The Group II Metabotropic Glutamate Receptor Agonist
(\(-\))2-Oxa-4-aminobicyclo[3.1.0.]hexane-4,6-dicarboxylate
(LY379268) and Clozapine Reverse Phencyclidine-Induced
Behaviors in Monoamine-Depleted Rats

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ABSTRACT
Recent studies have indicated that the selective group II metabotropic glutamate (mGlu) receptor agonist (\(-\))2-oxa-4-aminobicyclo[3.1.0.]hexane-4,6-dicarboxylate (LY379268) shares common biochemical and pharmacological effects with the atypical antipsychotic clozapine. The present study aimed to further investigate these similarities (or differences) in monoamine-depleted animals by using the phencyclidine (PCP) model. Animals were pretreated 24 h before PCP administration with (i.p.) vehicle, \(\alpha\)-methyl-\(\alpha\)-tyrosine methyl ester (\(\alpha\)-MPT; 400 mg/kg), or \(\alpha\)-p-chlorophenylalanine methyl ester (PCPA; 300 mg/kg) injections. \(\alpha\)-MPT and PCPA pretreatment significantly and selectively reduced catecholamine (dopamine and norepinepherine) or 5-hydroxytryptamine (5-HT, serotonin) and 5-hydroxyindoleacetic acid levels, respectively, in whole brain tissue. Both LY379268 and clozapine (s.c.) blocked PCP-evoked ambulatory activity and fine movements in control, \(\alpha\)-MPT-, and PCPA-treated animals. In contrast, the typical antipsychotic haloperidol (s.c.) attenuated PCP behaviors in control and PCPA-pretreated animals, but was without effect in subjects pretreated with \(\alpha\)-MPT. The \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate-selective antagonist \((3S, 4aR, 6R, 8aR)-6-[(2-\{1(2)OH\}tetrazole-6-yl]decahydroisoquinoline-3-carboxylic acid (LY293558) attenuated locomotor activity in \(\alpha\)-MPT-treated animals only, whereas the 5-HT\(_{2A/C}\)-selective antagonist ketanserin was effective at reducing ambulations and fine movements in control and \(\alpha\)-MPT-pretreated animals. Taken together, these data indicate an important role for glutamatergic and serotonergic mechanisms for PCP-evoked behaviors in catecholamine-depleted animals and suggest that like clozapine, LY379268 is more effective than typical antipsychotics in these models. This study further supports the potential use of group II mGlu agonists as novel therapeutic agents in the treatment of schizophrenia.

The evaluation of phencyclidine (PCP)-induced behaviors is regarded as a useful model of psychosis due to the ability of PCP to produce schizophrenia-like symptoms in healthy human subjects and exacerbate these symptoms in schizophrenic patients (Javitt and Zukin, 1991; Halberstadt, 1995; Steinpreis, 1996; Chavez-Noriega et al., 2002; Schoep and Marek, 2002). Moreover, PCP administration is known to elicit a number of behavioral effects in animals, which are thought to resemble both positive and negative symptoms of the disorder (Murray and Horita, 1979; Sturgeon et al., 1979; Steinpreis, 1996). Although an imbalance in the mesolimbic dopamine system has been suggested to underlie the pathology of schizophrenia (Creese et al., 1976; Seeman et al., 1976), it has become increasingly apparent that alterations in glutamate and 5-hydroxytryptamine (5-HT, serotonin) neurotransmission may be involved as well.

The primary impetus for a glutamatergic role in psychosis stems from the pharmacological actions of PCP within the central nervous system. PCP acts as an open channel blocker at the N-methyl-d-aspartate (NMDA) receptor (Anis et al., 1983), and changes in glutamate transmission via this mechanism have been linked to schizophrenia (Olney and Farber, 1995; Akbarian et al., 1996; Moghaddam and Adams, 1998). In addition, 5-HT mechanisms seem to contribute to behaviors associated with this model because antagonists to 5-HT\(_2\) receptors have been shown to attenuate some PCP-induced behaviors (Maurel-Remy et al., 1995; Krebs-Thomson et al., 1998; Millan et al., 1999). Serotonin transmission may also

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ABBREVIATIONS: PCP, phencyclidine; 5-HT, 5-hydroxytryptamine; NMDA, N-methyl-d-aspartate; mGlu, metabotropic glutamate; mPfc, medial prefrontal cortex; \(\alpha\)-MPT, \(\alpha\)-methyl-\(\alpha\)-tyrosine methyl ester; PCPA, \(\alpha\)-p-chlorophenylalanine methyl ester; NE, norepinepherine; DOPAC, dihydroxyphenylalanine; AMPA, \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; AMP, amphetamine; ANOVA, analysis of variance; LY379268, \((-\))2-oxa-4-aminobicyclo[3.1.0.]hexane-4,6-dicarboxylate; LY293558, \((3S, 4aR, 6R, 8aR)-6-[(2-\{1(2)OH\}tetrazole-6-yl]decahydroisoquinoline-3-carboxylic acid.

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be of clinical relevance because atypical antipsychotic agents (i.e., clozapine, olanzapine, and risperidone) ameliorate positive and negative schizophrenic symptoms, and they all reportedly possess antagonist activity at 5-HT$_{2A}$ receptors (Brunello et al., 1995; Millan et al., 1999). Thus, it seems likely that therapeutic strategies targeted to glutamate and/or 5-HT transmission may ameliorate some symptoms of this disorder.

Metabotropic glutamate (mGlu) receptors comprise a novel family of G protein-coupled receptors that have been classified into three groups based upon sequence homology, pharmacology, and the signal transduction mechanisms that they couple to in vitro (Nakanishi, 1992; Pin and Duvoisin, 1995). Group II (mGlu 2/3) mGlu receptors have recently emerged as targets of therapeutic value for psychiatric disorders due to their unique patterns of distribution (Ottersen and Landsend, 1997) and their ability to modulate glutamate neurotransmission (Moghaddam and Adams, 1998; Cartmell and Schoepp, 2000).

mGlu2/3 receptors exhibit moderate-to-high expression in forebrain regions that are commonly associated with schizophrenia such as prefrontal cortex, hippocampus, and nucleus accumbens (Ohishi et al., 1993a,b). Furthermore, group II mGlu receptors are generally expressed at extrasynaptic sites on neuron terminals where they influence the function of multiple neurotransmitter systems in an activity-dependent manner (Ohishi et al., 1994; Forsythe and Barnes-Davies, 1997). In this light, recent studies have indicated that group II mGlu receptors share common behavioral and biochemical effects with atypical antipsychotic drugs. For instance, systemic administration of either the selective group II mGlu receptor agonist (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268) or clozapine has been shown to block PCP- (ambulations and fine movements) and amphetamine (ambulations and rearing)-induced behaviors (Cartmell et al., 1999). Moreover, systemic administration of LY379268 has been shown to enhance monoamine release similarly to risperidone in the medial prefrontal cortex (mPfc) of freely moving rats (Cartmell et al., 2001) and increase dopamine turnover in mPfc tissue slices similar to risperidone and clozapine (Cartmell et al., 2000). Finally, like atypical antipsychotics, mGlu2/3 receptor agonists block the excitatory actions of 5-HT$_{2A}$ receptor activation (i.e., enhanced glutamate release) in the rat Pfc (Marek et al., 2000).

Taken together, these studies suggest that group II mGlu receptor agonists might offer a novel mechanism for the treatment of psychosis that parallels the actions of atypical antipsychotic drugs (Schoepp and Marek, 2002).

The present study aimed to compare the effects of LY379268 with the atypical antipsychotic clozapine in two monoamine-depleted models of PCP-induced behavioral activation. Whole brain tissue was subjected to high-performance liquid chromatography analysis 24 h after α-methyl-DL-p-tyrosine methyl ester (α-MPT) or DL-p-chlorophenylalanine methyl ester (PCPA) pretreatment to confirm depletion of monoamines. Behaviorally relevant monoamine depletion was evaluated in animals pretreated with α-MPT or PCPA 24 h before amphetamine administration in an automated photocell monitor. Either LY379268 or clozapine was systemically administered 30 min before PCP, and their effects on PCP-evoked behaviors in control animals were compared with those in animals selectively depleted of monoamines. In addition, the typical antipsychotic agent haloperidol was evaluated for its ability to effect PCP-induced behaviors across these models. Finally, the contribution of glutamate and serotonin transmission in monoamine-depleted models of PCP-evoked behaviors was evaluated by administering the selective AMPA/kainate antagonist (3S,4aR,6R,8aR)-6-[(1-2(12)OH-tetrazole-6-yl)decahydroisoquinoline-3-carboxylic acid (LY293558) or the selective 5-HT$_{2A/C}$ antagonist ketanserin, respectively, before PCP injection.

Materials and Methods

All experiments were conducted in strict accordance with animal care and use policies appointed by Eli Lilly & Co. (Indianapolis, IN), in conjunction with American Association for the Accreditation of Laboratory Animal Care-approved guidelines. Male Sprague-Dawley rats (250–300 g; Harlan, Indianapolis, IN) were group-housed (8 animals/cage) in a temperature-controlled facility where food and water were available ad libitum. All animals remained in the facility for at least 1 day before experimentation and rooms were set to a 12-h light/dark cycle (6:00 AM/6:00 PM) to maintain the animal photocycle. All experimentation was performed during the light cycle.

Monoamine Depletion and Neurochemical Quantification. Animals were administered i.p. injections of vehicle, α-MPT methyl ester (400 mg/kg), or PCPA (300 mg/kg) 24 h before neurochemical analysis. At 24 h post-treatment, animals were sacrificed via live decapitation and whole brains (with cerebellum removed and the spinal chord cut at the level of the obex) were rapidly dissected on ice and immediately frozen at −80°C. Samples were weighed on a Mettler balance (AE 163) and stored at −80°C in 5.0 ml of cold HCl (0.01 N) until further processing. The tissue was slow-thawed on ice and an additional 4.0 ml of 0.01 N HCl was added. Samples were sonicated and placed on ice at which time 1.0 ml of perchloric acid (1.5 M) was added to denature proteins. Contents were vortexed and let sit on ice for at least 1 h. One milliliter of denatured sample was centrifuged for 2 min (2000g) and clear supernatant was assayed for neurochemical levels.

The concentrations of norepinephrine (NE), dihydroxyphenylalanine (DOPAC), dopamine (DA), 5-HIAA (5-hydroxyindoleacetic acid), homovanilllic acid (HVA), and 5-HT were determined using high-performance liquid chromatography with electrochemical detection. Processed samples were spotted in replicate and 50 μl was injected via Autosampler (AS-100 HRLC; Bio-Rad, Hercules, CA) onto a reversed-phase column (3 × 150 mm, 4-μm ODS; Keystone Scientific, Bellefonte, PA) equipped with a 4-μm guard column (Keystone Scientific). The mobile phase consisted of 75 mM NaH$_2$PO$_4$, 0.5 mM EDTA (tetrasodium salt), 1.6 mM 1-octanesulfonic acid, 8% acetonitrile, and 0.8% tetrahydrofuran brought to pH 3.0 with 85% phosphoric acid. Neurochemicals were detected using an electrochemical detector (Princeton Applied Research, Oak Ridge, IN) set to the following parameters: A channel, $E = 720$ mV, range = 20 nA; and B channel, $E = 120$ mV, range = 20 nA. Brain samples were normalized to wet weight and quantified by comparing peak heights to an external standard curve. For all neurochemical peaks were collected using the EZChrome elite software package (Scientific Software, Inc., San Ramon, CA) and stored for analysis.

Behavioral Testing. Behavioral parameters were monitored in transparent shoebox cages that measured 45 × 25 × 20 cm, with a 1-cm depth of wood chips on the cage floor and a metal grill on top of the cage. Rectangular photocell monitors (Hamilton Kinder, Poway, CA) with a bank of 12 photocell beams (8 × 4 formation) surrounded each test cage. A lower rack of photocell beams was positioned 5 cm above the cage floor to enable detection of both body and head movements, whereas an upper bank positioned 10 cm above the first tabulated rearing activity. Ambulations (locomotor activity), fine
motor movements (an estimate of stereotyped behavior), and time at rest (total number of seconds in a 60-min session in which no beams were broken; taken at 1-s intervals) were recorded by computer and stored for each test session.

Subjects were placed in the test cage for a 30-min habituation period before testing to allow for acclimation to the testing environment. After this habituation period, animals were administered s.c. injections of phencyclidine (PCP) or amphetamine (AMP) and behavioral assessment began immediately after drug administration. In those studies that used LY379268, LY293558, haloperidol, clozapine, or ketanserin, animals were administered s.c. injections of these compounds in their test cages 30 min before PCP injection, and behavioral testing began immediately after PCP administration. Animals were monitored over a 60-min period in all instances, and data are expressed as total counts over the entire 1-h test period.

Statistical Analysis. Statistical analysis of neurochemicals and behavioral parameters were carried out using the GraphPad Prism statistical/graphing package (GraphPad, San Diego, CA). Neurochemical and behavioral data were analyzed using one-way analysis of variance (ANOVA), with the exception of the amphetamine experiment, which used a two-tailed unpaired t test. Upon discovery of statistical significance (P < 0.05), post hoc comparisons were performed using Tukey’s multiple comparisons test for each neurochemical (tissue analysis) or dose (behavioral studies) within a particular pretreatment test group versus control. In behavioral studies using antagonists, the antagonist plus PCP was also compared with PCP alone. Neurochemical analyses used 24-h pretreatment (control, MPT, and PCPA) as the main effect, and behavioral analyses included dosage or treatment (antagonist studies) as the main effect.

Materials. α-MPT methyl ester, PCPA, phencyclidine hydrochloride, and d-amphetamine sulfate were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in sterile saline. Clozapine, haloperidol, and ketanserin were obtained from Sigma/RBI (Natick, MA) and dissolved in 0.01 N HCl. Vehicle injections for these compounds consisted of 0.01 N HCl. LY379268 (Monn et al., 1997) and LY293558 (Ornstein et al., 1993) were synthesized as described previously (Lilly Research Laboratories, Eli Lilly & Co.) and were dissolved in sterile saline.

Results

Monoamine Depletion in Rat Whole Brain. Figure 1 (top) demonstrates that 24-h pretreatment with the tyrosine hydroxylase inhibitor α-MPT (400 mg/kg) or the tryptophan hydroxylase inhibitor PCPA (300 mg/kg) produced significant and selective alterations in the levels of norepinephrine (F2,27 = 31.58, P < 0.001), DOPAC (F2,27 = 29.74, P < 0.001), dopamine (F2,27 = 21.88, P < 0.001), HVA (F2,9 = 9.332, P < 0.001), 5-HIAA (F2,27 = 17.46, P < 0.001), and 5-HT (F2,27 = 26.05, P < 0.001) in whole brain tissue. Post hoc comparisons revealed that pretreatment with α-MPT significantly reduced levels of norepinephrine, DOPAC, dopamine, and HVA and elevated levels of 5-HIAA and 5-HT. In contrast, pretreatment with PCPA significantly reduced brain levels of 5-HIAA and 5-HT, whereas levels of NE, DOPAC, and DA were not significantly altered. Figure 1 (bottom) represents the same data expressed as percentage of control values. α-MPT reduced NE, DOPAC, and DA to 25, 29, and 29% of control values, respectively, whereas 5-HIAA and 5-HT were significantly elevated to 147 and 128% of control values, respectively. PCPA pretreatment significantly reduced 5-HIAA to 25% and 5-HT to 35% of control values.

To investigate whether the α-MPT regimen (i.e., dopamine depletion) used in this study could alter dopamine-mediated behaviors in the rat, amphetamine was administered to control, α-MPT-, and PCPA-treated animals (Fig. 2). Subcutaneous administration of a behaviorally activating dose (3 mg/kg; Cartmell et al., 1999) of AMP produced the expected robust and significant increases in ambulations (t8 = 5.424, P < 0.0006), fine motor movements (t8 = 10.54, P < 0.0001), and rearing (t8 = 4.375, P < 0.0024), and reduced time at rest (t8 = 14.6, P < 0.0001) in control animals. Ambulations (t8 = 23.60, P < 0.0001), fine movements (t8 = 8.431, P < 0.0001), and time at rest (t8 = 7.077, P < 0.0001) were significantly altered to a comparable degree in animals depleted of 5-HIAA and 5-HT after 24-h pretreatment with PCPA. However, amphetamine was unable to significantly increase ambulations and fine movements or reduce time at rest in animals pretreated with α-MPT.

PCP-Induced Behaviors. Figure 3 illustrates the dose-response observed with subcutaneous administration of PCP on the ambulatory, fine movement, and rest time parameters of behavioral activation. Statistical analysis using an overall one-way ANOVA indicates that PCP evoked significant increases in ambulations (F4,10 = 9.004, P < 0.002) and fine movements (F4,10 = 17.05, P < 0.002) and a significant reduction in rest time (F4,10 = 19.68, P < 0.001) over all pretreatment groups. Total ambulations reached a maximum effect at 8 mg/kg within all pretreatment groups (saline, PCPA, α-MPT, and MPT).
655% of control; α-MPT, 1616% of control; and PCPA, 1164% of control), whereas saline and α-MPT-treated animals displayed lower ambulations at 10 mg/kg compared with those receiving 8 mg/kg. Interestingly, fine movements also reached a plateau at 8 mg/kg in all treatment groups and remained elevated at 10 mg/kg in saline-treated animals compared with those receiving 8 mg/kg. This observation is likely due to the onset of stereotyped behaviors that is characteristic of high doses of PCP (Sturgeon et al., 1979). Thus, the remaining studies aimed at examining the behavioral effects of selective antagonists used 8 mg/kg PCP because this dose produced robust alterations in all behavioral parameters within each pretreatment group. LY379268 has been previously shown by this laboratory to reduce PCP-induced behaviors over a range of PCP doses (Schoepp et al., 2001).

Subcutaneous administration of the selective mGlu2/3 receptor agonist LY379268, at a dose (3 mg/kg) shown to maximally suppress PCP and amphetamine behaviors without producing significant motor ataxia (Cartmell et al., 1999), produced significant effects on PCP-evoked ambulations ($F_{3,8} = 7.296, P < 0.0112$), fine motor movements ($F_{3,8} = 9.858, P < 0.0046$), and rest time ($F_{3,8} = 12.81, P < 0.002$) as revealed by an overall one-way ANOVA (Fig. 4). Post hoc comparisons performed within pretreatment groups confirm that LY379268 significantly blocked PCP-evoked ambulations and fine movements and increased rest time in control, α-MPT-, and PCPA-treated animals. LY379268 blocked PCP-evoked ambulations in control, α-MPT-, and PCPA-treated animals by 77, 93, and 72%, respectively, compared with PCP alone (subtracting basal activity from PCP alone and PCP plus LY379268 groups). LY379268 administration alone significantly attenuated basal levels of ambulatory activity and fine movements in control and PCPA pretreatment groups.

Figure 5 illustrates that an effective dose (3 mg/kg; Cartmell et al., 1999) of the combined dopamine D2/serotonin 5-HT2 receptor antagonist clozapine produced similar effects on PCP-evoked ambulations ($F_{3,8} = 14.10, P < 0.0015$), fine movements ($F_{3,8} = 35.08, P < 0.0001$), and rest time ($F_{3,8} = 37.14, P < 0.0001$) as revealed by overall one-way ANOVA. Post hoc comparisons performed within pretreatment groups confirm that clozapine significantly blocked PCP-evoked ambulations and fine movements and increased rest time in saline, α-MPT-, and PCPA-treated animals. Ambulations...
were attenuated with clozapine pretreatment compared with PCP alone by 84, 84, and 87% in control, α-MPT-, and PCPA-treated animals, respectively (subtracting basal activity from PCP alone and PCP plus clozapine groups).

Importantly, a moderate dose of the D1/D2-selective dopamine antagonist haloperidol significantly blocked all PCP-evoked behaviors in saline- and PCPA-treated animals, but was ineffective at significantly blocking ambulations in α-MPT-treated subjects (Fig. 6). Overall one-way ANOVA reveals significant effects on ambulations ($F_{3,8} = 15.61, P < 0.0010$), fine movements ($F_{3,8} = 15.68, P < 0.0010$), and rest time ($F_{3,8} = 20.50, P < 0.0004$) with haloperidol pretreatment. However, post hoc comparison within pretreatment groups indicated that haloperidol did not significantly reduce PCP-evoked ambulations in α-MPT-treated animals. In contrast, haloperidol blocked PCP-induced ambulations in control and PCPA-treated animals by 89 and 73%, respectively, compared with PCP alone (subtracting basal activity from PCP alone and PCP plus haloperidol groups). Fine movements and time at rest were significantly reduced by haloperidol pretreatment in all models tested. Administration of haloperidol alone resulted in significant attenuation in basal levels of locomotion and fine movements.

To investigate the role that glutamate plays in PCP-induced locomotion, the AMPA/kainate-selective glutamate receptor antagonist LY293558 was administered to animals receiving PCP injections at a dose (3 mg/kg s.c.; Schoepp et al., 1995) that has previously been shown to block AMPA receptor activation in vitro and in vivo (Fig. 7). Overall one-way ANOVA indicates significant effects on ambulations ($F_{3,8} = 7.388, P < 0.0108$), fine movements ($F_{3,8} = 13.85, P < 0.0016$), and rest time ($F_{3,8} = 16.09, P < 0.0009$). Post hoc comparisons showed that fine movements were not significantly affected by AMPA receptor blockade in α-MPT-treated animals and neither control nor PCPA-treated animals showed significant alterations in any behavioral parameter.

Fig. 4. Effect of LY379268 (3 mg/kg) on PCP (8 mg/kg)-induced behaviors as measured by an automated photocell monitor after 24-h pretreatment with saline, α-MPT (400 mg/kg), or PCPA (300 mg/kg). LY379268 was administered (s.c.) 30 min before PCP (s.c.) injection. Data (mean ± S.E.M.) are expressed as the total number of behaviors over the 60-min test period; $n = 6$ rats. *, $P < 0.05$ compared with vehicle injection (0 PCP/0 LY379268). #, $P < 0.05$ compared with PCP alone (8 PCP/0 LY379268).

Fig. 5. Effect of clozapine (3 mg/kg) on PCP (8 mg/kg)-induced behaviors as measured by an automated photocell monitor after 24-h pretreatment with saline, α-MPT (400 mg/kg), or PCPA (300 mg/kg). Clozapine was administered (s.c.) 30 min before PCP (s.c.) injection. Data (mean ± S.E.M) are expressed as the total number of behaviors over the 60-min test period; $n = 8$ rats. *, $P < 0.05$ compared with vehicle injection (0 PCP/0 clozapine). #, $P < 0.05$ compared with PCP alone (8 PCP/0 clozapine).
tested. However, akin to a role for glutamate transmission in the locomotor effects of PCP, LY293558 significantly attenuated PCP-evoked ambulations by 52% in α-MPT-treated animals compared with PCP alone (subtracting basal activity from PCP alone and PCP plus LY293558 groups).

Overall one-way ANOVA revealed significant effects of administration of the selective 5-HT2A/2C antagonist ketanserin on ambulations ($F_{3,8} = 6.485, p < 0.0155$), fine movements ($F_{3,8} = 10.83, p < 0.0034$), and rest time ($F_{3,8} = 13.98, p < 0.0015$) (Fig. 8). Post hoc comparisons indicate that ketanserin significantly attenuated ambulations and fine movements elicited by PCP in control and α-MPT-treated animals. Ambulations were reduced by 53 and 61% in control and α-MPT-treated subjects, respectively (subtracting basal activity from PCP alone and PCP plus ketanserin groups). Ketanserin failed to significantly affect any behavioral parameter tested in PCPA-treated animals.

**Discussion**

In the present study, both LY379268 and clozapine administration significantly attenuated PCP-induced locomotor activity and fine movements in control, catecholamine-, and 5-HT-depleted animals. In contrast, the typical antipsychotic agent haloperidol was ineffective at blocking PCP-induced locomotion in animals depleted of catecholamines. The AMPA/kainate-selective antagonist LY293558 attenuated PCP-evoked locomotion only in α-MPT-treated animals, whereas the 5-HT2A/2C selective antagonist ketanserin blocked PCP-induced behaviors in both control and α-MPT-treated (but not PCPA-treated) subjects. Despite reports that have indicated that group II mGlu receptor agonists are unable to affect some discriminative and sensorimotor gating properties associated with PCP administration (Schreiber et al., 2000), these data support the hypothesis that group II mGlu agonists represent a novel therapeutic approach to the treatment of schizophrenia.

Much of the early work on schizophrenia focused upon abnormal dopaminergic transmission based, in part, on the finding that typical neuroleptic agents (i.e., haloperidol) act by blocking postsynaptic dopamine D2 receptors (Creese et al., 1976; Seeman et al., 1976). However, the fact that not all schizophrenic patients respond to dopamine blocking agents and dopaminergic agonists can only mimic the paranoid form
of psychosis (Seeman, 1995) is suggestive of the involvement of multiple neurotransmitter systems in this disorder. Indeed, NMDA antagonists can elicit locomotor activation in animals in the absence of dopamine (Carlsson and Carlsson, 1989; Carlsson, 1995), and atypical antipsychotic agents (i.e., clozapine) have been successful at improving positive and negative schizophrenic symptoms presumably, in part, through their preferential action at 5-HT2A receptors (Brunello et al., 1995).

Carlsson et al. (1989, 1995) have demonstrated that haloperidol failed to significantly reverse PCP-induced behaviors in α-MPT-treated animals in our study, it should be mentioned that there was a tendency toward a blockade of PCP effects in these animals. However, this observation might be explained by the activity of haloperidol at 5-HT receptors, because recent data indicate that haloperidol and clozapine display nearly equal binding affinity and functional antagonism at 5-HT2 receptors in vivo (ED50 = 1.5 mg/kg (μmol/kg); Zhang and Bymaster, 2000). With this in mind, the fact that both LY379268 and clozapine (but not haloperidol) are capable of blocking PCP-induced behaviors in animals lacking a functional dopaminergic system indicates a role for LY379268 modulation of glutamate neurotransmission in reversing behaviors associated with PCP administration.

It is important to note that the dose of LY379268 used in this study (3 mg/kg) significantly reduced basal levels of locomotor activity. However, Cartmell et al. (1999) have reported that although LY379268 reduced basal levels of locomotion at this dose in their study, it did not produce motor impairment as measured on the rotorod apparatus. In that study, the mGlu2/3 receptor agonist LY379268 and clozapine produced motor impairment on the rotorod at higher doses than those that blocked PCP-related behaviors. Furthermore, studies have shown that repeated administration of LY379268 led to tolerance to motor impairment, whereas the efficacy of the agonists ability to block PCP behaviors was retained (Cartmell et al., 2000). Thus, it is unlikely that LY379268 reverses evoked behaviors associated with PCP administration via nonspecific effects. This notion is supported by the fact that PCP-induced locomotion was not significantly affected by haloperidol at a dose (0.1 mg/kg) that also significantly reduced basal levels of spontaneous locomotor activity (this study) and that has been previously shown to produce motor ataxia on the rotorod apparatus in rats (Cartmell et al., 1999). Furthermore, PCP can elicit a
full increase in locomotor activation in the presence of doses of diazepam (10–30 mg/kg) that greatly suppress spontaneous locomotion and motor performance on the rotorod apparatus (unpublished observations; C. S. Swanson, B. G. Johnson, D. D. Schoepp, manuscript in preparation). In the present study, LY379268 and the AMPA/kainate receptor antagonist LY293558 were shown to possess different pharmacological profiles. Specifically, LY293558 significantly attenuated PCP-induced locomotor activity only in those animals that were depleted of catecholamines, whereas LY379268 significantly attenuated locomotor activity in control, α-MPT-, and PCPA-treated animals. There are several lines of reasoning that might explain this result. For example, mGlu receptors exhibit distinct distributions that allow for modulation of glutamatergic transmission at specific synapses within the central nervous system (Schoepp et al., 2001). In contrast, AMPA receptors are more ubiquitously expressed in the brain and play an integral role in driving synaptic events via fast excitatory transmission. Moreover, many studies have indicated that mGlu receptors may influence ion channel conductances and, thereby, may alter the sensitivity of postsynaptic neurons (Anwyl, 1999). The selective 5-HT2A/2C antagonist ketanserin significantly attenuated ambulations and fine movements in both control and α-MPT-treated animals. This finding corroborates previous studies suggesting a role for 5-HT mechanisms in some PCP-evoked behaviors (Maurel-Remy et al., 1995; Krebs-Thomson et al., 1998; Millan et al., 1999). Moreover, because α-MPT pretreatment resulted in a significant increase in 5-HT and 5-HIAA in this study, it is likely that 5-HT mechanisms are particularly important in producing PCP effects in these animals. Collectively, these data indicate that glutamate and 5-HT neurotransmission are important in mediating some behaviors associated with PCP administration under circumstances that may be related to brain catecholamine levels. Interestingly, both LY293558 and ketanserin administration were only partially effective at reducing PCP-induced behaviors in any model tested. Thus, the near complete blockade noted in control, catecholamine-, and 5-HT-depleted subjects by LY379268 indicates that group II mGlu receptor agonists may reverse PCP-evoked behaviors through concerted actions on dopamine, glutamate, and 5-HT neurotransmission. Similarly, the fact that clozapine was as effective as LY379268 at reducing PCP behaviors in control and monoamine-depleted animals (particularly 5-HT-depleted animals) indicates that this compound may also exert its effects on evoked behaviors via actions on multiple neurotransmitter systems. This latter notion is supported by work from Phillips et al. (2001) in animals sensitized to repeated PCP administration. In these studies, clozapine was effective at reducing locomotor activity produced by a challenge injection of PCP after withdrawal from repeated administration, whereas the 5-HT2A antagonist ketanserin was ineffective at reversing PCP effects in sensitized animals. The fact that ketanserin attenuates acute but not sensitized ambulatory behavior elicited by PCP brings up an interesting point regarding whether acute or chronic PCP administration is more appropriate for modeling schizophrenic symptoms. It is clear that acute administration of NMDA antagonists produce psychomimetic effects in humans; however, it is less clear what aspects of schizophrenia repeated administration of PCP models in animals. That being said, it has recently been shown that LY379268 reverses the expression but not the development of sensitized behavioral responses to repeated PCP administration in rats (Clark et al., 2002). The precise mechanism of LY379268 action in reversing PCP-mediated behaviors remains unclear because systemic administration of either LY379268 or PCP alone results in enhanced 5-HT and DA release/turnover in relevant brain regions (Cartmell et al., 2000, 2001). It is apparent from the data presented herein that a significant portion of PCP-induced behaviors is mediated by DA transmission because functional depletion of DA (α-MPT treatment) resulted in a 50% or greater reduction in the absolute magnitude of PCP-induced behavioral activation. However, it is important to note that the relative effects of PCP on behavioral activation are much more robust in α-MPT-treated animals when an increase over basal activity is considered. Taken together, these results suggest that higher doses of PCP (8–10 mg/kg) may be linked to DA-mediated behaviors because these doses of PCP seemed to be more affected by α-MPT treatment. Given the efficacy of LY379268 in blocking PCP behavior in dopamine intact animals, it is likely that mGlu2/3 receptor agonists exert some of their effects via modulation of dopamine transmission (Cartmell and Schoepp, 2000). However, a role for glutamatergic transmission is also evident by the finding that LY293558 is able to significantly block PCP responses in α-MPT-pretreated animals. Recently, Aghajanian and Marek (2000) have proposed that although 5-HT2 agonists and PCP act through different mechanisms to promote schizophrenic symptoms, they may share increased glutamate release in the prefrontal cortex as a common final pathway for eliciting these effects. This postulate is of particular interest given the ineffectiveness of dopamine blocking agents in reversing psychotic symptoms in some forms of the disorder. According to this hypothesis, LY379268 would be able to curtail enhanced glutamate release regardless of the mechanism involved through activation of group II mGlu autoreceptors on glutamatergic terminals. It has been demonstrated, for example, that systemic administration of a structurally distinct group II mGlu receptor agonist, (1S,2S,5R,6S)-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740), blocked PCP-induced behaviors that were associated with a reduction in PCP-induced glutamate release in the mPfc (Moghaddam and Adams, 1998). Furthermore, studies have indicated that PCP administration enhances 5-HT levels in Pfc (Martin et al., 1998) and that 5-HT2A activation induces excitatory postsynaptic potentials/currents in this structure in vitro (Marek et al., 2000). This latter effect can be suppressed by LY379268 and LY354740 and enhanced by application of the selective group II mGlu antagonist LY34495, indicating a role for endogenous glutamate in modulating 5-HT-induced excitatory postsynaptic currents. In summary, the results of the present study indicate that both LY379268 and clozapine display similar abilities to block PCP-induced behaviors in control and monoamine-depleted animals, likely via concerted actions on DA, glutamate, and 5-HT transmission. This notion is supported by the inability of haloperidol to attenuate PCP behaviors in catecholamine-depleted animals and suggests that LY379268 and clozapine may act by modulating glutamate and 5-HT mechanisms, respectively, in this study. Thus, the fact that LY379268 reverses PCP-induced behaviors in similar man-
ner to clozapine in a model where a typical antipsychotic agent is less effective further supports a role for group II mGlu receptor agonists in the treatment of schizophrenia.

References


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