists have been tested in both acute and chronic pain models, however there was no analgesic effect in acute pain models (Spooren et al., 2003). We tested the antinociceptive effects of mGluR ligands in the mouse formalin test. FTIDC inhibited formalin-induced nociceptive behavior. Conversely, FTIDC did not inhibit acute thermal pain in the same dosage (see Supplemental data), suggesting that mGluR1 could be involved in the sensitization process elicited by chemical stimuli, rather than acute pain responses to thermal stimulation. Our results are supported by studies with EMQMCM (Sevostianova and Danysz, 2006). Antinociceptive effects of MPEP have been well studied (Zhu et al., 2004; Varty et al., 2005) and are consistent with our results. Although antinociceptive responses of LY379268 were reported in the rat formalin test (Simmons et al., 2002), activation of mGluR2/3 did not show analgesic effects comparable to those with FTIDC or MPEP in the present study with mice, which might be due to a lack of presynaptic regulation of glutamate transmission by mGluR2/3 in the pain pathway. This could be supported by immunocytochemical findings that mGluR2/3 is distributed mainly in lamina IIi and virtually absent in laminae I and IIo in the rat dorsal horn, where the primary afferent neurons from dorsal root ganglia terminate and mGluR1- and mGluR5-positive cells have been detected (Jia et al., 1999).

In summary, the present study clarified the contribution of postsynaptic mGluR1 and mGluR5 to pharmacological effects of mGluR2/3 agonist, which is considered to regulate

release of glutamate. Anxiolytic effects of mGluR2/3 may mainly relate to the reduction of synaptic transmission *via* mGluR5. However, mGluR1 may also be involved in anxiolytic effects of mGluR2/3 when state of anxiety is high. Analgesic effects were pronounced by blockade of mGluR1 or mGluR5, rather than action of mGluR2/3, probably due to existence of mGluR1 and mGluR5, but not mGluR2/3, in the superficial layer of the spinal dorsal horn. Finally, reductions in neural activity *via* mGluR1 may participate in antipsychotic effects originally demonstrated by mGluR2/3 agonists. Antipsychotic effects of mGluR2/3 agonist were somewhat limited, but such effects might be related to balance between pharmacological blockades of postsynaptic mGluR5 and mGluR1 that caused opposite effects. Antipsychotic-like effects by blockade of mGluR1 come without catalepsy. These results indicate that blockade of mGluR1 might offer a new approach to the treatment of schizophrenia without extrapyramidal side effects.

ACKNOWLEDGEMENTS

We thank Ms. Mikiko Hata for measurements of plasma and brain exposure levels of FTIDC. We wish to thank Ms. Noriko Akaogi and Ms. Satomi Yoshinaga for technical assistance.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Effects of mGluR ligands and diazepam in the elevated plus maze. **A-D)** Effects on time spend in an each arm and center area. **E-H)** Effects on number of entries into each arm. **A and E)** Effects of LY379268. **B and F)** Effects of FTIDC. **C and G)** Effects of MPEP. **D and H)** Effects of diazepam. Data are presented as mean ± SEM. Open, closed and hatched columns indicate results obtained in open, closed and center areas, respectively. Experimental numbers in each group are indicated in parentheses. *p < 0.05 and **p < 0.01 versus vehicle-treated group (one-way analysis of variance followed by Dunnett's test).

Figure 2. Effects of mGluR ligands and diazepam on numbers of ultrasonic vocalizations. **A)** Effects of LY379268. **B)** Effects of FTIDC. **C)** Effects of MPEP. **D)** Effects of diazepam. Data are presented as mean ± SEM. Experimental numbers of each group are indicated in parentheses. **p < 0.01 versus vehicle-treated group (one-way analysis of variance followed by Dunnett's test).

Figure 3. Effects of mGluR ligands and diazepam on stress-induced hyperthermia (SIH). **A)** Effects of LY379268. **B)** Effects of FTIDC. **C)** Effects of MPEP. **D)** Effects of diazepam. Data are presented as mean ± SEM (n = 7-8). ΔT means SIH, representing the difference between basal core temperature and stress-induced hyperthermia. *p < 0.05 and **p < 0.01 versus vehicle-treated group (one-way analysis of variance followed by Dunnett's test).

Figure 4. Effects on the formalin-induced licking behavior in the mouse. **A)** Effects of LY379268. **B)** Effects of FTIDC. **C)** Effects of MPEP. **D)** Effects of morphine. Data are presented as mean ± SEM. LY, LY379268; veh, vehicle. Doses are given in milligrams/kilogram. Experimental numbers of each group are indicated in parentheses.

*p < 0.05 and **p < 0.01 versus vehicle-treated group at each time point (one-way analysis of variance followed by Dunnett's test).

Figure 5. Effects on spontaneous locomotor activities (**A-D**) and methamphetamine-induced hyperlocomotion (**E-H**). **A and E)** Effects of LY379268. **B and F)** Effects of FTIDC. **C and G)** Effects of MPEP. **D and H)** Effects of haloperidol. Data are presented as mean ± SEM. MAP, methamphetamine; veh, vehicle; LY, LY379268; HAL, haloperidol. Experimental numbers of each group are indicated in parentheses. *p < 0.05 and **p < 0.01 versus vehicle-treated group, and #p < 0.05 and ##p < 0.01 versus methamphetamine-treated group (one-way analysis of variance followed by Dunnett's test).

Figure 6. Effects of haloperidol and mGluR ligands on methamphetamine-induced PPI disruption. **A)** Effects of LY379268. **B)** Effects of FTIDC. **C)** Effects of MPEP. **D)** Effects of haloperidol. Data are presented as mean ± SEM. Experimental numbers of each group are indicated in parentheses. Doses are in milligrams/kilogram. VEH, vehicle; MAP, methamphetamine (3 mg/kg, s.c.); LY, LY379268; HAL, haloperidol. *p < 0.05 and **p < 0.01 versus vehicle-treated group, #p < 0.05 and ##p < 0.01 versus methamphetamine-treated group (one-way analysis of variance followed by Dunnett's test).

Figure 7. Effects on rota-rod performance in mice. **A)** Effects of FTIDC. **B)** Effects of haloperidol. Data are presented as mean ± SEM (n = 9-10). Doses are in milligrams/kilogram. Some upper error bars are omitted to simplify the figure. *p < 0.05 and **p < 0.01 versus vehicle-treated group at each time point (one-way repeated measures analysis of variance followed by Dunnett's test).

Figure 8. Effects on grip strength in mice. **A)** Effects of FTIDC (n = 8). **B)** Effects of diazepam (n = 5). Data are presented as mean ± SEM. Doses are in milligrams/kilogram. $^{**}p < 0.01$ versus vehicle-treated group and diazepam (10 mg/kg)-treated group, respectively (one-way repeated measures analysis of variance followed by Dunnett's test).

JPET Fast Forward. Published on May 16, 2008 as DOI: 10.1124/jpet.108.138107
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Table 1

Basal core temperature in stress-induced hyperthermia under various drug treatments. Values are mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test.

	Dose (mg/kg, i.p.)	Basal core temperature (°C)	*n*
Vehicle	-	36.3 ± 0.1	7
LY379268	0.3	36.7 ± 0.2	7
LY379268	1	37.1 ± 0.3	8
LY379268	3	37.2 ± 0.3	8
Vehicle	-	35.9 ± 0.2	7
FTIDC	1	36.2 ± 0.1	8
FTIDC	3	36.4 ± 0.1	8
Vehicle	-	36.1 ± 0.1	7
MPEP	10	36.6 ± 0.2	7
MPEP	30	34.4 ± 0.3**	8
Vehicle	-	36.9 ± 0.2	7
Diazepam	0.3	36.8 ± 0.1	7
Diazepam	1	36.7 ± 0.2	8

** $p < 0.01$ versus corresponding vehicle control group.

JPET Fast Forward. Published on May 16, 2008 as DOI: 10.1124/jpet.108.138107
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Table 2

Effects of mGluR ligands and haloperidol on spontaneous prepulse inhibition in rats. Values are mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test.

	Dose	Prepulse intensities above background noise			
	(mg/kg, i.p.)	3 dB	6 dB	12 dB	*n*
Vehicle	-	9.3 ± 10.6	36.4 ± 8.7	52.3 ± 6.4	10
LY379268	1	20.3 ± 5.0	48.9 ± 4.1	60.4 ± 3.2	10
LY379268	3	9.3 ± 6.2	36.4 ± 5.0	50.5 ± 5.5	10
Vehicle	-	16.5 ± 6.1	37.1 ± 3.9	54.9 ± 3.4	8
FTIDC	10	6.1 ± 4.2	18.2 ± 5.4**	36.0 ± 5.3**	8
FTIDC	30	5.2 ± 2.6	16.9 ± 3.6**	34.1 ± 4.7**	8
Vehicle	-	13.5 ± 7.5	43.9 ± 4.9	60.4 ± 4.1	10
MPEP	10	15.1 ± 6.3	39.9 ± 8.8	53.9 ± 8.2	10
MPEP	30	-10.6 ± 13.1	29.8 ± 7.0	37.3 ± 9.7	10
Vehicle	-	21.7 ± 6.6	40.1 ± 10.1	56.1 ± 6.9	10
Haloperidol	0.1[1)]	20.4 ± 4.9	41.8 ± 4.2	50.4 ± 4.5	11
Haloperidol	0.3[1)]	14.8 ± 4.9	44.7 ± 5.6	61.7 ± 4.7	11

1) Doses of haloperidol were given as mg/kg, s.c.

** $p < 0.01$ versus corresponding vehicle control group.

Table 3

Duration of catalepsy induced by FTIDC and haloperidol.

Values are mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test.

	Dose	Duration of catalepsy (s)	*n*
Vehicle	-	0.9 ± 0.3	5
FTIDC	100 mg/kg, i.p.	1.0 ± 0.1	5
Vehicle	-	2.9 ± 0.9	15
Haloperidol	0.03 mg/kg, s.c.	1.4 ± 0.3	5
Haloperidol	0.1 mg/kg, s.c.	7.0 ± 1.5	5
Haloperidol	0.3 mg/kg, s.c.	45.0 ± 4.5**	10

** $p < 0.01$ versus corresponding vehicle control group

Fig. 1.

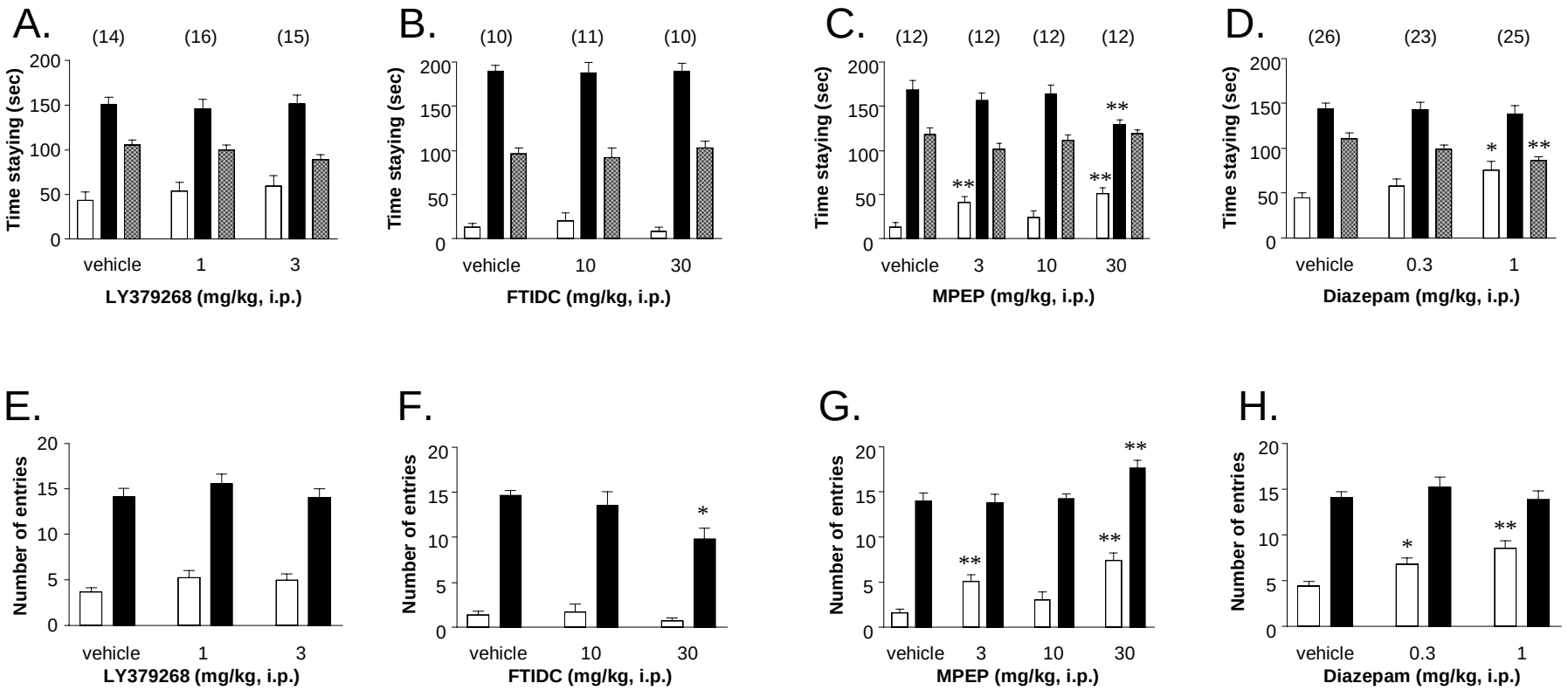
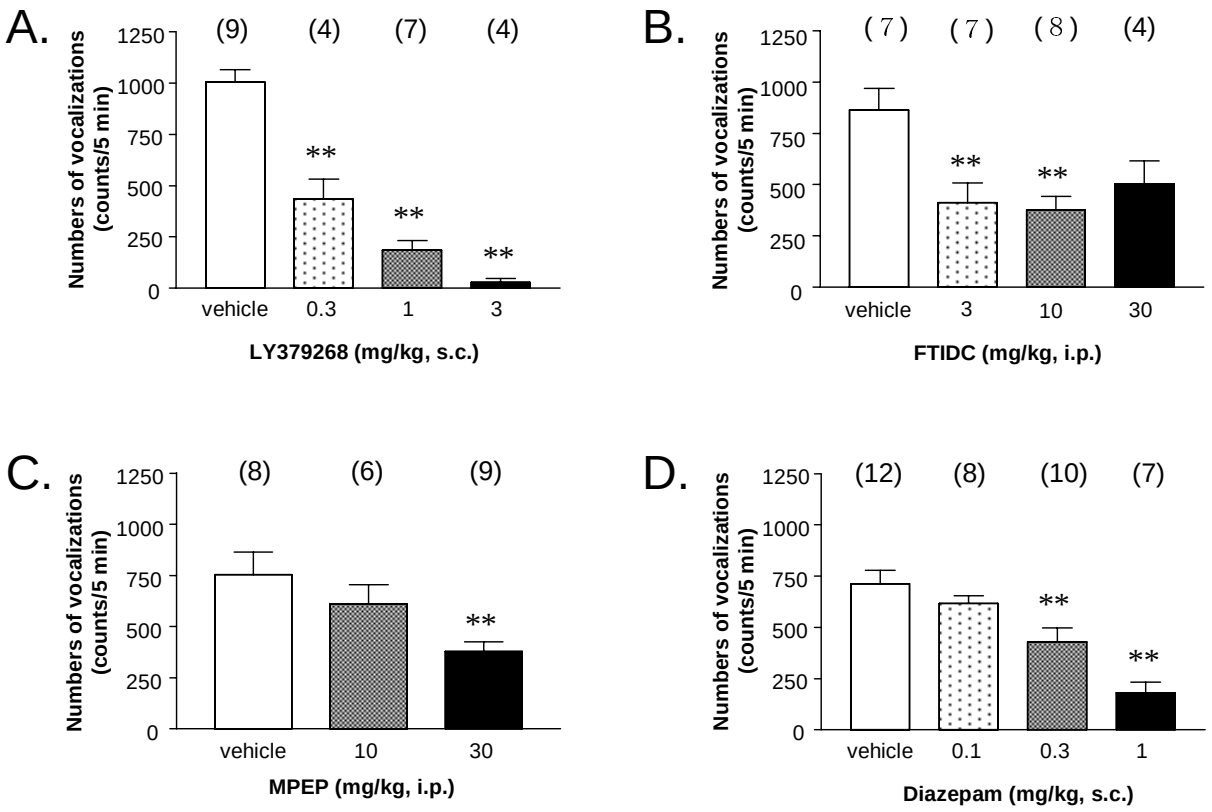


Fig. 2.



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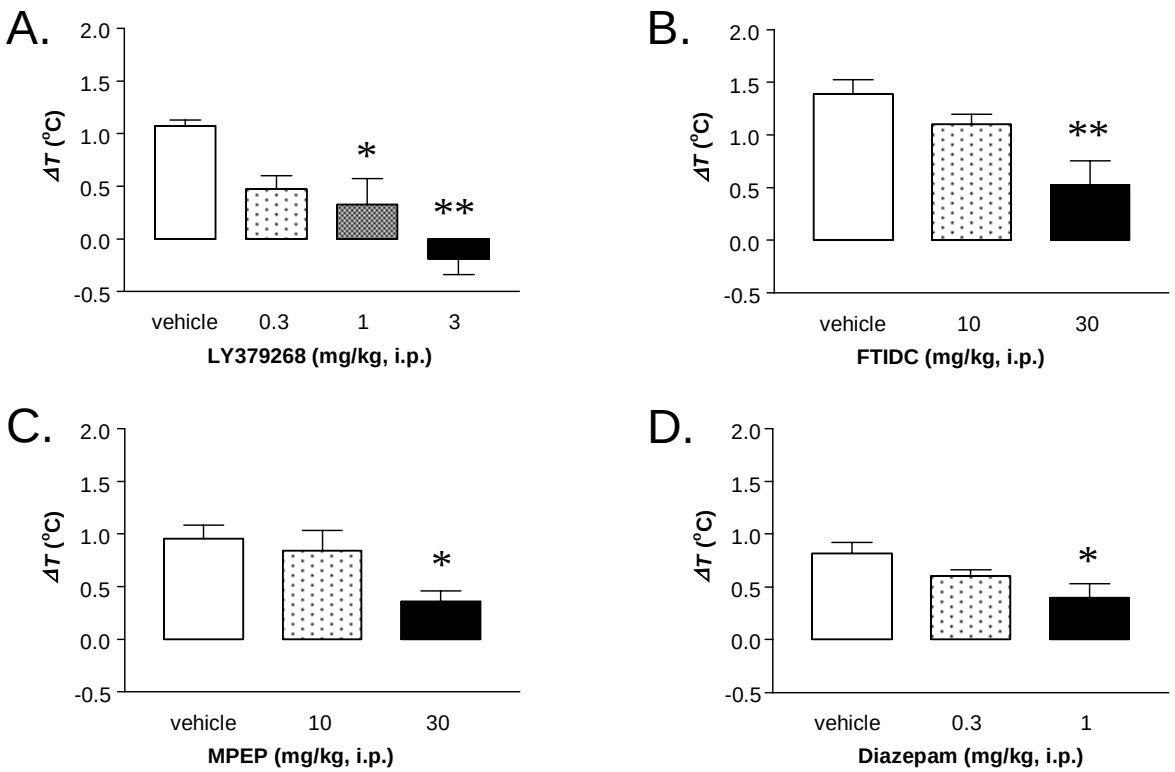

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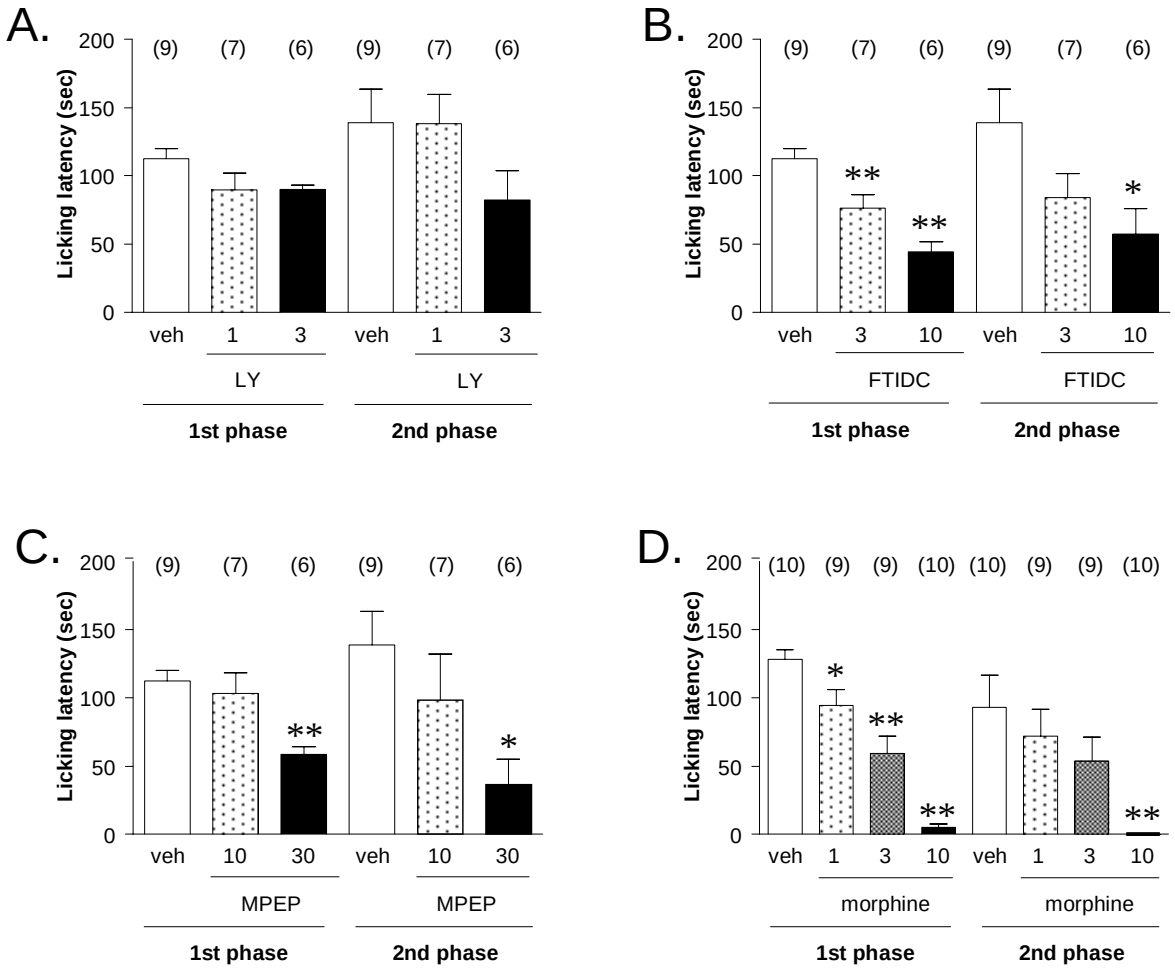

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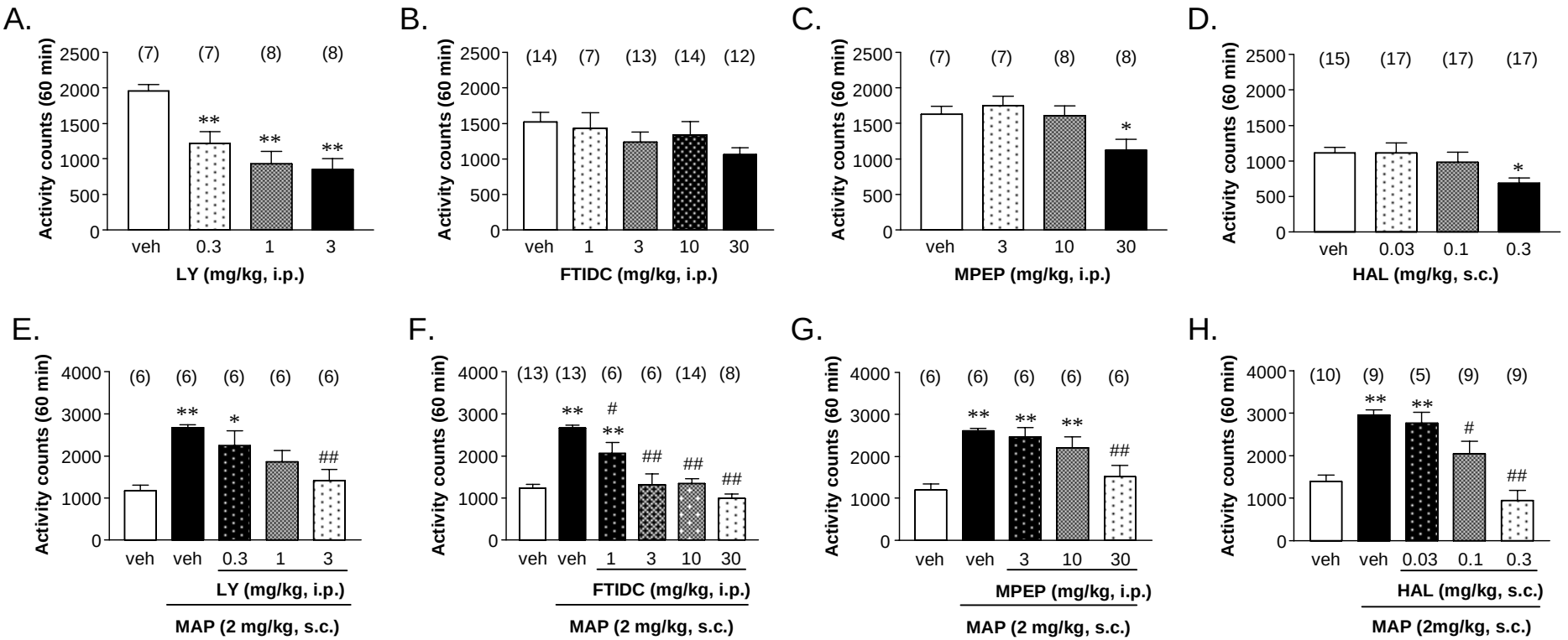

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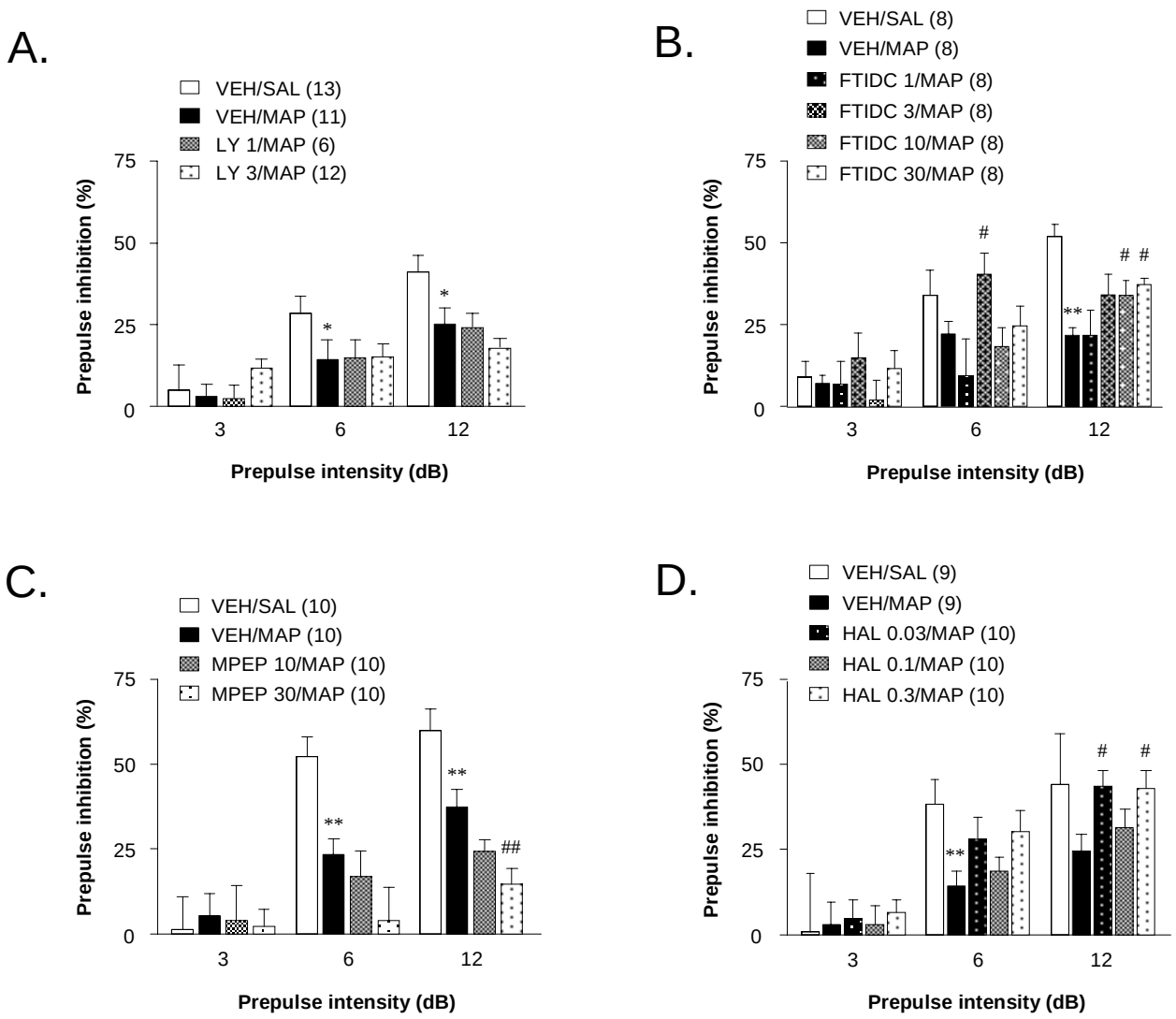

Fig. 7.

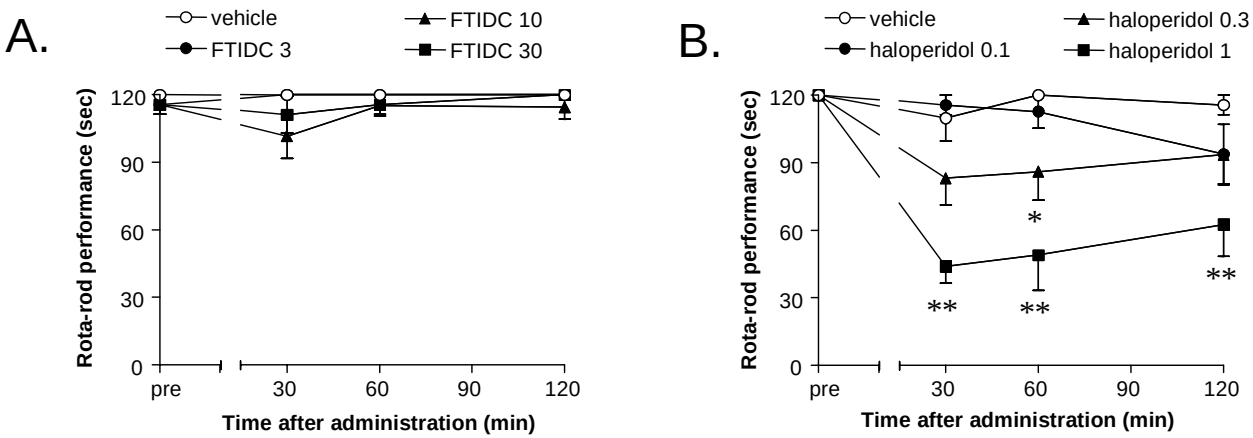

Fig. 8.