The Metabotropic Glutamate 2/3 Receptor Agonists LY354740 and LY379268 Selectively Attenuate Phencyclidine versus d-Amphetamine Motor Behaviors in Rats

JAYNE CARTMELL, JAMES A. MONN, and DARRYLE D. SCHOEPP
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana
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ABSTRACT

Previous animal studies have indicated that drugs targeted at metabotropic glutamate (mGlu) receptors may be useful for treatment of psychosis. In this article, the effects of the novel, potent, and selective mGlu2/3 receptor agonists LY354740 and LY379268, and the clinically effective agents clozapine and haloperidol, were investigated using phencyclidine (PCP; 5 mg/kg)- versus d-amphetamine (AMP; 3 mg/kg)-evoked motor activities. LY354740 (1–10 mg/kg s.c.), LY379268 (0.3–3 mg/kg s.c.), clozapine (1–10 mg/kg s.c.), and haloperidol (0.03–1 mg/kg s.c.) reversed the increases in ambulations, fine motor (nonambulatory) movements, and decreased time at rest evoked by PCP. Furthermore, the inhibitions of the PCP response by the mGlu2/3 agonist LY379268, but not by clozapine, were completely reversed by the selective mGlu2/3 receptor antagonist LY341495. Doses of LY354740 and LY379268 that blocked the effects on PCP had no effects on rotorod performance, and (with the exception of rearing behavior) had minimal effects on AMP-evoked motor activities. Clozapine blocked AMP-induced rearing but enhanced AMP-induced ambulations and fine movements at the lower doses (1 and 3 mg/kg). Unlike the mGlu2/3 agonists, the highest dose of clozapine tested (10 mg/kg) impaired animals on the rotorod. Haloperidol potently blocked all PCP and AMP effects, but only at doses associated with motor impairment. These data demonstrate that mGlu2/3 receptor agonists act via a unique mechanism to selectively block PCP-induced behaviors. Moreover, the marked mGlu2/3 receptor-mediated inhibitions of PCP-evoked behaviors by LY354740 and LY379268, with minimal effects on AMP, may indicate potential antipsychotic effects in humans in the absence of dopamine mediated extrapyramidal side effects.

Metabotropic glutamate (mGlu) receptors are a novel family of at least eight G protein-coupled receptor subtypes, which have been subclassified into three groups based on shared structural homology, coupling mechanisms, and pharmacological properties. Group I mGlu receptors (mGlu1 and mGlu5) are coupled to phospholipase C activation, whereas the group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) mGlu receptors are coupled negatively to adenylate cyclase (Nakanishi, 1992; Pin and Duvoisin, 1995; Conn and Pin, 1997). Current knowledge of the pharmacology of these receptors, along with studies on their localizations and physiological/pathological functions in the central nervous system (CNS) suggest that mGlu receptors are novel drug targets for psychiatric and neurological disorders (Nicoletti et al., 1996; Knopfel and Gasparini, 1996).

Modulation of glutamatergic synaptic transmission by group II (mGlu2/3) receptor agonists is of interest as a novel mechanism with which to regulate neuronal activity within specific synapses and neuronal circuits. MGlU2 and mGlU3 receptors are highly expressed in forebrain regions (i.e., prefrontal cortex and hippocampus; Ohishi et al., 1993a,b; Neki et al., 1996) where pathologically enhanced glutamate transmission has been implicated in a variety of CNS disorders. Interestingly, mGlu2 receptors are located presynaptically but outside of the active zone of glutamate release (in the periphery of the synapse) where they function to reduce glutamate excitations in an activity-dependent manner (Ohishi et al., 1994; Shigemoto et al., 1997; Forsythe and Barnes-Davies, 1997). Brain synapses where mGlu2/3 agonists have been shown to suppress glutamate transmission include the mossy fiber (Scanziani et al., 1997) and perforant pathways (Macek et al., 1996; Kilbride et al., 1998) of the hippocampus, the locus ceruleus (Dube and Marshall, 1997), nuclei of the amygdala (Neugebauer et al., 1997), the cortico-striatal pathway (Loving and McCool, 1995), and the medial prefrontal cortex (Marek and Aghajanian, 1998). Thus, mGlu2/3 receptor activation represents a novel mechanism for limiting excitatory synaptic transmission under conditions of excessive (pathologically) enhanced glutamate transmission in a regional, selective, and synapse-specific manner.

ABBREVIATIONS: mGlu, metabotropic glutamate; PCP, phencyclidine; AMP, d-amphetamine; MCPG, (RS)-α-methyl-4-carboxyphenylglycine.
LY354740 is a nanomolar potent, receptor-selective, and systemically active mGlu2/3 receptor agonist (Monn et al., 1997; Schoepp et al., 1997; Battaglia et al., 1997). Thus, LY354740 has served as a useful in vivo pharmacological tool for investigating the role of mGlu2/3 receptors in multiple disorders of the CNS. LY354740 has been shown to be active in animal models of seizures (Monn et al., 1997), global ischemia (Bond et al., 1998), anxiety (Monn et al., 1997; Helton et al., 1998b), and drug withdrawal states (Helton et al., 1997; Helton et al., 1998a). Each of these conditions have in common pathologically enhanced glutamate transmission in the CNS (Danyz et al., 1995), and thus provide the theoretical basis for investigations of the therapeutic prospects for mGlu2/3 agonists.

With this in mind, recent animal studies with phencyclidine (PCP) have suggested that group II mGlu receptors represent a novel target for the treatment of psychosis. Pharmacological studies in humans and animals suggest that PCP represents a useful model of psychosis, and serves as the basis for the “glutamate hypothesis of schizophrenia”. PCP has been reported to produce schizophrenia-like symptoms in healthy subjects and worsen psychosis in schizophrenic patients (Javitt et al., 1991; Halberstadt, 1995; Steinpreis, 1996). The mechanism(s) by which PCP induces psychosis in humans is currently an active area of research, and may be the key to understanding the actions of current and future antipsychotic drugs. PCP is open channel blocker of the N-methyl-D-aspartate subclass of ionotropic glutamate receptors (Anis et al., 1983) that has multiple behavioral effects in animals, which include increased motor behaviors, stereotypy, and cognitive disruptions (Murray and Horita, 1979; Sturgeon et al., 1979; Steinpreis, 1996). These actions of PCP in animals have been linked to enhanced release of a number of CNS neurotransmitters, including glutamate (Adams and Moghaddam, 1998), as well as dopamine and serotonin (Hernandez et al., 1988; Honda et al., 1994; Lillrank et al., 1994). Recently, Moghaddam and Adams (1998) reported that LY354740 blocked PCP-evoked increases in prefrontal cortex glutamate levels, while having no effects on basal or PCP-evoked extracellular dopamine levels. This effect of LY354740 was associated with reductions in PCP-evoked locomotor activity and inhibition of PCP-induced stereotypies. These data indicate that mGlu2/3 receptor agonists may block PCP-mediated behaviors via a novel mechanism (reduction in PCP-evoked glutamate release), which, unlike typical and atypical antipsychotic drugs, might be independent of the dopamine system.

Amphetamine interacts with catecholamine systems (dopamine, serotonin, and norepinephrine) in the CNS to produce a variety of behaviors in animals and humans (Seiden et al., 1993). In rats, d-amphetamine (AMP) produces increased locomotion and stereotypies, the expression of which are highly dependent on the dose of AMP tested (Kuczenski and Segal, 1989; Seiden et al., 1993). Antipsychotic drugs, including typical drugs such as haloperidol, and atypical drugs, such as clozapine, have in common postsynaptic dopamine receptor-blocking properties (Iversen, 1975). Because these compounds reverse certain AMP-induced behaviors in animals (Del Rio and Fuentes, 1969; Tschanz and Rebec, 1989; Mueller, 1993), as well as schizophrenic symptoms in humans, a role for an abnormally activated dopamine system in schizophrenia has been proposed. However, motor disturbances such as extrapyramidal Parkinsonian signs have also been linked to dopamine blockade by antipsychotic drugs (Seeman, 1995). Thus, the discovery of novel antipsychotic mechanisms (e.g., drugs that directly target glutamate receptor mechanisms) may be desirable.

In this article, we further investigated a potential role of mGlu2/3 receptors in the treatment of drug-induced psychosis and possibly schizophrenia using both PCP and AMP models in rats. The systemically active mGlu2/3 receptor agonist LY354740 (Monn et al., 1997; Schoepp et al., 1997) and a newer structurally different mGlu2/3 receptor agonist compound, LY379268 (Monn et al., 1999), were examined for their abilities to influence PCP- versus AMP-induced motor activations, by using an automated behavioral system that monitors both gross ambulatory locomotion and nonambulatory fine movements in a home-cage environment. To test for sedation/ataxia and thus determine whether the effects of these agents were related to motor impairment of the animals, a rotorod apparatus was used. The actions of known antipsychotics, clozapine and haloperidol, were also examined in the same test systems. To further establish that the effects of mGlu receptor agonists in these test systems were mGlu2/3 receptor mediated, we used the potent and highly selective mGlu2/3 receptor antagonist compound, LY341495 (Kingston et al., 1998).

**Experimental Procedures**

All experiments were performed in accordance with Eli Lilly and Company animal care and use policies. Male Sprague-Dawley rats (250–300 g) were group-housed (maximum of seven rats per cage) under standard laboratory conditions with ad libitum access to food and water (12 h light/dark cycle), for at least 1 day before use.

**Activity Assessment.** Behaviors were monitored in transparent, plastic shoebox cages of the dimensions 45 × 25 × 20 cm, with 1 cm depth of wood chips as bedding, and a metal grill on top of the cage. Motor monitors (Hamilton Kinder, Poway, CA) consisted of a rectangular rack of 12 photobeams arranged in a 8 × 4 formation. Shoe box cages were placed inside these racks, enabling the activity of the rat to be monitored in a home-cage environment. The lower rack was positioned at a height of 5 cm, which allowed the detection of PCP-induced head movements in addition to movements of the body of the rat. Rearing activity was detected by a second rack placed 10 cm above the first.

Rats were placed in the cage for an acclimation period of 30 min, then were removed, administered a s.c. injection of LY354740, LY379268, clozapine, haloperidol, or sterile water (1 ml/kg), and then returned to the same cages. Antagonist studies using the selective group II receptor antagonist LY341495 at a dose of 1 mg/kg consisted of coinjection of LY341495 with LY379268, clozapine, or sterile water. After 30 min, the rats were given an s.c. injection of PCP, AMP, or sterile water (1 ml/kg), and once again returned to the cages. Motor activity was monitored for the following 60 min. Software analysis of beam breaks, under the definitions of Hamilton Kinder, resulted in the measurement of three different parameters: ambulations (pattern of beam breaks indicating that the animal has relocated its entire body), fine movements (nonambulatory beam breaks), and time at rest (total seconds in a 60-min session in which no new beams were broken, measured at 1-s intervals). An indication of rearing activity was determined by the total number of ambulations detected in the upper rack of photobeams.

**Rotorod Performance.** An automated rotorod apparatus (Rotorod; San Diego Instruments Inc., San Diego, CA) was used as a test for motor impairment/ataxia. Ninety minutes before drug administration, rats were trained to stay on the rotorod, rotating at 4 rpm,
over four successive trials. Those rats that remained on the rod for a consecutive 60-s period were retested 30 min before drug administration. Rats successful in the retesting session were then given s.c. injections of LY354740, LY369268, clozapine, haloperidol, or sterile water (1 ml/kg). After an additional 30 min, these rats were again tested on the rotorod for a period of up to 60 s. Data were expressed as the number of seconds in which the animal remained on the rotorod apparatus.

Statistical Analysis. Statistical analyses of behaviors were carried out using the GraphPad PRISM statistical program (GraphPad, San Diego, CA). Data were analyzed by a one-way ANOVA, and then post hoc comparisons for each dose group versus control or PCP alone or PCP and test compound were made using Newman-Keuls Multiple Comparison Test. A $P < .05$ was considered significant. ED$_{50}$ values were calculated from mean data using the median effect plot of Chou and Talalay (1983). For this analysis, data at each dose of test drug were converted percentage of inhibition (or percentage of reversal) of PCP or amphetamine-induced changes in activity. ED$_{50}$ values generally represent three increasing doses of drug producing at least one value with <50% effect and one value with >50% effect and a linear regression coefficient ($r^2$) of >0.9.

Materials. PCP, hydrochloride, and AMP sulfate were obtained from Sigma (St. Louis, MO). Clozapine and haloperidol were purchased from Research Biochemicals International (Natick, MA). LY354740 and LY379268 were synthesized as described previously (Monn et al., 1997, 1999). LY341495 (Ornstein et al., 1998) was provided by Dr. Paul L. Ornstein at Lilly Research Laboratories, Indianapolis.

Results

PCP-Induced Motor Activities. PCP evoked dose-dependent increases in ambulations and fine motor (nonambulatory) movements, at doses between 3 and 10 mg/kg (Fig. 1). Interestingly, maximal effects of PCP on total ambulations and fine movements were observed at different doses. Total ambulations reached a maximum at doses of around 3 and 5 mg/kg (approximately 750% of basal values), whereas fine movements reached a plateau at 8 to 10 mg/kg (750% increase).

Subcutaneously administered LY354740 (0.1–10 mg/kg) and LY379268 (0.3–3 mg/kg) produced dose-related inhibitions of PCP (5 mg/kg s.c.) evoked total ambulations, fine movements, and reductions of time at rest (Fig. 2). LY354740 and LY379268 inhibited PCP-elicited ambulations by 82 and 89%, respectively, at the highest doses tested (10 and 3 mg/kg, respectively). PCP-induced increases in fine movements were also greatly decreased at the higher doses of LY354740 (60% reduction at 10 mg/kg) and LY379268 (83% reduction at 3 mg/kg). Calculated ED$_{50}$ values for reversal of PCP behaviors by LY354740 were 1.7 mg/kg s.c. (total ambulations), 4.0 mg/kg (fine movements), and 5.0 mg/kg s.c. (time at rest). LY379268 ED$_{50}$ values for reversal of PCP behaviors were 1.4 mg/kg s.c. (total ambulations), 1.7 mg/kg (fine movements), and 1.6 mg/kg s.c. (time at rest). Likewise, clozapine (1–10 mg/kg s.c.) and haloperidol (0.03–0.3 mg/kg) also produced marked dose-related inhibitions of PCP (5 mg/kg s.c.), evoked total ambulations, fine movements, and reductions of time at rest (Fig. 3). As was observed for LY354740 and LY379268, all three PCP-induced parameters were about equally affected by clozapine and haloperidol. Calculated ED$_{50}$ values for reversal of PCP by clozapine were 1.7 mg/kg s.c. (total ambulations), 4.2 mg/kg (fine movements), and 3.8 mg/kg s.c. (time at rest). Haloperidol ED$_{50}$ values for reversal of PCP behaviors were 0.07 mg/kg s.c. (total ambulations), 0.09 mg/kg (fine movements), and 0.10 mg/kg s.c. (time at rest).

To further examine the nature of its actions, LY379268 was tested for the ability to reverse a higher dose of PCP (8 mg/kg s.c.), where as shown in Fig. 1, PCP fine movements were further enhanced. In this experiment, LY379268 (3 mg/kg s.c.) produced highly significant reversals of PCP (8 mg/kg s.c.)-induced ambulations (99 ± 1% inhibition), fine movements (54 ± 6% inhibition), and decreased time at rest (63 ± 5% reversal). This indicates...
that in this model, LY379268 suppression of motor activities induced by the lower PCP dose (5 mg/kg s.c.) were likely not due to the enhancement of other PCP behaviors.

The selective mGlu2/3 receptor antagonist, LY341495 (Kingston et al., 1998), at a dose of 1 mg/kg, was without significant effects on basal activity, or each of the three behavioral parameters evoked by 5 mg/kg PCP. However, this dose of LY341495 completely reversed the effects of 3 mg/kg LY379268 on PCP-elicited total ambulations, fine movements, and reduced time at rest (Fig. 4). Moreover,
when tested against a dose of clozapine (10 mg/kg) that produced the same extent of inhibition of the PCP response as 3 mg/kg 379268, 1 mg/kg LY341495 was without significant effect on each of the three parameters measured (Fig. 4).

**AMP-Induced Motor Activities.** AMP produced a clear dose-related biphasic effect on total ambulations (Fig. 5). A dose of 3 mg/kg AMP maximally increased total ambulations by 590% of basal, a value comparable with the maximal effect induced by PCP (Fig. 1). The higher doses of AMP (10 and 30 mg/kg) produced significantly smaller increases in total ambulations when compared with the 3 mg/kg dose ($P < .05$). In fact, at the highest dose tested (30 mg/kg), significant AMP-induced increases in total ambulations were no longer observed (Fig. 5). Similar to ambulations, AMP also increased rearing activity in a biphasic manner, peaking at a dose of 3 mg/kg, with no significant increases at the higher doses of 10 or 30 mg/kg (Fig. 5). At the 3 mg/kg dose, rearing activity was increased over 30-fold the basal values (Fig. 5). In contrast, AMP-evoked increases in fine movements were maximal at 1 mg/kg and remained significantly elevated at doses up to 30 mg/kg AMP. The maximal increases in AMP-evoked fine movements were lower than those observed for PCP (330% of basal at 3 mg/kg s.c. AMP). These behavioral effects also resulted in a dose-related reductions in time spent at rest over a range of 1 to 30 mg/kg AMP.

Overall, doses of LY354740 and LY379268, which significantly reduced PCP-evoked motor activities produced little or relatively modest reductions in AMP (3 mg/kg s.c.)-evoked total ambulations, fine movements, or reduced time at rest (Fig. 6). Even at the dose of LY354740, which almost completely abolished actions of PCP (10 mg/kg), no significant effects on AMP-induced total ambulations, fine movements, or reduced time at rest were observed. Certain doses of LY379268 produced statistically significant decreases in AMP-evoked total ambulations (29 and 42% reductions at 1 and 3 mg/kg, respectively) or fine movements (30 and 33% reductions at 1 and 3 mg/kg, respectively). However, these same doses had no effect AMP-evoked reductions in time at rest (Fig. 6). In contrast, both LY354740 and LY379268 greatly reduced AMP-elicited rearing activity. At 10 mg/kg of LY354740, AMP-evoked rearing activity was inhibited by 55%, whereas a dose of 3 mg/kg LY379268 produced 91% inhibition of AMP-rearing activity (Fig. 6).

Similar to mGlu2/3 agonists, clozapine failed to inhibit fine movements elicited by AMP, but almost completely abolished rearing activity at 10 mg/kg. Interestingly, fine movements were actually enhanced by the lower dose (1 mg/kg s.c.) of clozapine, to 160% of the control value. Likewise, clozapine (1 mg/kg s.c.) significantly increased total ambulations (to 215% of basal). Doses of 1 and 3 mg/kg clozapine also significantly enhanced AMP-evoked increases in fine movements and decreases in time at rest. However, the highest dose of clozapine (10 mg/kg s.c.) reduced AMP-evoked ambulations by 81%, but had no effects on either fine movements or time at rest (Fig. 7). In contrast, rearing activity evoked by AMP was reduced by clozapine in a dose-related manner over the range of 1 to 10 mg/kg clozapine (ED$_{50}$ 4.0 mg/kg).

In common with its effects on PCP-evoked behaviors, haloperidol (0.03–0.3 mg/kg) produced dose-related reductions in all AMP-induced behaviors, including ambulations (ED$_{50}$ < 0.1 mg/kg), fine movements (ED$_{50}$ 0.05 mg/kg), reduced time at rest (ED$_{50}$ 0.05 mg/kg), and rearing activity (ED$_{50}$ 0.05 mg/kg) (Fig. 7).

**Effect on Basal Motor Activities.** The effects of LY354740, LY379268, clozapine, and haloperidol on basal activities were determined under the same conditions used...
when studying AMP- or PCP-evoked increases in motor activities (Table 1). Animals were adapted to the cage environment for 30 min, drugs or saline vehicle were then administered, and motor activities were monitored 30 min later for a 60-min period (Table 1). LY354740 and clozapine (up to doses of 10 mg/kg) did not significantly affect any basal activities of the animals. In contrast, haloperidol significantly reduced total ambulations, fine movements, and rearing activity at doses of 0.1 mg/kg and greater; total ambulations and rears were completely abolished at 0.3 mg/kg. In contrast to LY354740, LY379268 at 1 and 3 mg/kg significantly reduced basal total ambulations and fine movements, and increased the animals’ time at rest (Table 1).

**Performance on Rotord.** LY354740 and LY379268, tested at doses up to 10 and 3 mg/kg, respectively, did not significantly affect the performance of rats on a rotorod apparatus, indicating that these compounds did not impair the motor abilities of the animals at all of the doses examined in

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Fig. 5. Effect of various doses of AMP (mg/kg) on behaviors as measured in an automated locomotor behavior monitor. Behaviors were monitored over a 60-min time period after s.c. injection of various doses of AMP or vehicle. Data (mean ± S.E.) are presented as the total number of behaviors expressed during 60 min; n = 3 rats. *P < .05, when compared with the corresponding vehicle control (V).

Fig. 6. Effect of various doses of LY354740 and LY379268 (mg/kg) on AMP (3 mg/kg)-evoked behaviors, as measured in an automated locomotor behavior monitor. Compounds were administered (s.c.) 30 min before AMP s.c. injection, behaviors were monitored over a 60-min time period immediately after injection of AMP. Data (mean ± S.E.) are presented as the total number of behaviors expressed during 60 min; n = 4 to 6 rats. *P < .05, when compared with AMP alone.
other tests (Table 2). However, the dose of 10 mg/kg s.c. of clozapine significantly inhibited rotorod performance by 52% (Table 2). Furthermore, haloperidol produced pronounced motor impairment (75% decreases in time on the rotorod) at doses of 0.1, 0.3, or 1 mg/kg s.c. (Table 2).

### Discussion

These studies examined PCP- and AMP-evoked motor activities in an automated motor behavior assessment system in which different parameters (ambulatory and nonambulatary/fine movements) were used. Our dose-response data with PCP and AMP indicate that this system discriminates between increases in locomotion or ambulations and other behaviors such as stereotypes that are not necessarily associated with increased locomotion. For example, unlike ambulations, which were maximal at 3 mg/kg, fine movements did not peak until higher doses of PCP (8 mg/kg). This difference might be due to the onset of ataxia at doses greater than 5 mg/kg PCP, resulting in limited ambulatory movements of the animals, but accentuating the expression of certain stereotypes (e.g., oral movements and head weaving). In any case, the reduction of time spent at rest, which was observed at all doses of PCP, indicates that the animals were still active to a comparable degree at the higher doses when ambulations had diminished. The difference between these measurements was even more pronounced with AMP, because ambulations peaked at 3 mg/kg, but were not significantly different from control animals at 30 mg/kg. However,
TABLE 2
Effect of compounds on rotorod performance

<table>
<thead>
<tr>
<th>Compound</th>
<th>Seconds on Rotorod</th>
</tr>
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<tbody>
<tr>
<td>Vehicle control</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>LY354740</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>0.01 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>59 ± 1</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>LY379268</td>
<td></td>
</tr>
<tr>
<td>0.003 mg/kg</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>0.03 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Clozapine</td>
<td></td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>29 ± 11*</td>
</tr>
<tr>
<td>Haloperidol</td>
<td></td>
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<tr>
<td>0.03 mg/kg</td>
<td>60 ± 0</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>13 ± 9*</td>
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<tr>
<td>0.3 mg/kg</td>
<td>8 ± 3*</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>4 ± 2*</td>
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</tbody>
</table>

* P < .05 when compared with vehicle control animals.

AMP increases in fine movements and reductions in time at rest were still present at the 30 mg/kg dose. In our drug studies, we generally used doses of 5 mg/kg PCP and 3 mg/kg AMP, because these doses gave robust increases in each of the three parameters measured for both agents (ambulations, fine movements, and time at rest).

It should be noted that our study used an automated motor behavioral system that may not fully discriminate between the specific motor behaviors induced by PCP or AMP. Furthermore, in addition to stimulation of motor activities in rodents, PCP also induces nonstimulant drug actions, which include disruption of cognition and sensory gating functions (prepulse inhibition of startle reflexes; Geyer and Braff, 1987), and these effects of PCP are thought to be relevant to antipsychotic drug actions. Moreover, the acute actions of PCP in animals, although suggested as a useful model for studying mechanisms of antipsychotic drug actions, have not yet been validated as a model of human schizophrenia (Halberstadt, 1995; Thorberg and Saklad, 1996; Jentsch and Roth, 1999). However, LY354740 (10 mg/kg i.p.) has been reported to reverse PCP-induced disruption of working memory and stereotypy, as well as locomotion in rats (Moghaddam and Adams, 1998). This indicates that suppression of PCP-induced motor activations may be a useful and predictive test system for initial evaluation of novel agents against PCP behaviors. In any case, this requires further investigation of these agents in other PCP models, and, ultimately, clinical studies are required to validate any animal model.

All compounds examined in this study reduced ambulations and fine motor movements evoked by 5 mg/kg PCP with the relative potency order of haloperidol > LY379268 ≈ clozapine > LY354740. Thus, in the present model, haloperidol was highly potent, and our work supports earlier reports by others (Del Rio and Fuentes, 1969; Ljungberg and Ungerstedt, 1985) who showed that similar doses of haloperidol reduced locomotor activity and stereotypies evoked by AMP. Haloperidol also potently blocked the actions of PCP in this study. However, these doses of haloperidol greatly impaired animals, as indicated by both decreased spontaneous basal motor activities and severe impairments on the rotorod. Thus, the nonselective properties of haloperidol on different aspects of stimulated and basal motor functions make it difficult to ascribe antipsychotic activities to haloperidol based on these data.

In contrast to haloperidol, clozapine selectively inhibited the actions of PCP at doses that did not produce motor impairment. Interestingly, AMP fine movements were enhanced by lower doses of clozapine. Clozapine augmentation of AMP behaviors has been reported in other studies as well. Robertson and MacDonald (1984) showed that clozapine, at doses of 5 and 10 mg/kg, reduced locomotor activity in response to 2.5 mg/kg AMP, but increased the expression of stereotypies. Similarly, in another study, although haloperidol reduced all behaviors evoked by 2 mg/kg AMP, clozapine antagonized AMP-induced locomotion, but increased certain stereotypic behaviors (Ljungberg and Ungerstedt, 1985). In contrast, Tschanz and Rebec (1989) showed that clozapine, at doses of 1 and 5 mg/kg, did block some types of stereotypies, such as sniffing and oral movements, evoked by 1 mg/kg AMP. Thus, it would seem that the effects of clozapine on AMP-evoked stereotypic behaviors are dependent on the type of stereotypy assessed. Although clozapine failed to reduce fine motor movements in the current model, it is possible that the compound could have reduced oral stereotypic behaviors that were not specifically differentiated by our monitoring system. In any case, the lack of pronounced dose-related clozapine inhibition of fine movements evoked by AMP were in contrast to the potent reduction of behaviors by haloperidol. It has been suggested that the occurrence of extrapyramidal side effects of neuroleptics in the clinic can be predicted by their ability to inhibit certain AMP-induced behaviors indicative of interference of striatal dopaminergic neurotransmission. Thus, the selective actions of mGlu2/3 agonists and clozapine against AMP suggest that mGlu2/3 agonists, like clozapine, may have a low risk of extrapyramidal side effects.

Furthermore, like clozapine, LY354740 and LY379268 had selective actions on PCP-evoked motor activations. LY379268 was more potent when compared with LY354740 in these behavioral studies. At doses that greatly reduced the actions of PCP, LY354740 was without effect on either spontaneous motor activities or rotorod performance. However, the more potent compound LY379268 did reduce basal motor activities at 1 and 3 mg/kg, doses that were also highly effective against PCP. The reasons for this difference between LY354740 and LY379268 are not clear, but might be related to subtle differences in mGlu2 versus mGlu3 receptor selectivity. In binding and functional studies in vitro, LY354740 has a 2- to 6-fold selectivity for mGlu2 versus mGlu3 receptors, whereas LY379268 has about equal activity at both receptors (Monn et al., 1999). Nevertheless, at these doses of LY379268, the animals’ motor abilities were not impaired, as demonstrated by normal performance on the rotorod and the ability of AMP to stimulate motor activity, albeit to a somewhat lesser degree, in the presence of the drug. Importantly, the effects of LY379268 (but not clozapine) on PCP were completely reversed by the selective mGlu2/3 receptor antagonist, LY341495, clearly indicating that, unlike clozapine, the effects of LY379268 in the PCP model are mediated via group II mGlu receptors.

Previous studies have reported that direct intracerebral
injections of less selective mGlu2/3 receptor agonists can produce motor impairments. Krontahler and Schmidt (1996, 1998) showed that infusion of the nonselective mGlu receptor agonists, 1S,3R-1-amino cyclopentanone-1,3-dicarboxylic acid (1S,3R-ACPD) or (2S,3S,4S)-a-carboxycyclopentylglycine, into the lateral ventricle of rats resulted in a moderate level of motor impairment described as catalepsy. However, we found no evidence for catalepsy in LY354740- or LY379268-treated animals. Furthermore, LY354740 has been reported to block haloperidol-induced catalepsy in rats at doses of 5 and 10 mg/kg (Konicezny et al., 1998), suggesting that mGlu2/3 agonists reverse rather than induce drug-induced Parkinson symptoms. Collectively, these data suggest that 1S,3R-ACPD and (2S,3S,4S)-a-carboxycyclopentylglycine induction of catalepsy is mediated by actions at mGlu subtypes other than mGlu2/3 receptors. At doses that greatly suppressed PCP-induced behaviors, LY354740 and LY379268 had minimal effects on PCP-evoked increases in ambulations and fine movements. However, like clozapine, LY354740 and LY379268 greatly reduced AMP-evoked rearing activity. This suggests that this behavior might be mediated by a mechanism distinct from that underlying AMP-evoked locomotion. Interestingly, Attarian and Alamir (1997) have shown that the less selective mGlu agonist 1S,3R-ACPD potentiates locomotor hyperactivity evoked by amphetamine. Kim and Vezina (1997) have reported that injection of relatively low doses of the group I/II mGlu receptor antagonist, (RS)-a-methyl-4-carboxyphenylglycine (MCPG) into the rat nucleus accumbens augments amphetamine-induced locomotion. In contrast, higher concentrations of MCPG reversed the increase in locomotion by amphetamine. These biphasic effects of MCPG might be indicative of the dose-dependent recruitment of distinct subgroups of mGlu receptors. Our data would suggest that this increase in AMP-evoked locomotor activity might be mediated via group I, rather than group II mGlu receptors. In summary, LY354740 and LY379268 effectively reversed PCP-evoked motor activations, at doses that had minimal effects on AMP-induced locomotor behaviors, and no gross impairment of the animals’ motor abilities. Because the effects of LY354740 and LY379268 in this study more closely resemble clozapine than haloperidol, this indicates that mGlu2/3 receptor agonists, like clozapine, may possess antipsychotic actions with a low risk of extrapyramidal side effects. Furthermore, the robust inhibitions of PCP behaviors by these novel compounds, but their more selective effects on AMP behaviors, provides a basis for further investigation of the neurochemical mechanism(s) by which mGlu2/3 agonists exert their behavioral actions.

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References


