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A Selective Allosteric Potentiator of Metabotropic Glutamate (mGlu) 2 Receptors Has Effects Similar to an Orthosteric mGlu2/3 Receptor Agonist in Mouse Models Predictive of Antipsychotic Activity

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ABSTRACT

Recent studies suggest that agonists of group II metabotropic glutamate (mGlu) receptors (mGlu2/3) have potential utility as novel therapeutic agents for treatment of psychiatric disorders such as anxiety and schizophrenia. Agonists of mGlu2/3 receptors block amphetamine- and phencyclidine (PCP)-induced hyperlocomotor activity in rodents, two actions that may predict potential antipsychotic activity of these compounds. We now report that LY487379 [*N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine], a recently described selective allosteric potentiator of mGlu2 receptor, has behavioral effects similar to mGlu2/3 receptor agonists. LY487379 and LY379268 [(−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate], an ortho-steric mGlu2/3 receptor agonist, induced similar dose-dependent reductions in PCP- and amphetamine-induced hyperlocomotor activity in C57BL6/J mice at doses that did not significantly alter spontaneous locomotor activity. These effects were blocked by the mGlu2/3 receptor antagonist LY341495 [(2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid]. LY487379 had a short duration of action compared with LY379268. Furthermore, unlike the mGlu2/3 agonist, LY487379 reversed amphetamine-induced disruption of prepulse inhibition of the acoustic startle reflex. When LY379268 was given chronically, it failed to block amphetamine- and PCP-induced hyperlocomotor activity. The finding that the effects of an orthosteric mGlu2/3 receptor agonist in these models can be mimicked by a selective allosteric potentiator of mGlu2 suggests that these effects are mediated by the mGlu2 receptor subtype. Furthermore, these data raise the possibility that a selective allosteric potentiator of mGlu2 receptor could have utility as a novel approach for the treatment of schizophrenia.

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) and is responsible for generation of fast excitatory synaptic responses at the vast majority of CNS synapses (Dingledine et al., 1999). Fast synaptic responses at glutamatergic synapses are mediated by activation of a well characterized family of glutamate receptor cation channels referred to as the ionotropic glutamate receptors. In addition, glutamate activates metabotropic glutamate (mGlu) receptors, which are coupled to effector systems through GTP-binding proteins (Conn and Pin, 1997). A family of eight mGlu receptor subtypes has been identified in mammalian brain, and these subtypes are classified into three major groups (Conn and Pin, 1997). A large body of preclinical studies now suggests that ligands for specific mGlu receptor subtypes have potential for treatment of a range of CNS disorders, including depression, anxiety, chronic pain, epilepsy, Alzheimer's disease, Parkinson's disease, and schizophrenia among others (Schoepp et al., 2001; Marek, 2004).

Group II mGlu receptors include mGlu2 and mGlu3 subtypes. These receptors are localized primarily presynaptically in the cortex, thalamus, striatum, amygdala, and hippocampus (Ohishi et al., 1993). These areas of the brain are thought to play a critical role in anxiety disorders (Walker and Davis, 2002) and psychosis (Moghaddam and Adams, 1998; Schoepp and Marek, 2002) among other CNS disorders. Hyperactivity of glutamatergic transmission in these structures is thought to be associated with the pathogenesis of anxiety (Walker and Davis, 2002) and schizophrenia

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ABBREVIATIONS: CNS, central nervous system; mGlu, metabotropic glutamate; PCP, phencyclidine; PPI, prepulse inhibition; LY487379, *N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine; LY379268, (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; ANOVA, analysis of variance.

(Moghaddam and Adams, 1998; Lorrain et al., 2003). It has been postulated that agonists at mGlu2/3 receptors could reduce anxiety- and psychotic-like behaviors by decreasing transmission at glutamatergic synapses in these brain regions (Moghaddam and Adams, 1998; Lorrain et al., 2003a). The anxiolytic effects of mGlu2/3 agonists have now been documented in a broad range of animal models, including fear-potentiated startle, elevated plus maze, stress-induced hyperthermia, and the Vogel conflict test (for review, see Schoepp et al., 2003). Furthermore, although the anatomical site of mGlu2/3 agonists is not definitively established, multiple clinical studies indicate that mGlu2/3 receptor agonists have antianxiety effects in humans (Grillon et al., 2003). More recently, it has been hypothesized that mGlu2/3 agonists might be effective in the treatment of psychoses. For example, studies have indicated that an mGlu2/3 agonist blocks hyperactivity in rats (Cartmell et al., 1999, 2000a) and attenuates memory deficits in humans (Krystal et al., 2005) induced by amphetamine and PCP.

The relative contributions of mGlu2 and mGlu3 to the effects of mGlu2/3 agonists are not fully understood. Since the glutamate binding site is highly conserved across mGlu receptor subtypes (Conn and Pin, 1997), it has been difficult to develop highly selective orthosteric agonists of mGlu2 or mGlu3.

Recently, highly selective mGlu2 receptor allosteric potentiators have been developed (Johnson et al., 2003; Lorrain et al., 2003b; Schaffhauser et al., 2003; Pinkerton et al., 2004). These small molecules do not activate the mGlu2 receptor directly but act at an allosteric site on the receptor to potentiate glutamate-induced activation of the receptor (Schaffhauser et al., 2003). It is possible that this will offer an advantage to orthosteric agonists by preserving activity dependence of glutamate physiological functions and perhaps reduce receptor desensitization and adverse effects relative to those that occur with orthosteric receptor agonists. Interestingly, initial studies suggest that mGlu2 receptor potentiators may mimic some of the behavioral effects of mGlu2/3 agonists in animal models used to predict anxiolytic and antipsychotic activity (Johnson et al., 2003, 2005; Pinkerton et al., 2004). However, the behavioral effects of these compounds have not been systematically and rigorously characterized across a range of animal models and doses to gain a clear view of their effects in comparison with orthosteric mGlu2/3 agonists.

Amphetamine- and PCP-induced hyperlocomotor activity and disruption of prepulse inhibition (PPI) deficits are standard procedures commonly used to evaluate the antipsychotic-like activity of compounds (Geyer and Ellenbroek, 2003). Amphetamine and *N*-methyl-D-aspartate receptor antagonists increase locomotor activity and disrupt PPI in many species (Geyer and Ellenbroek, 2003), including humans (Krystal et al., 1994), and these effects are reduced by typical and atypical antipsychotic agents (for review, see Geyer and Ellenbroek, 2003). We now report a series of acute and chronic studies aimed at comparing the effects of LY487379, a selective mGlu2 receptor allosteric potentiator, and LY379268, an orthosteric mGlu2/3 receptor agonist, in these behavioral models of antipsychotic-like effects in mice.

Materials and Methods

Drugs. LY487379 was synthesized as previously described (Johnson et al., 2003). The product was characterized by NMR (^{1}H and ^{13}C) and electrospray ionization-mass spectrometry (M+H, 453.2). Elemental analysis of the product was performed by Atlantic Microlab, Inc. (Norcross, GA; calculated C, 55.75%; H, 4.23%; and N, 6.19%; found C, 55.66%; H, 4.21%; and N, 6.16%). LY379268 was a generous gift from Dr. James Monn (Eli Lilly & Co., Indianapolis, IN). LY341495 was obtained from Tocris Cookson Inc. (Ellisville, MO). PCP hydrochloride and amphetamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). LY487379 was dissolved in 10% Tween 80 (Sigma-Aldrich) and 1% lactic acid (Sigma-Aldrich), and the pH was adjusted with 1 M sodium hydroxide (Mallinckrodt, St. Louis, MO). LY379268, LY341495, PCP, and amphetamine were dissolved in water.

Subjects. Experiments were conducted in 2- to 5-month-old C57BL6/J male mice. Subjects were group-housed in plastic cages under 12-/12-h light/dark schedule and humidity-controlled rooms and had ad libitum access to water and food (PMI Nutrition International, Brentwood, MO). Animals were handled according to the Guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council (Department of Health, Education, and Welfare, National Institutes of Health Publication No. 85-23, revised 1996).

Apparatus. Open field chambers (27 × 27 × 20 cm) (MED Associates, St. Albans, VT) equipped with 16 horizontal (*x*- and *y*-axes) and 16 vertical (*z*-axis) infrared photobeams located 1 and 5.5 cm above the floor of the chamber, respectively, were used to detect locomotor activity. Movements were detected through photobeam breaks (i.e., counts) and were recorded with a Pentium I computer equipped with a mouse activity-monitoring system software (MED Associates).

For studies of the acoustic startle reflex, sound-attenuating acoustic startle cubicles (MED Associates) were equipped with two speakers, a mouse holder, and a transducer system (a platform with load cells) through which startle responses were recorded. Chambers were connected to an amplifier and to a Pentium IV computer equipped with Startle Reflex software (MED Associates).

Locomotor Activity (Acute Studies). Studies were designed to assess the effects and dose dependence of LY487379, a selective mGlu2 allosteric potentiator, and LY379268, an mGlu2/3 agonist, on amphetamine- and PCP-induced hyperlocomotor activity. Experiments were conducted using a within-subject counterbalanced design. For example, experiments were conducted every 3 to 4 days in the same mice. Specifically, animals were placed in the open field for 60 min (habituation period). Thereafter, on each session, one-half of the mice received an acute injection of vehicle or water (10 ml/kg i.p.) with amphetamine (3.2 mg/kg s.c.) or PCP (5.6 mg/kg s.c.), and the remaining one-half received LY487379 (10, 32, and 100 mg/kg i.p.) or LY379268 (0.3, 1, and 3 mg/kg i.p.) with the same dose of amphetamine or PCP. Locomotor activity was then measured for an additional 120 min. In a separate study, the duration of action of the largest dose of LY487379 (100 mg/kg i.p.) and LY379268 (3 mg/kg i.p.) was evaluated in the same mice by administering these compounds 2, 4, 8, and 16 h prior to amphetamine. In addition, blockade of the effects of LY379268 and LY487379 was also evaluated in the same mice. Mice were placed in the open field, and LY341495 (3 mg/kg i.p.), a competitive mGlu2/3 antagonist, was given 20 min before the end of the 60-min habituation period. Thereafter, mice randomly received in separate occasions LY487379 (100 mg/kg i.p.) or LY379268 (3 mg/kg i.p.) with amphetamine (3.2 mg/kg s.c.) or PCP (5.6 mg/kg s.c.). In addition, experiments assessed whether LY487379 (32, 100 mg/kg) and LY379268 (1, 3 mg/kg) disrupt spontaneous locomotor activity in nonhabituated naive mice, using a between-subject design. In these experiments, compounds were given immediately prior to the 60-min habituation period, and locomotor activity was studied for 180 min.

Locomotor Activity (Chronic Studies). The effects of repeated administration of LY379268 were evaluated in naive mice using a between-subject counterbalanced design (i.e., tests were conducted only once in each mouse). LY379268 (3 mg/kg i.p.) was administered

once daily for 7 consecutive days. Twenty-four hours after the last administration, mice were placed in the open field for 60 min. Thereafter, one-half of the mice received an injection of LY379268 (3 mg/kg i.p.) and amphetamine (3.2 mg/kg s.c.), and the remaining one-half received vehicle and amphetamine (3.2 mg/kg s.c.). The effects of repeated administration of LY487379 were not evaluated because of the short duration of action of this compound.

PPI of Startle Reflex. The effects of LY487379 (32 mg/kg i.p.) or LY379268 (3 mg/kg i.p.) on amphetamine-induced (3.2 mg/kg s.c.) or PCP-induced (5.6 mg/kg s.c.) disruption of PPI were studied using a within-subject design. Six different treatment conditions were assigned to 16 naive mice in each session. Vehicle, LY487379, and LY379268 were administered 30 min, whereas water, amphetamine, and PCP were given 15 min prior to the beginning of the session. These pretreatment times were chosen according to the open field studies and to the literature. Each session started with a 5-min acclimatization period, during which a 65-db background noise was continuously present, and included a total of 54 trials. Six different trial types were randomly assigned and delivered (every 15–20 s on average) for nine times throughout the session: 40-ms broadband 120-db burst (pulse only), 65-db background noise (noise only), and 20-ms prepulse of 70, 76, 82, and 88 db followed by 100 ms of 120-db pulse. Sessions were conducted every 3 to 4 days.

Statistical Analysis. Locomotor activity data were expressed as mean counts (i.e., photobeam brakes) ± S.E.M. Time course and antagonist studies were analyzed using repeated measures ANOVA with treatment and time (i.e., 120 min postinjection) as within-group factors followed by analyses of simple main effects and, when appropriate, post hoc analysis with least significant difference test. The acute effects of LY487379 and LY379268 on spontaneous locomotor activity in naive mice and the effects of repeated administration of LY379268 in naive mice were analyzed with one-way ANOVA. An effect was considered statistically significant when $p \leq 0.05$.

To quantify the potency and duration of action of LY487379 and LY379268, locomotor activity data were calculated as the average counts/5 min that occurred in the first 60 min postinjection (i.e., after habituation time) and were expressed as percent control (i.e., average of three determinations of amphetamine or PCP effects by itself). ED_{50} values were calculated by nonlinear regression or by interpolation (when only two data points were available) for each mouse and were expressed as mean ± S.E.M.

Startle amplitude data were expressed as the mean value ± S.E.M. during presentation of the background noise only (i.e., 65 db) and the pulse alone (i.e., 120 db) and were analyzed with one-way repeated measures ANOVA. Levels of PPI were determined by the formula [100 − ((prepulse pulse/pulse alone) × 100)] and were expressed as percent PPI ± S.E.M. Data were analyzed using repeated measures ANOVA with the prepulse intensity and treatment as within-group factors followed by analyses of simple main effects and, when appropriate, post hoc analysis with least significant difference test. An effect was considered statistically significant when $p \leq 0.05$.

Results

Locomotor Activity (Acute Studies). Consistent with previous studies, amphetamine (3.2 mg/kg s.c.) and PCP (5.6 mg/kg s.c.) induced a robust increase in locomotor activity. Furthermore, both LY487379 and LY379268 induced a dose-dependent reduction in the hyperlocomotor responses to PCP and amphetamine (Fig. 1). Thus, two-way repeated measures ANOVA indicated that there was a significant treatment effect [Fig. 1a, $F(5, 30) = 17.02$, $p < 0.05$; Fig. 1b, $F(4,24) = 6.85$, $p < 0.05$; Fig. 1c, $F(5,30) = 8.52$, $p < 0.05$; and Fig. 1d, $F(5,25) = 5.26$, $p < 0.05$]. Post hoc analysis indicated that amphetamine (Fig. 1, a and c) and PCP (Fig. 1, b and d) significantly increased locomotor activity compared with vehicle and that LY487379 (32 and 100 mg/kg i.p.) and LY379268 (1 and 3 mg/kg i.p.) dose dependently reduced these effects. Systemic administration of LY341495 (3 mg/kg i.p.), an mGlu2/3 antagonist, significantly blocked the effects of the largest dose of LY487379 (100 mg/kg i.p.) and LY379268 (3 mg/kg i.p.). Two-way repeated measures ANOVA also indicated that there was a time effect [Fig. 1a, $F(23,138) = 24.58$, $p < 0.05$; Fig. 1b, $F(23,138) = 39.72$, $p <$

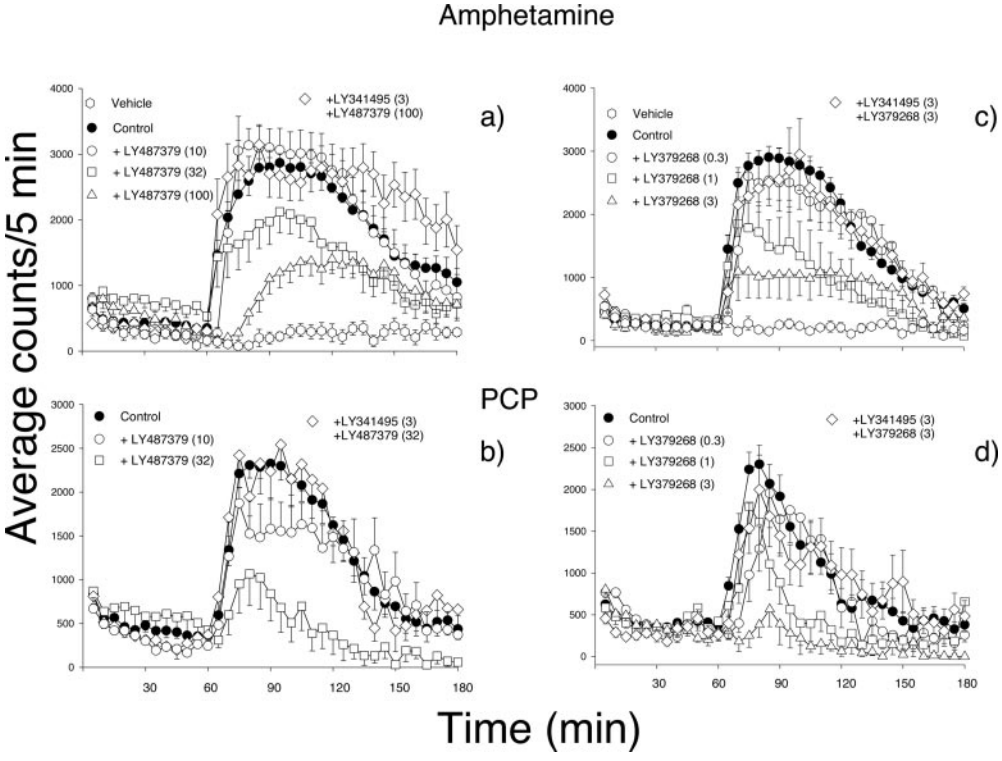

Fig. 1. Dose-effect curves and time courses of LY487379 (a and b) and LY379268 (c and d) for the blockade of PCP- and amphetamine-induced hyperlocomotor activity (n = 7–8 per dose). Data are expressed as mean ± S.E.M. Ordinate, average counts/5 min. Abscissa, time in minutes. Control data represent the average of three determinations of amphetamine or PCP effects by itself.

0.05; Fig. 1c, $F(23,138) = 43.13$, $p < 0.05$; and Fig. 1d, $F(23,115) = 25.38$, $p < 0.05$] and treatment × time interaction [Fig. 1a, $F(115,690) = 4.83$, $p < 0.05$; Fig. 1b, $F(92,552) = 4.68$, $p < 0.05$; Fig. 1c, $F(115,690) = 5.34$, $p < 0.05$; and Fig. 1d, $F(115,575) = 3.57$, $p < 0.05$].

The ED_{50} values of LY379268 and LY487379 for blocking the effects of amphetamine were 1.7 ± 0.4 and 50.7 ± 10.9 mg/kg, respectively. For blocking the effects of PCP, the ED_{50} values were 1.3 ± 0.4 and 29.2 ± 6.2 mg/kg, respectively (Fig. 2).

We next determined the effects of LY487379 and LY379268 on the hyperlocomotor response to amphetamine. In these experiments, LY487379 or LY379268 was administered simultaneously with (i.e., time 0 h) and 2, 4, 8, and 16 h prior to amphetamine. One-way repeated measures ANOVA indicated that LY487379 (100 mg/kg i.p.) blocked amphetamine-induced hyperlocomotor activity only when it was coadministered (i.e., time 0 h), and these effects were no longer present 2 h later [$F(4,24) = 11.99$, $p < 0.05$; Fig. 3]. In contrast, LY379268 (3 mg/kg i.p.) significantly blocked the hyperlocomotor activity induced by amphetamine up to 8 h [$F(6,36) = 6.13$; $p < 0.05$; Fig. 3].

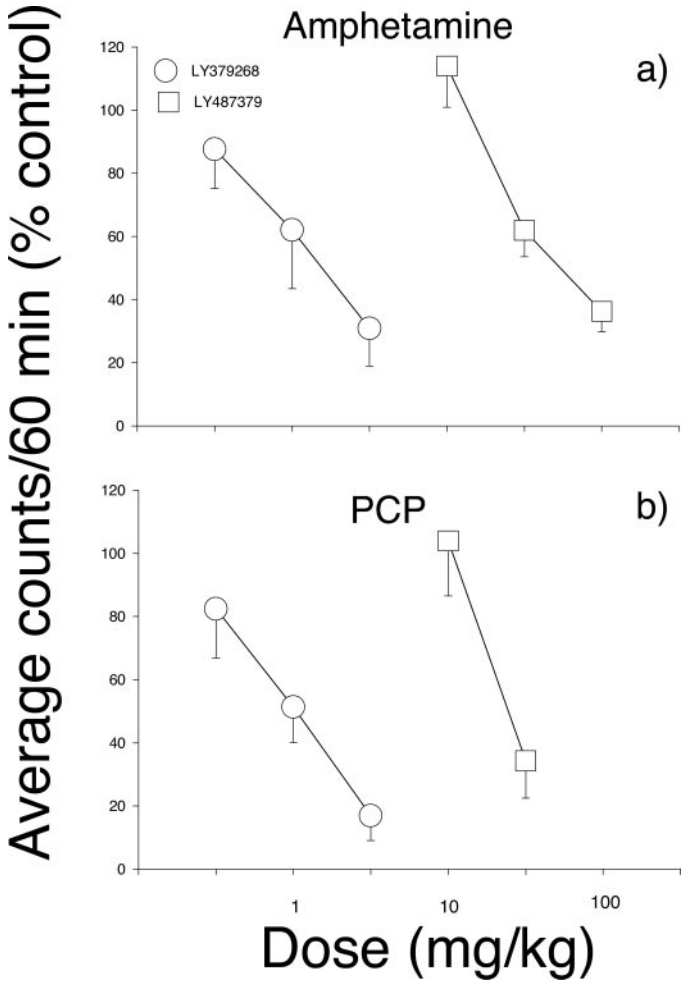

Fig. 2. Potency of LY487379 and LY379268 in blocking amphetamine-induced (a) and PCP-induced (b) hyperlocomotor activity. Average counts/60 min, expressed as mean percentage ± S.E.M of the average of three determinations of amphetamine or PCP effects (i.e., percent control), are plotted as a function of LY487379 and LY379268 dose expressed in milligrams per kilogram (n = 7–8 per dose).

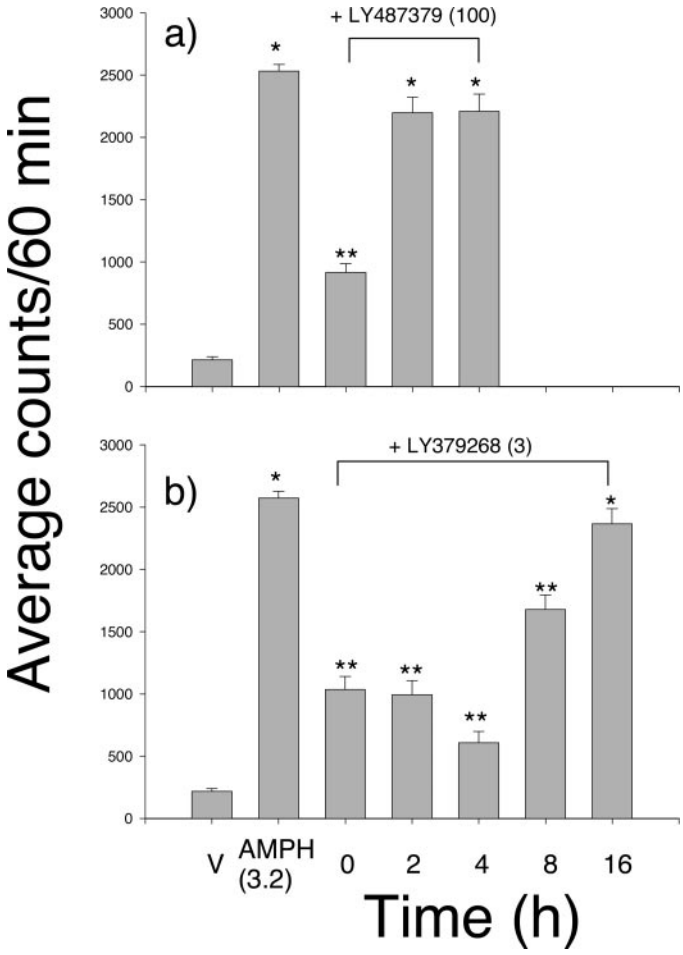

Fig. 3. Duration of action of LY487379 (a) and LY379268 (b) on amphetamine-induced hyperlocomotor activity. LY487379 or LY379268 was administered with (i.e., at time 0 h) and 2, 4, 8, and 16 h prior to amphetamine. Data are expressed as mean ± S.E.M. Average counts/60 min are plotted as a function of time in hours (n = 7–8 per condition). *, $p < 0.05$ indicates statistical difference from vehicle (V). **, $p < 0.05$ indicates statistical difference from amphetamine (AMPH; 3.2 mg/kg).

We next determined the effect of LY487379 and LY379268 on basal locomotor activity (i.e., without PCP or amphetamine) (Fig. 4). One-way ANOVA indicated that there was a main treatment effect on spontaneous locomotor activity in naive mice [Fig. 4a, $F(2,20) = 9.15$, $p < 0.05$; and Fig. 4b, $F(2,21) = 7.65$]. Only the highest dose of LY487379 (100 mg/kg i.p.) and LY379268 (3 mg/kg i.p.) significantly decreased locomotor activity. There was also a significant time effect [Fig. 4a, $F(35,700) = 49.34$, $p < 0.05$; and Fig. 4b, $F(35,735) = 48.38$] and a treatment × time interaction [Fig. 4a, $F(70,700) = 7.21$, $p < 0.05$; and Fig. 4b, $F(70,735) = 3.36$].

Locomotor Activity (Chronic Studies). A significant problem that often limits the use of receptor agonists is development of tolerance after chronic administration. However, most previous studies with mGlu2/3 receptor agonists have only evaluated the action of these compounds acutely. Thus, we administered LY379268 chronically over 7 days to determine whether tolerance would develop to the ability of this compound to block amphetamine- and PCP-induced hyperlocomotor activity. Interestingly, two-way ANOVA indicated that LY379268 did not reduce, after chronic dosing, amphetamine-induced [Fig. 5a, $F(1,14) = 2.01$, $p = 0.17$] or PCP-induced [Fig. 5b, $F(1,14) = 2.11$, $p = 0.16$] hyperloco-

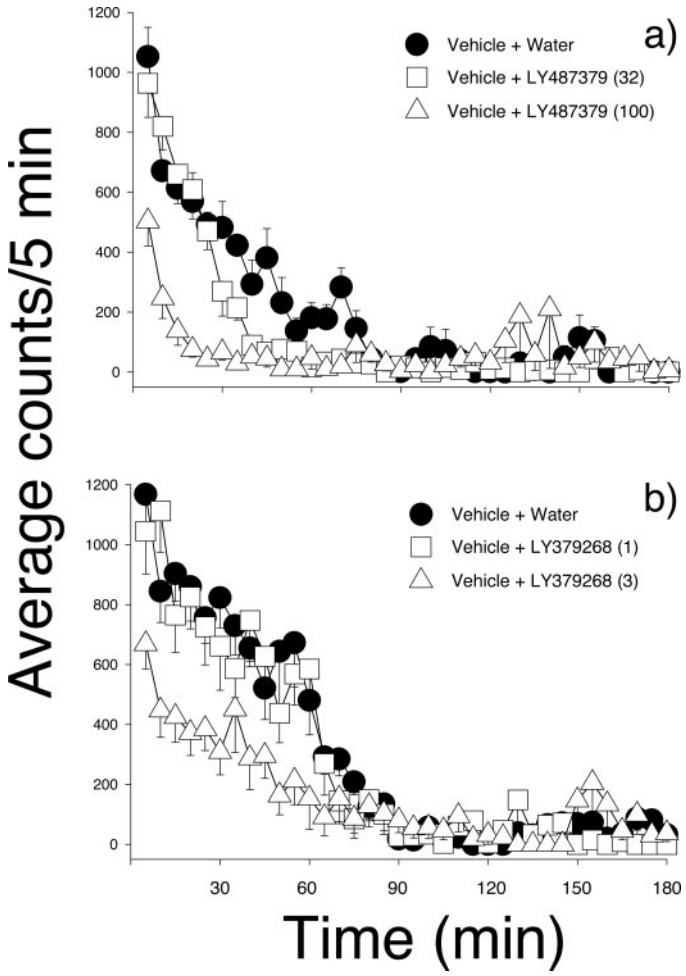

Fig. 4. Dose-effect curves and time courses of LY487379 (a) and LY379268 (b) on spontaneous locomotor activity. Data are expressed as mean ± S.E.M. Average counts/5 min is plotted as a function of time in min (n = 7–8 per condition).

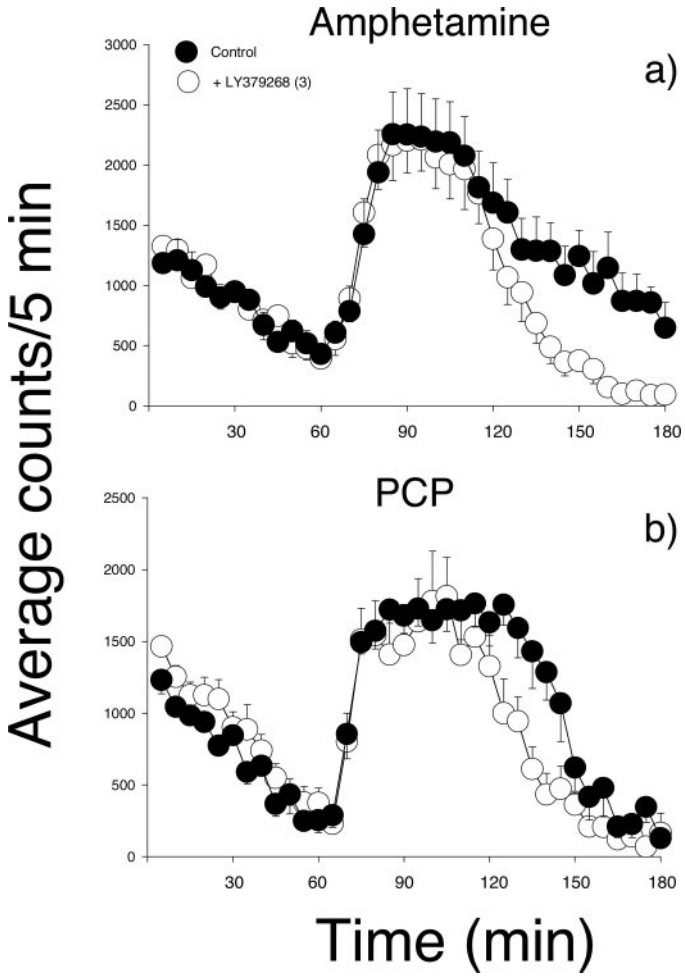

Fig. 5. Time course of amphetamine (a) and PCP (b) before and after 7-day chronic treatment with LY379268. Data are expressed as mean ± S.E.M. Average counts/5 min are plotted as a function of time in minutes (n = 7–8 per condition).

motor activity. There was a significant time effect [Fig. 5a, $F(23,322) = 32.91$, $p < 0.05$; and Fig. 5b, $F(23,322) = 40.7$, $p < 0.05$] and a significant treatment × time interaction [Fig. 5a, $F(23,322) = 2.43$, $p < 0.05$; and Fig. 5b, $F(23,322) = 2.22$, $p < 0.05$].

PPI of Startle Reflex. Another commonly used model of antipsychotic-like drug action is disruption of PPI, a measure of sensorimotor gating that is disrupted in schizophrenic patients. We determined the effects of LY379268 and LY487379 on disruption of PPI induced by amphetamine and PCP. Two-way repeated measures ANOVA indicated that there was a treatment effect [Fig. 6a, $F(5,75) = 15.8$, $p < 0.05$; and Fig. 6b, $F(5,75) = 7.21$, $p < 0.05$]. In addition, there was a prepulse intensity effect (i.e., decibels) [Fig. 6a, $F(3,45) = 84.01$, $p < 0.05$; and Fig. 6b, $F(3,45) = 112.58$, $p < 0.05$] and a treatment × prepulse intensity interaction [Fig. 6a, $F(15,225) = 3.95$, $p < 0.05$]. Consistent with previous studies, post hoc analysis indicated that amphetamine and PCP significantly decreased percent PPI of startle reflex (Fig. 6, a and b). Furthermore, systemic administration of LY487379 significantly reversed amphetamine-induced disruption of PPI without affecting PCP effects (Fig. 6a). In contrast, the mGlu2/3 agonist LY379268 did not significantly reverse amphetamine- or PCP-induced disruption of PPI (Fig. 6b). One-way ANOVA indicated that there was a treatment effect on startle amplitude when the background noise (i.e., 65 db) was presented [Table 1, LY487379, $F(5,65) = 5.25$, $p < 0.05$; LY379268, $F(5,70) = 6.74$]; post hoc analysis indicated that amphetamine and PCP significantly increased startle amplitude. However, there was no treatment effect under all six treatment conditions when the pulse alone (i.e., 120 db) was presented [Table 1, LY487379, $F(5,65) = 1.05$, $p = 0.39$; LY379268, $F(5,70) = 0.71$, $p = 0.61$].

Discussion

The goal of these studies was to characterize the behavioral effects of LY487379, a selective mGlu2 allosteric potentiator, in preclinical mouse models predictive of antipsychotic activity. The results indicate that this mGlu2 receptor allosteric potentiator has a number of effects that are consistent with possible antipsychotic-like activity. Thus, LY487379 induced a dose-dependent reduction in amphetamine- and PCP-induced hyperlocomotor activity. Consistent with previous studies (Cartmell et al., 1999, 2000), the mGlu2/3 orthosteric agonist LY379268 also reduced the hyperlocomotor responses to amphetamine and PCP. These results suggest that the previously described effects of orthosteric mGlu2/3 agonists on stimulant-induced

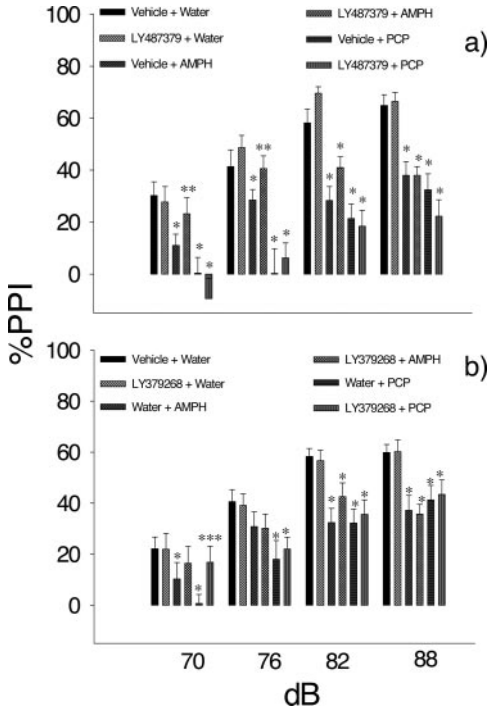

Fig. 6. Effects of LY487379 (a) and LY379268 (b) on amphetamine- and PCP-induced PPI deficits. Data are expressed as mean ± S.E.M. Percent PPI is plotted as a function of decibel intensity (n = 14–16/treatment). *, $p < 0.05$, indicates statistical difference from vehicle + water. **, $p < 0.05$, indicates statistical difference from vehicle + amphetamine. ***, $p < 0.05$, indicates statistical difference from vehicle + PCP.

locomotor activity are mediated by the mGlu2 receptor subtype and can be mimicked by an allosteric potentiator of this receptor. To the extent that the increase in locomotor activity induced by amphetamine and PCP is centrally mediated, blockade of these effects by LY487379 suggests that systemic administration of the allosteric potentiator might centrally modulate the activity of neurotransmitters such as glutamate in a manner similar to orthosteric mGlu2/3 agonists (Moghaddam and Adams, 1998; Lorrain et al., 2003a). The central activity of LY487379 is also consistent with a recent finding indicating that a chemically unrelated class of selective mGlu2 receptor allosteric potentiators blocked ketamine-induced hyperactivity when administered i.c.v. in rats (Pinkerton et al., 2004). These results also indicate that LY487379 and LY379268 have a relatively fast onset of action. For example, within 30 min, both compounds blocked PCP and amphetamine effects. However, the duration of action of the potentiator was shorter than the competitive agonist; presumably, this is associated with a shorter half-life. For example, the effects of LY487379 were no longer present 2 h after administration, whereas the effects of LY379268 lasted up to 8 h, and these results appear to be consistent with the relatively long duration of action and half-life of this and structurally related mGlu2/3 agonists in rats (Cartmell et al., 1999; Johnson et al., 2002).

It is important to note that the potentiator and orthosteric agonist blocked the effects of amphetamine and PCP at doses that did not disrupt spontaneous locomotor activity. Thus, the decrease in responses to PCP and amphetamine is not likely to be solely due to a generalized behavioral depression. However, a small increase in dose decreased spontaneous locomotor activity, suggesting that the effective window between these two responses for two chemically and mechanistically unrelated classes of compounds is small. Importantly, the effects of the potentiator and the agonist were blocked by LY341495, an mGlu2/3 antagonist, and mGlu2/3 agonists do not block PCP-induced hyperlocomotor activity in mGlu2 knockout mice (Spooren et al., 2000), providing strong evidence that the behavioral effects of LY487379 and LY379268 in this model are mediated by mGlu2 receptors.

The results of this study indicate that doses of amphetamine and PCP that significantly increased locomotor activity in mice also disrupted PPI of the startle reflex, a result that is consistent with other studies conducted in mice and other species (Geyer and Ellenbroek, 2003). We were surprised to find that LY487379 reversed the amphetamine-induced disruption of PPI at doses that did not disrupt startle amplitude. In contrast, the orthosteric agonist LY379268 failed to reverse either PCP- or amphetamine-induced disruption of PPI. This is consistent with previous studies showing that mGlu2/3 agonists are without effects on PPI (Schreiber et al., 2000; Henry et al., 2002). This finding raises the exciting possibility that selective stimulation of the mGlu2 receptor subtype might have beneficial effects on sensorimotor gating or cognitive-related deficits associated with psychoses that are not observed with nonselective mGlu2/3 receptor agonists. Although speculative, it is conceivable that mGlu3 receptor activation has an action that counteracts the effect of mGlu2 receptor activation in this model. To the extent that amphetamine-induced disruption of PPI is asso-

TABLE 1
Amplitude of acoustic startle reflex
Data are expressed as mean ± S.E.M. when the background noise stimulus (i.e. 65 db) or the pulse stimulus (i.e. 120 db) was presented by itself.

	Startle Amplitude	
	65 db	120 db
Vehicle + water	154.5 ± 20.8	717.5 ± 80.0
LY487379 (32) + water	121.3 ± 13.6	768.0 ± 104.2
Vehicle + amphetamine (3.2)	248.4 ± 28.4*	801.3 ± 65.0
LY487379 (32) + amphetamine (3.2)	236.5 ± 25.0*	737.8 ± 94.8
Vehicle + PCP (5.6)	228.2 ± 22.3*	613.0 ± 83.4
LY487379 (32) + PCP (5.6)	226.2 ± 15.9	695.8 ± 76.0
Vehicle + water	165.5 ± 16.9	767.9 ± 72.0
LY379268 (3) + water	155.4 ± 17.6	726.0 ± 57.8
Vehicle + amphetamine (3.2)	249.6 ± 17.5*	622.1 ± 38.0
LY379268 (3) + amphetamine (3.2)	230.1 ± 19.7*	649.7 ± 59.1
Vehicle + PCP (5.6)	161.6 ± 18.4	734.1 ± 86.1
LY379268 (3) + PCP (5.6)	153.9 ± 17.8	745.2 ± 80.6

* $p < 0.05$ compared with control (i.e., vehicle + water).

ciated with sensory motor gating and cognitive or preattentive deficits (Ellenbroek, 2004), these results raise the possibility that mGlu2 allosteric potentiators could have a better efficacy than competitive mGlu2/3 agonists.

The behavioral effects of the mGlu2/3 receptor agonist were evaluated after repeated administration to determine whether efficacy can be maintained over time. Unfortunately, the very short duration of action of LY487379 prevented any chronic studies with the selective mGlu2 potentiator. However, the results indicate that tolerance developed to the ability of LY379268 to block amphetamine- and PCP-induced hyperlocomotor activity. These results are in contrast with another study in which tolerance only developed to the motor impairments induced by a competitive mGlu2/3 agonist (Cartmell et al., 2000b). This different outcome could be explained by the use of a different species (i.e., rats), route of administration (i.e., p.o.), or duration of the treatment (i.e., 3 days). The mechanism by which tolerance developed to the behavioral effects of LY379268 is not clear. One possibility is that repeated stimulation of the mGlu2 receptor results in receptor desensitization and tolerance (Barchfeld and Medzihradsky, 1984; Parolaro et al., 1993). However, it is possible that pharmacokinetic tolerance might have developed after chronic administration of the agonist. Regardless of the mechanism by which tolerance developed, these results raise the possibility that the effects of mGlu2/3 agonists on some behavioral responses might not be maintained after repeated administration. In future studies, it will be important to determine whether tolerance develops to selective mGlu2 allosteric potentiators. If not, this could represent an advantage of allosteric potentiators over mGlu2/3 agonists. However, answering this question must await discovery of new allosteric potentiators of this receptor that have a longer half-life and more prolonged action in acute studies.

In summary, LY487379 reversed amphetamine- and PCP-induced hyperlocomotor activity and reversed amphetamine-induced disruption of PPI in mice. The selective mGlu2 potentiator had a short duration of action and was less potent than the competitive mGlu2/3 agonist in reversing amphetamine- and PCP-induced hyperlocomotor activity. However, tolerance developed to the competitive mGlu2/3 agonist. Collectively, these results indicate that the effects of selective mGlu2 allosteric potentiators could be an alternative approach for the treatment of CNS disorders that are associated with altered glutamate transmission.

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