0022-3565/06/3162-905–913$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 316:905–913, 2006

Vol. 316, No. 2
91926/3071693
Printed in U.S.A.

Improved Bioavailability of the mGlu2/3 Receptor Agonist LY354740 Using a Prodrug Strategy: In Vivo Pharmacology of LY544344

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Received July 1, 2005; accepted October 12, 2005

ABSTRACT

Numerous studies have indicated that selective agonists of group II metabotropic glutamate (mGlu) receptors, such as LY354740 [(1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate] and LY379268 [(−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate], may be useful in the treatment of many psychiatric disorders, including psychosis, anxiety, and drug withdrawal. Although animal and human studies demonstrate potential therapeutic utility, poor oral bioavailability is a limiting factor in the clinical development of these compounds. Therefore, a novel prodrug approach is being pursued to increase exposure levels of active compound after oral administration. Here, we demonstrate a 10-fold increase in brain, plasma, and cerebrospinal fluid levels of LY354740 after oral prodrug administration. Furthermore, we compare the oral efficacy of the mGlu2/3 receptor agonist LY354740 and its prodrug LY544344 [(1S,2S,5R,6S)-2-[(2′*S*)-(2′-amino)propionyl]aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid hydrochloride] in rodent models of psychosis and anxiety. Phencyclidine (PCP)-induced hyperlocomotion was dose dependently inhibited in rats receiving oral administration of 30 or 100 mg/kg LY544344, whereas LY354740 did not significantly reverse PCP-mediated behaviors at doses up to 100 mg/kg. Orally administered LY544344 (30 mg/kg) and subcutaneously administered LY354740 (10 mg/kg) attenuated stress-induced hyperthermia in DBA/2 mice, with the prodrug producing anxiolytic effects at lower oral doses than the parent compound. Although oral administration of LY354740 did not significantly affect fear-induced suppression of operant responding in rats, subcutaneously administered LY354740 (10 or 20 mg/kg) and orally administered LY544344 (10 or 30 mg/kg) produced significant anxiolytic effects in this model. The present data confirm that mGlu2/3 receptor agonists produce antipsychotic and anxiolytic effects in animal behavioral models and demonstrate that oral bioavailability of LY354740 was substantially increased using a prodrug strategy.

Glutamate is the primary excitatory neurotransmitter in the brain. Ionotropic glutamate receptors are ligand-gated ion channels that mediate fast synaptic excitatory neurotransmission via NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate receptors (Feldman et al., 1997). In contrast, metabotropic glutamate (mGlu) receptors are G-protein-coupled receptors that seem to have evolved to modulate neuronal excitability (Feldman et al., 1997; Schoepp, 2001). Numerous studies indicate that many neurological and psychiatric disorders may be linked to excessive neuronal excitability, including psychosis, anxiety, drug dependence, ischemia, and epileptic seizures (Parsons et al., 1998; Vandergriff and Rasmussen, 1999; Moghaddam, 2002; Baptista et al., 2004; Bergink et al., 2004). Because mGlu receptors are linked to modulatory second messenger systems, pharmacological manipulation of these receptors may reduce excessive glutamatergic excitatory activation without affecting global CNS functionality or producing unwanted side effects, as have been observed with NMDA antagonists and benzodiazepines (Gudex, 1991; Moghaddam and Adams, 1998; Cartmell et al., 1999; Schoepp and Marek, 2002). The emergence of mGlu receptor subtype-selective

Some portions of the data have been previously presented in poster form at the annual meeting of the International Behavioral Neuroscience Society (2004 Jun 16–20; Key West, FL).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.105.091926.

ABBREVIATIONS: mGlu, metabotropic glutamate receptor; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate; LY544344, (1*S*,2*S*,5*R*,6*S*)-2[(2′S)-(2′-amino)propionyl]aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid hydrochloride; LY379268, (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate; LY341495, (2*S*-2-amino-2-(1*S*,2*S*-2-carboxylcyclopropyl-1-yl)-3-(xanth-9-yl)propanoic acid; MK-801, dizocilpine; ANOVA, analysis or analyses of variance; CNS, central nervous system; CS, conditioned stimulus; SPE, solid phase extraction; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine.

compounds with agonist and antagonist properties suggests that they may be promising therapeutic targets for the treatment of many psychiatric disorders (Schoepp et al., 1999).

There are currently eight known mGlu receptor subtypes that can be divided into three classes based on structural sequence homology, receptor coupling, and pharmacology. Group I mGlu receptors (mGlu1 and mGlu5) are coupled to phosphoinositide hydrolysis, whereas group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) receptors are negatively coupled to cyclic AMP production (Schoepp et al., 1999). Group II mGlu2/3 receptors are localized primarily in limbic and forebrain regions, such as the frontal cortex, hippocampus, amygdala, and locus coeruleus (Ohishi et al., 1993a,b; Dube and Marshall, 1997). This pattern of localization suggests that mGlu2/3 receptors may be important targets for pharmacological treatment of disorders such as anxiety and psychosis (Schoepp, 2001; Moghaddam, 2002; Swanson et al., 2005). Thus, precise manipulation of specific receptor subtypes in restricted brain areas may allow for more direct therapeutic intervention of psychiatric disorders as well as reduced propensity for side effects.

Although located in both presynaptic and postsynaptic sites, mGlu2/3 receptors function primarily as presynaptic autoreceptors (Dube and Marshall, 1997; Cartmell and Schoepp, 2000). Activation of these receptors by selective mGlu2/3 agonists, such as LY354740 and LY379268, dampens glutamate hyperexcitability by reducing its presynaptic release in the prefrontal cortex and locus coeruleus (structure of LY354740 shown in Table 1; Moghaddam and Adams, 1998; Vandergriff and Rasmussen, 1999; Homayoun et al., 2005). mGlu2/3 agonists, including LY354740, have demonstrated efficacy in animal behavioral models of anxiety, psychosis, and drug withdrawal. For example, studies in rats have demonstrated that LY354740 decreased fear-potentiated startle responses, increased open arm time in the elevated plus-maze, increased the number of punished responses in conflict tests, and reversed behavioral and psychotomimetic actions of NMDA antagonists, such as phencyclidine (PCP) and MK-801 (Helton et al., 1998; Moghaddam and Adams, 1998; Klodzinska et al., 1999; Schoepp et al., 1999; Vandergriff and Rasmussen, 1999; Schoepp and Marek, 2002; Tizzano et al., 2002; Homayoun et al., 2005). Likewise, LY354740 exerted an anxiolytic-like effect in a test of fear-potentiated startle in humans (Grillon et al., 2003). A possible role for LY354740 in drug dependence was supported by electrophysiological and behavioral evidence of attenuation of morphine withdrawal in rats (Vandergriff and Rasmussen, 1997; Klodzinska et al., 1999). Unlike GABAergic agonists, LY354740 produced these behavioral effects at doses that did not produce sedation or impair motor performance or cognitive function (Helton et al., 1998; Cartmell et al., 1999; Klodzinska et al., 1999).

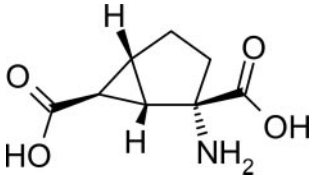
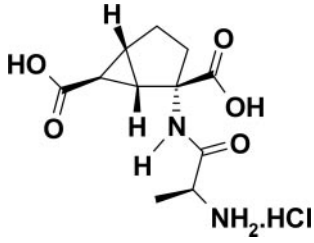
Although animal and human studies demonstrate the potential therapeutic utility of mGlu2/3 agonists in several models of psychiatric/clinical disorders, low levels (~10%) of LY354740 are absorbed after oral administration, thus requiring larger doses in clinical populations (Johnson et al., 2002). Therefore, a prodrug approach is being pursued to obtain an mGlu2/3 agonist with acceptable oral bioavailability. Instead of relying on passive diffusion through the intestinal epithelium, the prodrug LY544344 is actively transported out of the intestinal tract via the peptide transporter PepT1. Once transported from the intestinal tract into the CNS, enzymatic processes metabolize LY544344 into the active mGlu2/3 receptor agonist LY354740. Using this approach, 85% oral bioavailability has been achieved in animal studies (Bueno et al., 2005).

The present experiments were conducted to directly compare the oral efficacy of the mGlu2/3 receptor agonist LY354740 and its prodrug LY544344 in animal models of psychosis and anxiety, including reversal of PCP-induced hyperlocomotion, stress-induced hyperthermia, and fear-

TABLE 1

Plasma, brain, and CSF concentrations of LY354740 after an oral dose of LY354740 or LY544344

Summary of LY354740 pharmacokinetic parameters in brain, plasma, and CSF of male Sprague-Dawley rats after a single oral administration of 100 mg/kg LY354740 or 10 mg/kg LY544344. Data are presented as the mean of 3 values per time point. C_{max} and $AUC_{0\text{-}t}$ are rounded to no more than three significant figures.

	Compound Administered					
	LY354740 (100 mg/kg p.o.)			LY544344 (10 mg/kg p.o.)		
	Plasma	Brain	CSF	Plasma	Brain	CSF
Structure						
LY354740 measured						
C_{max} (ng/ml)	2120	75.9	14.3	3300	84.4	24.2
$AUC_{0\text{-}t\ (ng \cdot h/ml)}$	24,300	64,00	346	25,700	7160	554
t_{max} (h)	3	24	3	0.5	12	3
$t_{1/2}$ (h)	6.8	69	33	4.4	74	35
% Oral bioavailability (F) of LY354740	10[a]			85[b]		

C_{max} = maximal concentration; $AUC_{0\text{-}t}$ = area under the concentration versus time curve from 0 to the last quantifiable time point; t_{max} = time to C_{max}; $t_{1/2}$ = half-life.
[a] From Johnson et al. (2002); bioavailability following 30 mg/kg (p.o.) dose of LY354740.
[b] From Bueno et al. (2005); bioavailability following 5 mg/kg (p.o.) dose of LY544344.

induced suppression of operant behavior (Stanhope and Dourish, 1996; Cartmell et al., 1999; Rorick-Kehn et al., 2005).

Materials and Methods

Animals. Male Sprague-Dawley rats weighing approximately 250 to 350 g (Harlan, Indianapolis, IN) were tested in the PCP-induced hyperlocomotion and fear-induced suppression of operant responding experiments. Rats were pair-housed with water available ad libitum and maintained on a 12-h light/dark cycle (lights on at 6:00 AM). A total of 48 rats were used to assess the effects of LY354740 and LY544344 on spontaneous locomotor activity. A total of 25 rats were used in the PCP-induced hyperlocomotion experiment (LY354740, $n = 9$; LY544344, $n = 16$). For these experiments, rats were food-fasted for 12 to 18 h before the experiment. For the operant tests, a total of three different groups of rats were used. An initial test using subcutaneous administration of LY354740 was conducted ($n = 7$) followed by a second experiment using LY354740 ($n = 14$) and a third experiment using LY544344 ($n = 14$). For the second LY354740 experiment, oral and subcutaneous dose-response assessments were measured in the same group of 14 rats, with 1 week between experiments. Therefore, some of the rats used in these experiments had previous drug exposure. Rats were maintained at 85% of their free-feeding weights for the duration of each experiment. Stress-induced hyperthermia experiments were conducted using male DBA/2 mice ($n = 6–8$/group) weighing approximately 25 to 35 g (Taconic, Germantown, NY). For these experiments, 1 week elapsed between testing of subsequent compounds. All mice were individually housed, with food and water available ad libitum, and maintained on a 12-h light/dark cycle (lights on at 6:00 AM). All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 85-23, 1985 and were approved by the Eli Lilly Institutional Animal Care and Use Committee.

Surgical Procedures. Radiotelemetric analysis of stress-induced hyperthermia in DBA/2 mice required the implantation of small radiotransmitters approximately 1 week before experimental procedures. All surgical procedures were performed under aseptic conditions. Mice were anesthetized using a 1.5% concentration of isoflurane and shaved along the ventrum. A 1.5-cm incision was made through the skin and abdominal wall along the midline through which a sterile radiotelemetric transmitter (Data Sciences International, Arden Hills, MN) was implanted into the peritoneal cavity. The abdominal wall and skin were then sutured, and mice were returned to the home cage for recovery. After surgery, animals were administered 0.05 mg/kg buprenorphine (s.c.), individually housed, and allowed 5 to 7 days to recover from surgery.

Drugs. PCP was obtained from Sigma-Aldrich (St. Louis, MO). LY354740 and LY544344-HCl (LY544344) were synthesized at Lilly Research Laboratories. LY354740 was solubilized in sterile water by the dropwise addition of NaOH, whereas LY544344 and PCP were dissolved in sterile water. Sterile water was administered to vehicle groups. All drugs were mixed fresh before use and administered orally, unless otherwise stated. Mice were dosed with a volume of 10 ml/kg. Rats were dosed with a volume of 1 ml/kg, unless otherwise stated.

Pharmacokinetics of LY354740: Oral Administration of LY354740 or LY544344. Male Sprague-Dawley rats weighing approximately 330 to 375 g (Harlan) were given a single oral dose of LY544344-HCl (10 mg/kg) or LY354740 monohydrate (100 mg/kg). Doses were administered by oral gavage in aqueous solution (5 ml/kg). Plasma, cerebrospinal fluid (CSF), and brain samples were collected after euthanasia from 3 rats/group at 0.5, 3, 12, 24, 48, 72, 96, 120, 144, and 168 h after dosing. Whole-blood samples were collected by cardiac puncture into tubes containing EDTA at each time point, and plasma was obtained by centrifugation. CSF was collected from the cisterna magna, and whole brains were collected after whole-body perfusion, with 45 ml of isotonic saline. Plasma, CSF, and brain samples were stored at approximately −70°C until analysis. Rats were euthanized by CO_2 fixation during the terminal blood collection.

Concentrations of LY354740 were determined by liquid chromatography/mass spectrometry/mass spectrometry. LY354740 was extracted from either 150 μl of plasma or 50 μl of CSF by cation-exchange solid phase extraction (SPE). Whole brains were homogenized in purified water (2 ml of H_2O/g brain). LY354740 was extracted from 300 μl (0.1 g) of brain homogenate by protein precipitation followed by cation-exchange SPE and anion-exchange SPE. Samples were chromatographically separated using a Restek Ultra intrinsically base deactivated column [2.1 × 100 mm; 3 μm) (Restek, Bellefonte, PA) with a water/methanol/acetic acid (90/10/0.2, v/v) mobile phase at approximately 50°C]. The flow rate was 0.3 ml/min. LY354740 were detected using selective reaction monitoring/ESI/TurboIonSpray on a PE-Sciex API 3000 instrument (Applied Biosystems, Foster City, CA).

Pharmacokinetic parameters were calculated using noncompartmental analysis with WinNonlin, version 3.1 (Pharsight, Mountain View, CA). Concentrations of LY354740 (brain, plasma, and CSF) below the lower limit of quantification (10 ng/g for brain, 5 ng/ml for plasma, and 1 ng/ml for CSF) were assigned a value of zero for calculation purposes.

Spontaneous Locomotor Activity and PCP-Induced Hyperlocomotion. Behavioral parameters of spontaneous locomotor activity and PCP-induced hyperlocomotion were monitored in transparent cages (45 × 20 × 20 cm; Ancarez, Bellmore, NY) containing a thin layer of wood chips (1 cm) on the cage floor and a metal grill on top of the cage. Rectangular photocell monitors (Hamilton Kinder, Poway, CA) were placed around each test cage during the experiment. One grid of 12 photocell beams arranged in an 8 × 4 configuration was positioned 5 cm above the cage floor to enable detection of body movements (ambulations) and head movements, whereas another grid positioned 15 cm above the cage floor detected rearing activity. Ambulations, head movements, and rearing were recorded by a computer for offline analysis.

To assess the effects of LY354740 and LY544344 on spontaneous locomotor activity, animals received an oral gavage of vehicle (sterile water, 1 ml/kg) or test compound (100 mg/kg LY354740; or 3, 10, 30, or 100 mg/kg LY544344) and returned to their homecage. After 60 min, rats were placed in the test cage for a 90-min assessment of spontaneous locomotor activity.

On the test day, rats were administered a randomly assigned dose of LY354740 (10, 30, or 100 mg/kg), LY544344 (3, 10, 30, or 100 mg/kg), or sterile water vehicle (1 ml/kg) and returned to their home cage for 30 min. Rats were then placed in the test cage for a 30-min habituation period to allow for acclimation to the test cage environment and to measure baseline locomotor activity. After the habituation period, animals were administered a challenge dose of PCP (5 mg/kg, s.c.) or 0.9% NaCl vehicle (1 ml/kg, s.c.) and returned to the test cage for an additional 60 min, during which stimulant-induced behavioral activity was recorded.

Stress-Induced Hyperthermia in DBA/2 Mice. Mice were administered a randomly assigned dose of LY354740 (3, 10, or 30 mg/kg), LY544344 (3, 10, or 30 mg/kg), or vehicle (sterile water, 10 ml/kg). Mice were then returned to their home cages, and each cage was placed onto a receiver configured to the specifications of each transmitter, which relayed core body temperature (°C) measurements to the computer once every five min. After placement on the receiver, the lights were turned off and the experimenter left the room for a 60-min baseline assessment period. After the baseline period, the lights were turned on and mice were removed from their home cage and placed in a novel cage (20 cm × 20 cm × 29 cm; Ancare) containing soiled shavings and feces from a rat cage (approximately 5 cm in depth). Body temperature was recorded in 5-min increments for an additional 60 min. For comparison purposes,

an additional experiment was conducted in which mice received an s.c. injection of either vehicle (0.9% NaCl) or LY354740 (10 mg/kg). These experiments replicate the results of a previous stress-induced hyperthermia experiment using LY354740 (Rorick-Kehn et al., 2005).

Fear-Induced Suppression of Operant Behavior. Operant conditioning was monitored in standard operant boxes (Coulbourn Instruments, Allentown, PA) consisting of two stainless steel and two Plexiglas walls. The grid floor was composed of stainless steel bars, 0.5 cm in diameter located approximately 1.5 cm apart (center to center). An operant lever was positioned in the middle of one stainless steel wall, approximately 10 cm above the floor grid.

Food-restricted rats were first trained to lever press for food reward (45-mg sucrose pellets; BioServ, Frenchtown, NJ) on a variable interval-30-s (± 10 s) schedule of reinforcement during daily 32-min sessions. Once a stable level of responding was reached (approximately 2–3 weeks), rats were transferred to fear-suppressed operant conditioning. During this phase of the experiment, rats received daily 32-min sessions consisting of two trials. Each trial consisted of a 10-min baseline period, during which no stimuli were presented, followed by a 2-min conditioned stimulus (CS) period, during which a 5-watt house light was illuminated. During the last 0.5 s of the CS period, rats received a 0.3-mA coterminating footshock. Thus, the CS signaled the impending inescapable footshock. Each session ended after an additional baseline period after the second trial. Throughout the session, lever presses made by the animal were reinforced on a variable interval-30-s schedule. On test days in the first experiment, rats received subcutaneous administration of vehicle (0.9% NaCl, 2 ml/kg) or LY354740 (5, 10, or 20 mg/kg, and 2 ml/kg) 30 min before the session in a within-subject, ascending dose-response design. In later experiments, rats were orally administered a dose of LY354740 (10 or 30 mg/kg in a volume of 2 ml/kg), LY544344 (3, 10, or 30 mg/kg), or sterile water vehicle (2 or 1 ml/kg, respectively) 60 min before the test session, using ascending doses in a within-subject design. For comparison purposes, an additional experiment was conducted in which rats received either vehicle (0.9% NaCl s.c. administration, 30-min pretreatment time) or LY354740 (10 mg/kg s.c.). At least 3 days elapsed between doses of each experimental drug, and in the second LY354740 experiment, 1 week elapsed between subsequent dose-response assessments. Behavioral measures recorded were the number of operant responses made during the 2-min CS period, the number of operant responses made during the last 2 min of the baseline period, and the suppression ratio [CS responses/(CS responses + Baseline responses)].

Statistics. In the spontaneous locomotor activity test, a repeated measures analysis of variance (ANOVA) was calculated for the first 30 min of the behavioral assessment, with Dose as the between-subject factor and Time as the within-subject repeated measure. Significant ANOVA were followed by one-way ANOVA at each time point and a Dunnett's post hoc comparison. In the PCP-induced hyperlocomotion test, data were analyzed for each compound using one-way ANOVA, with Dose as a between-subject factor. For the stress-induced hyperthermia test, a one-way ANOVA was calculated on the prestress baseline body temperatures and a mixed-design ANOVA was calculated on the difference scores (post-stress − prestress body temperature) during the 60-min stress period, with time as the within-subject factor. For fear-induced suppression of operant responding, data for each experimental compound were analyzed using one-way ANOVA, with Dose as the within-subject factor. Significant ANOVA were followed by post hoc comparisons using the Dunnett's t test ($\alpha = 0.05$). In the PCP-induced hyperlocomotion test, post hoc Dunnett's t tests were conducted using both the veh/veh and veh/PCP groups as the control group.

Results

Pharmacokinetic Properties of LY354740: Oral Administration of LY354740 or LY544344. The chemical structures and mean pharmacokinetic parameters of the mGlu2/3 receptor agonist LY354740 after oral administration of LY354740 or the prodrug LY544344 are reported in Table 1. LY544344 was rapidly absorbed and converted to LY354740 after oral administration, leading to a marked increase in relative systemic availability of LY354740. The rate of increase in LY354740 concentrations was faster in the LY544344-dosed group, consistent with rapid intestinal absorption of LY544344 via active PepT1-mediated transport. Initial uptake of LY354740 into the brain was similarly rapid, but a lag period of at least several hours existed between the peak concentrations in plasma and brain. However, the true lag time between plasma and brain peaks is difficult to determine based on the current sampling schedule. Despite the rapid appearance of LY354740 in the CNS, concentrations were relatively low in brain and CSF compared with plasma, indicating limited pas-

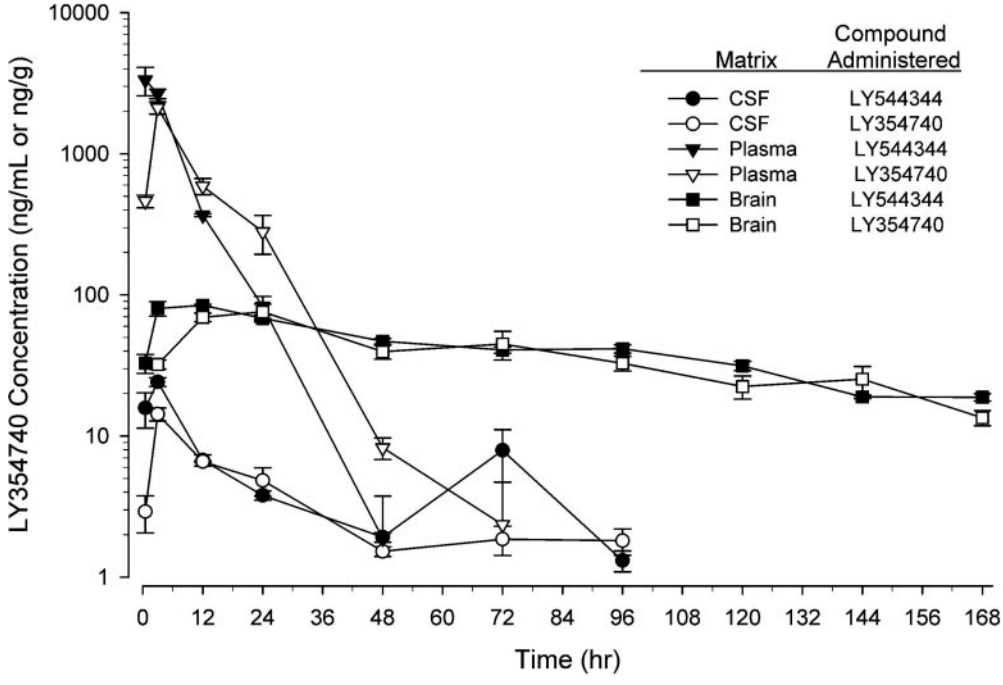


Fig. 1. Concentrations of LY354740 in plasma, CSF, and whole brain of Sprague-Dawley rats following oral administration of LY544344 (10 mg/kg) or LY354740 (100 mg/kg). Values represent the mean (±S.E.M.) of three samples per time point.

sive penetration of LY354740 through the blood-brain barrier and blood-CSF barrier (Fig. 1). In both dose groups, plasma exposure to LY354740 (AUC) was approximately 4-fold higher than brain and 50–70-fold higher than CSF. The similarity of CNS exposure at similar plasma concentrations indicates that the prodrug itself does not have any direct contribution to brain penetration of LY354740 at efficacious doses.

The elimination profiles of LY354740 in plasma and brain were characterized by a single compartment, with elimination half-lives ($t_{1/2}$) ranging from approximately 4 to 74 h, respectively. In contrast, the elimination of LY354740 in CSF displayed multiple phases, mirroring plasma concentrations at early time points and elimination from brain tissue at later times (Fig. 1). Although CSF and brain concentrations seem to be at equilibrium beyond 48 h, the substantially lower concentrations in CSF suggest a sequestering of LY354740 within the brain tissue. The underlying cause of prolonged brain retention and the sites of brain deposition in rats have not been fully elucidated.

Spontaneous Locomotor Activity and PCP-Induced Hyperlocomotion. Baseline locomotor activity decreased throughout the 90-min behavioral assessment (Fig. 2A). Oral administration of 30 or 100 mg/kg LY544344 produced a significant decrease in spontaneous locomotor activity during the first 30 min of the behavioral assessment, $F(5,35) = 3.67$; $p < 0.005$. One-way ANOVA conducted at each time point revealed a significant difference between groups for each 5-min interval (all p values <0.005). Dunnett's t tests indicated that rats administered 30 or 100 mg/kg LY544344 exhibited significantly decreased locomotor activity at each 5-min interval for the first 25 min of the assessment (all p values <0.05). In addition, rats administered 100 mg/kg LY544344 exhibited decreased spontaneous locomotor activity at the 30-min time point ($p < 0.01$). No other significant differences were observed.

PCP evoked a significant increase in locomotor activity that was partially reversed by oral administration of LY354740 (Fig. 2B), as indicated by a significant ANOVA, $F(4,72) = 6.53$, $p < 0.001$. Post hoc analysis (Dunnett's t test) confirmed that rats administered 5 mg/kg PCP (veh/PCP group) demonstrated a significant increase in ambulatory movements, relative to vehicle controls (veh/veh group). However, oral administration of LY354740, at doses up 100 mg/kg, only partially reversed PCP-induced hyperlocomotion. Although rats administered 30 and 100 mg/kg LY354740 did not differ significantly from the PCP group, these groups were not significantly different from the veh/veh group, indicating a partial reversal.

In contrast to the results of LY354740, PCP evoked a significant increase in locomotor activity, which was dose dependently reversed LY544344 (Fig. 2C), $F(5,100) = 7.69$; $p < 0.001$. Specifically, 5 mg/kg PCP evoked a significant increase in locomotor activity compared with the vehicle control group, whereas oral administration of LY544344 (30 and 100 mg/kg) significantly attenuated the PCP-induced hyperlocomotion by approximately 59 and 72%, respectively. Similar results were observed in measures of time at rest and head movements (data not shown, all p values <0.001).

Stress-Induced Hyperthermia in DBA/2 Mice. Table 2 details the effects of orally administered LY354740 and LY544344 and subcutaneously administered LY354740 on core body temperatures during the last 5-min block of the 60-min baseline period. Administration of LY354740 did not significantly affect core body temperatures during baseline, regardless of whether it was administered orally or subcutaneously (p values >0.05; Table 2). Oral administration of LY354740 produced a mild attenuation of stress-induced changes in core body temperature, relative to the last 5-min block of the baseline period (Fig. 3A). However, this reduction was not statistically significant ($p = 0.06$). For comparison purposes, the inset of Fig. 3A shows that subcutaneous administration of 10 mg/kg LY354740 resulted in a significant attenuation of stress-induced hyperthermia, $F(11,132) = 3.37$; $p < 0.001$. These latter results confirm our previous findings with LY354740 in a dose-response study (Rorick-Kehn et al., 2005).

Oral administration of LY544344 produced a significant, dose-dependent reduction in stress-induced hyperthermia [Fig.

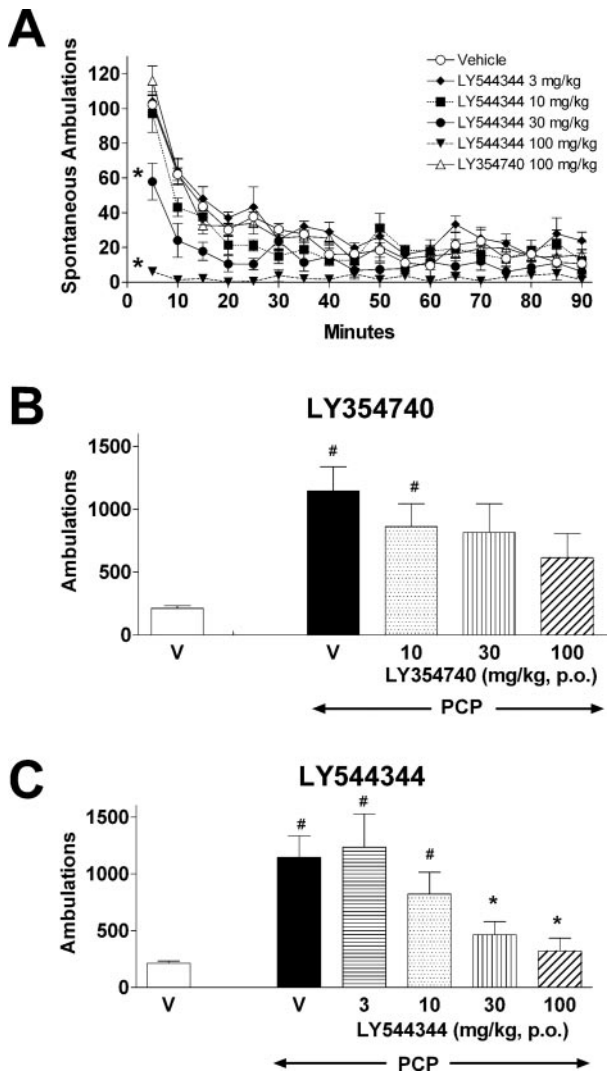


Fig. 2. Spontaneous locomotor activity after oral administration of the mGlu2/3 receptor agonist LY354740 or the mGlu2/3 receptor agonist prodrug LY544344 (A); *, $p < 0.05$ versus vehicle group; $n = 8$/group. Effect of oral administration of various doses of LY354740 (B) or LY544344 (C) on PCP-induced (5 mg/kg s.c.) increases in locomotor activity. Behaviors were monitored over a 60-min time period after injection of PCP or vehicle. Experimental compounds were administered 60 min before PCP injection. Values represent the mean (±S.E.M.) number of ambulations ($n = 9$ –15/group). #, $p < 0.05$ versus veh/veh control group. *, $p < 0.05$ versus veh/PCP group.

TABLE 2

Effects of the mGlu2/3 receptor agonist LY354740 or the mGlu2/3 prodrug LY544344 on baseline body temperature (in °C) in the stress-induced hyperthermia model

Data are presented as the mean (±S.E.M.) prestress core body temperature, measured at the end of the 60-min baseline period (i.e., prior to presentation of the stressor) in male DBA/2 mice.

Compound	Dose	*n*	Prestress Body Temperature
	mg/kg		*mean ± S.E.M.*
LY354740 (p.o.)	0	8	36.20 (0.22)
	3	7	36.23 (0.31)
	10	6	36.09 (0.18)
	30	7	36.30 (0.34)
LY544344 (p.o.)	0	6	36.02 (0.28)
	3	6	36.92 (0.18)
	10	6	36.60 (0.14)
	30	6	37.45 (0.15)*
LY354740 (s.c.)	0	7	36.41 (0.23)
	10	7	36.58 (0.40)

* Significant difference from vehicle group ($p < 0.05$).

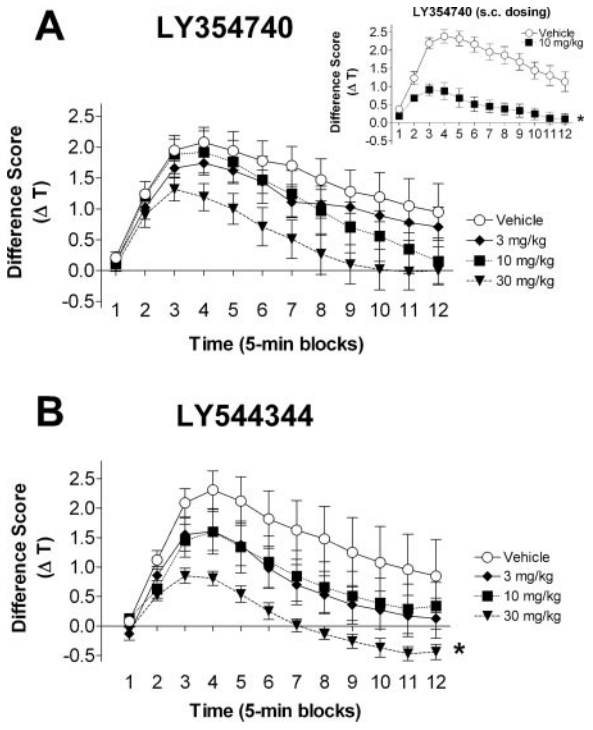


Fig. 3. Time course of stress-induced hyperthermia after placement in a novel cage containing soiled shavings from a rat cage in male DBA/2 mice receiving oral administration of the mGlu2/3 receptor agonist LY354740 (A) or the mGlu2/3 prodrug LY544344 (B). Values represent changes in core body temperature in °C (±S.E.M.) relative to the prestress baseline period ($n = 6–8$/group). For comparison purposes, the inset figure shows the mean (±S.E.M.) stress-induced hyperthermia observed in mice subcutaneously administered vehicle (0.9% NaCl) or LY354740 (10 mg/kg). *, $p < 0.05$ versus vehicle group.

3B; $F(33,220) = 1.64$; $p = 0.02$]. Post hoc comparisons confirmed that attenuation of the stress-induced hyperthermic response was significant in mice receiving 30 mg/kg. However, the 30 mg/kg dose of LY544344 also significantly increased core body temperatures during the baseline period, $F(3,23) = 4.98$, $p = 0.01$ (Table 2). Post hoc tests confirmed that mice receiving an oral dose of 30 mg/kg had significantly higher prestress body temperatures compared with the vehicle control group.

Fear-Induced Suppression of Operant Behavior. An initial experiment ($n = 7$) indicated that subcutaneous administration of LY354740 produced a dose-dependent increase both in the number of lever presses during the 2-min CS period and in the suppression ratio relative to the vehicle control day [$F(3,18) = 6.64$; $p < 0.005$ and $F(3,18) = 9.47$; $p < 0.001$, respectively; Fig. 4A]. A suppression ratio of 0 indicates a complete fear-induced suppression of operant responding during the CS period, whereas a suppression ratio of 0.5 indicates no suppression of operant behavior. Post hoc tests confirmed that the increased responding was significant at the 10 and 20 mg/kg doses for both measures (all p values <0.05). In addition, the total number of lever presses made during the 2-min pre-CS baseline period was decreased in rats receiving LY354740, $F(3,18) = 5.78$; $p < 0.01$. Post hoc tests confirmed that the attenuation of baseline responding was observed at all doses of LY354740.

In an additional experiment, oral administration of LY354740 had no significant effect on fear-induced suppression of operant behavior or on the number of lever presses during the baseline and CS periods, as illustrated in Fig. 4B (N.S. > 0.05). In a follow-up experiment in the same rats, subcutaneous administration of 10 mg/kg LY354740 produced a significant increase in the number of lever presses during the CS period and in the suppression ratio, indicating anxiolytic effects [$F(1,12) = 8.9$; $p < 0.02$ and $F(1,12) = 13.63$; $p < 0.005$, respectively; Fig. 4B]. In addition, the total number of lever presses during the 2-min pre-CS baseline period was decreased in rats receiving 10 mg/kg LY354740, $F(1,12) = 12.72$; $p < 0.005$.

As illustrated in Fig. 4C, oral administration of LY544344 significantly and dose dependently increased the number of lever presses made during the CS period, as well as the suppression ratio [$F(3,39) = 14.59$; $p < 0.001$ and $F(3,39) = 21.61$; $p < 0.001$, respectively]. Post hoc tests indicated that the 30 mg/kg dose of LY544344 increased the number of lever presses made during the CS period relative to the vehicle control. Post hoc tests also determined that the 10 and 30 mg/kg doses of LY544344 produced significantly higher suppression ratios than observed on the vehicle control day. That is, rats administered 10 and 30 mg/kg LY544344 demonstrated significantly decreased fear-induced suppression during the CS period, indicating anxiolytic effects. In addition, LY544344 produced a reduction in the number of lever presses observed during the last 2 min of the baseline period, which was significant in rats administered 3 mg/kg [$F(3,39) = 2.92$; $p < 0.05$].

Discussion

The present findings replicate and extend earlier reports that group II mGlu receptor agonism produces robust efficacy in animal behavioral models of psychosis and anxiety. In addition, our findings demonstrate the viability of a prodrug strategy in increasing oral bioavailability of LY354740. The paradigms used here are widely accepted in vivo paradigms that demonstrate predictive validity for clinical utility in psychiatric disorders. Although several studies indicate that LY354740 is active in animal models of psychosis and anxiety with minimal potential for side effects (Helton et al., 1998; Moghaddam and Adams, 1998; Cartmell et al., 1999; Klodzinska et al., 1999; Homayoun et al., 2005), it has low bioavailability following oral administration (~10%) because of poor gastrointestinal absorption (Johnson et al., 2002). Therefore, a prodrug approach was eval-

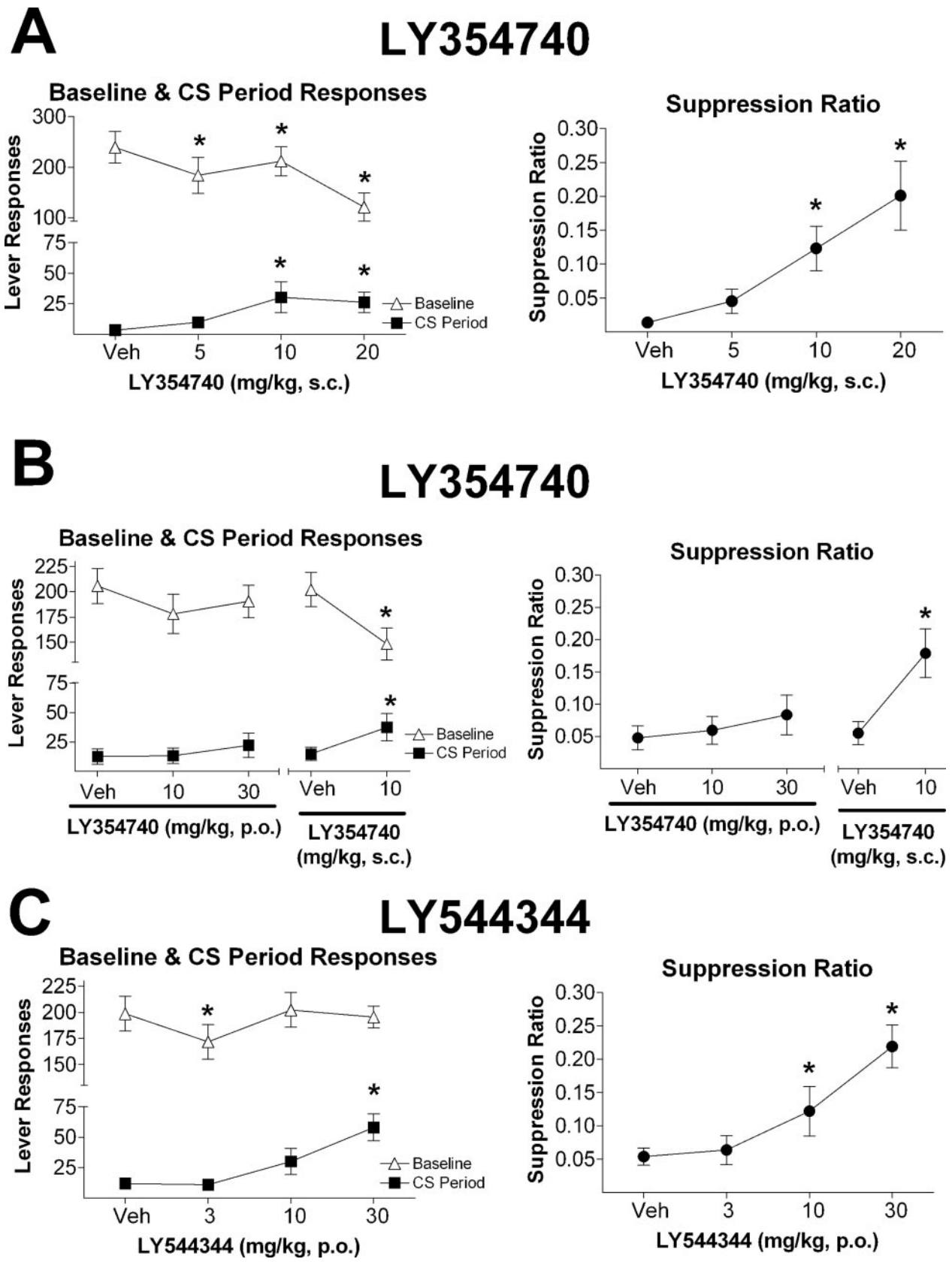


Fig. 4. Initial experiment ($n = 7$) evaluating the effect of subcutaneous administration of the mGlu2/3 receptor agonist LY354740 on fear-induced suppression of operant responding (A). Follow-up experiments examining the effect of oral versus subcutaneous administration of LY354740 (B) and oral administration of the mGlu2/3 prodrug LY544344 (C) on fear-induced suppression of operant responding ($n = 14$) are shown. Values represent mean (±S.E.M.) number of lever presses observed during the 2-min baseline and CS periods (left) and fear-induced suppression ratios (right). A suppression ratio of 0 indicates complete suppression of operant responding during the CS period, whereas a suppression ratio of 0.5 indicates no fear-induced suppression of operant behavior (i.e., anxiolytic effects). *, $p < 0.05$ versus vehicle group.

uated to increase oral bioavailability of the mGlu2/3 receptor agonist LY354740. In brief, the prodrug LY544344 is transported from the intestinal lumen via PepT1 transporters followed by rapid and extensive biotransformation into the parent compound LY354740 (Bueno et al., 2005). The resulting oral bioavailability of LY354740 in rats is approximately 85%, with no detectable prodrug levels 30-min postadministration (Bueno et al., 2005).

In the present pharmacokinetic experiments, oral administration of the prodrug LY544344 to rats resulted in approximately 10-fold greater exposure (relative to dose) to LY354740 in plasma, brain, and cerebrospinal fluid. The CNS penetration of LY354740 was relatively low but is clearly driven by plasma concentration (Fig. 1). These findings are consistent with previous reports that oral administration of the prodrug significantly increased intestinal uptake and subsequent bioavailability of the parent compound in rats (Bueno et al., 2005). In that study, LY544344 was completely bioconverted to the parent compound in vivo and activity in the fear-potentiated startle paradigm was increased significantly relative to LY354740. Moreover, the prodrug had no affinity for mGlu2 or mGlu3 receptors, suggesting that the anxiolytic effects resulted from bioconversion of the prodrug to the parent compound. The current results support and extend previous findings by demonstrating that oral prodrug administration produced robust antipsychotic and anxiolytic effects in animal models of psychosis and anxiety.

PCP-induced hyperlocomotion was dose dependently and completely inhibited in rats receiving oral administration of the mGlu2/3 prodrug LY544344 (30–100 mg/kg), whereas the mGlu2/3 agonist LY354740 did not fully reverse PCP-induced hyperlocomotion at doses up to 100 mg/kg p.o. Previous research indicated that LY354740 effectively reduced PCP-induced hyperlocomotion at doses as low as 10 mg/kg when administered intraperitoneally and 0.1 mg/kg when administered subcutaneously (Moghaddam and Adams, 1998; Cartmell et al., 1999). Thus, the current results are consistent with evidence that poor gastrointestinal absorption of LY354740 results in low CNS concentrations (Johnson et al., 2002). In contrast to the parent compound, oral administration of LY544344 significantly reversed PCP-induced hyperlocomotion at doses as low as 30 mg/kg. These results, along with evidence that LY544344 is effectively converted into LY354740 in vivo (Bueno et al., 2005), indicate that the prodrug approach results in a marked improvement in exposure levels of the mGlu2/3 receptor agonist in the CNS.

The reversal of PCP-induced hyperlocomotion by LY544344 demonstrated here provides further support that activation of mGlu2/3 receptors produces antipsychotic effects in animal models of psychosis. Similar to all clinically active antipsychotics, LY544344 inhibited spontaneous locomotor activity at 30–100 mg/kg, which also effectively reversed PCP-induced hyperlocomotion (Arnt, 1995). However, at these doses, mGlu2/3 receptor agonists do not affect normal behavior, such as rotarod performance, suggesting decreased propensity for extrapyramidal side effects associated with such typical antipsychotics as haloperidol (Ogren and Goldstein, 1994; Cartmell et al., 1999). Indeed, LY354740 has been shown to reverse haloperidol-induced catalepsy in rats (Konieczny et al., 1998), suggesting that its effects are not attributable to nonspecific sedative effects.

Oral administration LY544344 and subcutaneous administration of LY354740 attenuated stress-induced hyperthermia in DBA/2 mice, with the prodrug producing anxiolytic effects at lower oral doses than the parent compound. The stress-induced hyperthermia model is a robust and reliable method of assessing anxiolytic efficacy, wherein reduction of stress-related increases in body temperature indicates anxiolytic properties (Bouwknecht and Paylor, 2002; Spooren et al., 2002; Rorick-Kehn et al., 2005). Previous reports indicated that intraperitoneally administered LY354740 (3–10 mg/kg) and orally administered LY314582 (10 mg/kg; racemic mixture of LY354740) produced a dose-dependent reduction in stress-induced hyperthermia (Spooren et al., 2002; Rorick-Kehn et al., 2005). In this experiment, although oral administration of LY354740 did not produce significant anxiolytic effects, subcutaneous administration of 10 mg/kg LY354740 significantly reduced stress-induced hyperthermia. In fact, this dose produced a greater attenuation than 30 mg/kg administered orally, reinforcing the necessity for improving bioavailability of this compound. In contrast to LY354740, orally administered LY544344 significantly reduced stress-related increases in body temperature at 30 mg/kg. Although pharmacokinetic data in the mouse are not available for these compounds, these behavioral results indicate greater bioavailability of LY354740 following oral administration of the prodrug.

At the highest dose tested, LY544344 increased body temperature during the baseline period, suggesting potential nonspecific effects on thermoregulatory mechanisms. The initial hyperthermia observed here was similar to that produced by some clinical anxiolytic compounds such as alprazolam and buspirone (Rorick-Kehn et al., 2005). Nonetheless, that report indicated that drug effects on basal body temperature and stress-induced hyperthermia may be dissociable. Consistent with this conclusion, LY544344 significantly attenuated the stress response regardless of minor effects on basal body temperature. Overall, the present results confirm that mGlu2/3 agonists produce anxiolytic effects in an animal model of anxiety and indicate that LY544344 effectively reduced stress-induced hyperthermia at a lower oral dose than LY354740.

Although oral administration of LY354740 did not significantly affect fear-induced suppression of operant responding, subcutaneously administered LY354740 (10 and 20 mg/kg) and orally administered LY544344 (10 and 30 mg/kg) produced significant anxiolytic effects. This model is a form of emotional conditioning in which ongoing behavior is suppressed in the presence of a signal predicting shock (CS). This conditioned fear is sensitive to the anxiolytic effects of diazepam, chlordiazepoxide, and ipsapirone, because these compounds alleviate fear-induced suppression of responding (Stanhope and Dourish, 1996; Mirza et al., 2005). Efficacious doses of subcutaneously administered LY354740 also produced a significant decrease in the number of responses during the baseline period, suggesting perhaps an effect on general motor or consummatory behavior. However, this reduction in baseline responding was mild and not likely to confound an anxiolytic interpretation. Consistent with this, LY354740 does not produce motor impairment in the rotarod or other tests of motor function (Helton et al., 1998; Konieczny et al., 1998; Cartmell et al., 1999) and similar effects on baseline responding were not observed at efficacious doses of LY544344. Regardless, if the compound were affecting general motor activity, one would expect to see an increase in operant suppression rather than the decrease that was ob-

served here. In summary, both LY354740 (10–20 mg/kg s.c.) and LY544344 (10–30 mg/kg p.o.) produced anxiolytic effects in this model. These data extend previous reports of the functional efficacy of mGlu2/3 agonists in animal models of anxiety (Helton et al., 1998; Klodzinska et al., 1999; Tizzano et al., 2002) and fear-potentiated startle in humans (Grillon et al., 2003; for review, see Swanson et al., 2005).

The models used in these experiments are widely used to screen novel pharmacological agents for psychosis and anxiety, and they demonstrate predictive validity of clinical efficacy. The current results complement previous reports that mGlu2/3 receptor activation produces anxiolytic and antipsychotic effects in these and other animal models (Helton et al., 1998; Moghaddam and Adams, 1998; Klodzinska et al., 1999; Schoepp et al., 1999; Schoepp and Marek, 2002; Tizzano et al., 2002; Homayoun et al., 2005). The reversal of the mGlu2/3 agonist effects by the selective mGlu2/3 receptor antagonist LY341495 in those studies indicates that the reported effects were mediated via mGlu2/3 receptor activation (Cartmell et al., 1999; Bueno et al., 2005). These receptors represent viable therapeutic targets, because they exert a modulatory influence on glutamatergic tone via altering intracellular signaling cascades in key limbic and forebrain areas associated with anxiety, cognition, and higher executive function (Ohishi et al., 1993a,b; Dube and Marshall, 1997; Swanson et al., 2005). Thus, activation of mGlu2/3 receptors may provide a means to fine-tune the excessive glutamatergic activation associated with several psychiatric disorders without altering fast synaptic transmission or producing unwanted side effects, as observed with NMDA antagonists and benzodiazepines (Gudex, 1991; Bergink et al., 2004).

Therapeutic drugs that can be taken orally greatly improve both convenience for the patient and level of compliance, because they do not require painful needlesticks or complicated delivery devices. Therefore, the possibility of improving the oral bioavailability of potentially beneficial drugs is of great interest. Growing preclinical literature indicates that group II mGlu receptor agonists, such as LY354740 and LY379268, may be effective novel treatments for several psychiatric disorders, including psychosis and anxiety (Swanson et al., 2005). Here, we demonstrated substantially improved oral bioavailability and efficacy of LY354740 using a prodrug strategy in various animal models of psychosis and anxiety. These data strongly suggest that the prodrug approach may be useful in the search for novel pharmacological treatments of many psychiatric disorders.

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