Abstract  
Rationale: Recent studies using phencyclidine (PCP) as a model for psychosis have implicated metabotropic glutamate (mGlu) receptors in schizophrenia. We have shown, using an automated motor activity monitoring system, that selective group II mGlu receptor agonists attenuate PCP (5 mg/kg)-evoked increases in ambulations and fine motor movements with similar profiles to the atypical antipsychotic, clozapine.

Objective and methods: Because the automated system does not discriminate between specific PCP-evoked behaviors, in this paper we examined the effects of the potent mGlu2/3 receptor agonist L Y379268 on PCP-evoked behaviors as assessed by observational methods. Furthermore, we have compared the actions of L Y379268 to the atypical antipsychotic clozapine.

Results: L Y379268 and clozapine reduced the expression of PCP-induced falling, turning and back pedaling in a dose-dependent manner. Thirty minutes post-PCP administration, 1 mg/kg L Y379269 reduced falls and turns by 89% and 53%, respectively, and 1 mg/kg clozapine attenuated turning by 70%. Interestingly, low doses of clozapine increased PCP-elicited falls. Back-pedaling was particularly sensitive to L Y379268 and clozapine, with 1 mg/kg of either agent completely abolishing back-pedaling 30 min after PCP administration. However, in contrast to L Y379268, attenuation of these behaviors by clozapine only occurred at doses that augmented PCP-evoked ataxia. Furthermore, L Y379268 did not affect PCP-evoked forepaw treading.

Conclusions: These results indicate that mGlu2/3 receptors do not mediate a generalized reduction in motor activity, but instead selectively modulate specific PCP behaviors, further implicating group II mGlu receptors as viable drug targets in the treatment of schizophrenia.

Key words  Metabotropic glutamate receptor · PCP · Psychosis · Behavior · Clozapine · L Y379268

Introduction

The non-competitive NMDA receptor channel blocker phencyclidine (PCP) is capable of evoking a reaction in humans which resembles an acute episode of schizophrenia, and hence PCP is widely used as a model for psychosis (for reviews, see Javitt and Zukin 1991; Halberstadt 1995). PCP is able to evoke both positive (delusions, paranoia, hallucinations) and negative (apathy, motor impairment, social withdrawal) symptoms of schizophrenia (Javitt 1987). Furthermore, an additional aspect of PCP intoxication in humans is the appearance of stereotyped (persistent repetitive) motor movements including repetitive rocking, grimacing and shaking of the head from side to side (Luby et al. 1959). Similar stereotypic behavior can also be evoked in animals upon administration of PCP. For example, PCP-evoked head-weaving, as well as increased locomotion and rotating behavior have been observed in rats (Murray and Horita 1979; Castellani and Adams 1981). Many of these behaviors are attenuated by neuroleptic drugs such as chlorpromazine, haloperidol, pimozide and risperidone (Murray and Horita 1979; Kitaichi et al. 1994).

A recent report by Moghaddam and Adams (1998) showed the reversal of PCP-evoked locomotion and head-weaving by a selective group II metabotropic glutamate (mGlu) receptor agonist, LY354740, indicating that mGlu receptors might play a role in schizophrenia. This family of receptors has been subdivided into three distinct groups on the basis of receptor molecular structure, second messenger coupling and pharmacology. Group I mGlu receptors (mGlu1, mGlu5) are coupled to phospholipase C, whereas groups II (mGlu2, mGlu3) and III (mGlu4, mGlu6, mGlu7, mGlu8) receptors are negatively coupled to adenyl cyclase (for review, see Conn and Pin 1997). A relatively high level of expression of mGlu2 and mGlu3 receptor mRNA is found in limbic ar-
eas of the rat brain (Ohishi et al. 1993a, 1993b), suggesting that these receptors may represent targets for novel psychiatric drugs.

We recently extended the study by Moghaddam and Adams (1998) to show that LY354740 and another novel nanomolar potent and selective mGlu2/3 receptor agonist LY379268 (Monn et al. 1999) dose-dependently reversed the increases in locomotion and fine motor activities elicited by PCP in an automated activity monitor (Cartmell et al. 1999). ED50 values obtained for LY354740 and LY379268 for the inhibition of PCP-induced motor activities (ambulations and fine movements) were between 2 and 4 mg/kg. Furthermore, the atypical antipsychotic clozapine also reduced PCP motor activities over a comparable dose range to the mGlu2/3 agonists. Although we observed similar profiles of selective group II mGlu receptor agonists and clozapine on PCP-evoked ambulations and non-ambulatory (fine motor) movements, the automated activity monitoring system used does not fully differentiate multiple specific behaviors elicited by PCP. Therefore, as LY379268 was the more potent of the two mGlu2/3 receptor agonists in our previous model, we further compared the effect of LY379268 and clozapine on individual behaviors evoked by 5 mg/kg PCP. In this study, identical doses of LY379268 and clozapine were used to those previously reported to be effective against PCP (5 mg/kg) in the automated activity monitor (0.3–3 mg/kg LY379268 and 1–10 mg/kg clozapine; Cartmell et al. 1999). Behaviors such as head-weaving, falling, turning and backward shuffling (back-pedaling) were assessed using a time-sampling method similar to that described by Kitaichi et al. (1994).

Materials and methods

Methods

All experiments were performed in accordance with Eli Lilly and Company animal care and use policies, each animal being used on only one occasion. Male Sprague-Dawley rats (250–300 g) were group-housed (maximum of seven rats per cage) under standard laboratory conditions (12-h light/dark cycle) with ad libitum access to food and water, for at least 1 day before use. Rats were assigned randomly to seven dosage groups (n=6 rats per treatment for tests on vehicle-treated rats; n=12 rats per treatment for tests on PCP-treated rats). The behaviors of 12 rats (with random treatments) were scored per experiment by one trained investigator, blinded to the drug treatment.

Behaviors were observed in transparent, plastic shoe-box cages of dimensions 45 x 25 x 20 cm, with 4 cm depth of wood chips as bedding, and a metal grill on top of the cage. Rats were placed in the cage (one rat per cage) for an acclimation period of 30 min, then were removed, administered a subcutaneous (SC) injection of LY379268, clozapine or sterile water (1 ml/kg), and then returned to the cages. After 30 min, the rats were given an SC injection of sterile water or 5 mg/kg PCP (1 ml/kg), and once again returned to the cages.

Observations were carried out over the subsequent 60 min using a time-sampling method, modified from Kitaichi et al. (1994). Evaluation of behaviors was determined for a 60-s period every 15 min (at time points 15, 30, 45 and 60 min) after water or PCP administration. The definitions of the behavioral activities were adapted from Iwamoto (1984): 1) head-weaving; completion of a left-right-left or right-left-right lateral movement of the head; 2) forepaw treading; reciprocal paddling movements of the front limbs; 3) falling; fall to the side or backwards from a resting, rearing or locomoting position; 4) back-pedaling; rapid backward shuffling using all four limbs; and 5) turning; completion of a full 360 degree turn within a relatively limited space. Each episode of these behaviors occurring during the 60-s time sample, was scored as “1”. Ataxia was assessed by means of a rating scale: 0 – locomoting freely, without falling; 1 – stationary or locomoting with falls; 2 – stationary, hind limb extension and some fine movements; 3 – stationary, severe hind limb extension and motionless. In order to limit the time rats were under the influence of test compounds and PCP, they were killed immediately upon termination of the experiment at the end of the 60-min time period.

Statistical analysis

Statistical analyses of PCP-evoked behaviors were carried out using the GraphPad Prism statistical program. Data were analyzed by a two-way analysis of variance (ANOVA) with time as a between-subject factor and with treatment as a within-subject factor. All post-hoc comparisons were conducted using the Neuman-Keuls multiple comparison test. Statistical significance in the Neuman-Keuls test is shown by asterisks in the figures (results were considered to be significant at P<0.05).

Results

Effect of LY379268 or clozapine on vehicle-treated rats

The behavioral activities assessed (falling, turning, back-pedaling, head-weaving and forepaw treading) were observed only in animals treated with PCP (5 mg/kg) (data not shown); therefore, data from vehicle-treated rats are not presented. ANOVA of clozapine ataxia data revealed a significant effect of treatment [F(3,20)=17.71, P<0.0001] but no significant effect of time [F(3,20)=1.782, P=0.1573], and no significant dose x time interaction. ANOVA of LY379269 ataxia data revealed a significant effect of treatment [F(3,20)=3.446, P=0.0205] but no significant effect of time [F(3,20)=0.7393, P=0.5317], and no significant dose x time interaction. Neuman-Keuls post-hoc analysis indicated that scores for ataxia in rats treated with LY379268, up to the maximal dose tested (3 mg/kg), were not significantly different from those animals treated with vehicle (Fig. 1A). The post-hoc test also showed that clozapine significantly increased ataxia scores, 10 mg/kg having significant effects from the 30-min time point, and 3 mg/kg increasing ataxia 45 and 60 min post-administration (Fig. 1B).

Time course for PCP-induced behaviors

The expression of PCP (5 mg/kg)-evoked behaviors was time-dependent (Fig. 2–6). Head-weaving was maximal