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Short communication

The $mGlu_{2/3}$ receptor agonist LY379268 selectively blocks amphetamine ambulations and rearing

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Abstract

We have previously reported that the specific group II metabotropic glutamate receptor agonist LY379268 inhibited phencyclidine (PCP)-induced motor activations in rats, but had mixed effects on behaviors produced by amphetamine. Here, LY379268 (1 mg/kg subcutaneous (s.c.)) attenuated amphetamine-induced ambulations and rearing but did not alter amphetamine-evoked fine motor movements. Consistent with a mechanism involving $mGlu_{2/3}$ receptors, the inhibitory actions of LY379268 on ambulations and rearing were reversed by LY341495, a $mGlu_{2/3}$ receptor antagonist. These data further suggest antipsychotic actions of $mGlu_{2/3}$ receptor agonists with a low propensity for extra-pyramidal side effects. © 2000 Published by Elsevier Science B.V.

Keywords: Glutamate receptor, metabotropic; Amphetamine; LY379268; Motor activity; Stereotypy

1. Introduction

The mGlu family consists of eight second messenger-linked receptors that vary in their primary transduction mechanisms and sensitivities to various pharmacological agents (Nakanishi, 1992; Conn and Pin, 1997). Group I (consisting of $mGlu_1$ and $mGlu_5$) couple to phospholipase C and are selectively activated by 3,5-dihydroxyphenylglycine. Groups II ($mGlu_2$ and $mGlu_3$) and III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) are negatively linked to adenylyl cyclase and are selectively activated by LY354740 or LY379268 and by L-(+)-2-amino-4-phosphonobutyric acid, respectively (for a recent pharmacological review, see Schoepp et al., 1999).

Using an automated behavioral monitoring system to examine psychomotor stimulant induced changes in ambulatory or non-ambulatory (fine movements) motor behaviors, we recently showed that the potent selective group II mGlu receptor agonist LY379268 (Monn et al., 1999) inhibited the increases in ambulations and fine motor movements in rats produced by 5 mg/kg phencyclidine (PCP) (Cartmell et al., 1999). Interestingly, we also observed that doses of LY379268 that greatly attenuated PCP behaviors had limited effects on fine motor movements induced by 3 mg/kg D-amphetamine. However, LY379268 did significantly attenuate D-amphetamine (3 mg/kg) ambulations and rearing activity (as measured by ambulations in an upper rack of photobeams). These data, along with previous work by others (Moghaddam and Adams, 1998), indicate that $mGlu_{2/3}$ receptor agonists might have antipsychotic potential in humans. However, in other animal models of schizophrenia such as pre-pulse inhibition of startle responding the less potent $mGlu_{2/3}$ receptor agonist LY354740 was not active (Schreiber et al., 2000). Therefore, further studies in animals are warranted to better understand the full behavioral profile and neurochemical actions of these agents as it might relate to psychiatric disorders in humans.

Many reports have shown that D-amphetamine produces dose-dependent behaviors, lower doses increase ambulatory activity and higher doses are associated with the expression of stereotypic behaviors (Randrup and Munkvad, 1974; Arnt, 1995; Antoniou et al., 1998). Therefore, in this study we have extended our initial studies (Cartmell et al., 1999) by (1) using an automated rearing frame system to quantify the number of rears induced by amphetamine (rather than upper rack ambulations), (2)

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testing LY379268 (1 mg/kg s.c.) against a full range of amphetamine doses where various behaviors (ambulations, fine movements, rears) predominate, and (3) examining the actions of the potent $mGlu_{2/3}$ receptor antagonist, LY341495 (Kingston et al., 1998) on the inhibitory effects of LY379268 in these tests.

2. Materials and methods

2.1. Animals and experimental design

Activity monitoring was performed as described in Cartmell et al. (1999). All experiments were performed in accordance with Eli Lilly 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. Behaviors were monitored in transparent, plastic shoe-box cages of 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, San Diego, CA) consisted of two rectangular racks of 12 photobeams arranged in an 8 × 4 formation. The lower rack was positioned at a height of 5 cm and rears were 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 with a subcutaneous (s.c.) injection of LY369268 and/or LY341495 or sterile water, and then returned to the same cages. After 30 min, the rats were given a s.c. injection of a dose of D-amphetamine between 0.3 and 30 mg/kg 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 (see Cartmell et al., 1999), resulted in the classification of four types of behavior, ambulations, fine motor movements (non-ambulatory beam breaks in which the animal has not relocated its body), time at rest (no beam breaks for a period of 1 s) and rears. In contrast to our previous study (Cartmell et al., 1999) that measured 'rearing activity' using the number of ambulations in the upper rack, here we were able to measure individual rears. A rear was defined as each time a beam in the upper rack was broken followed by all beams being released.

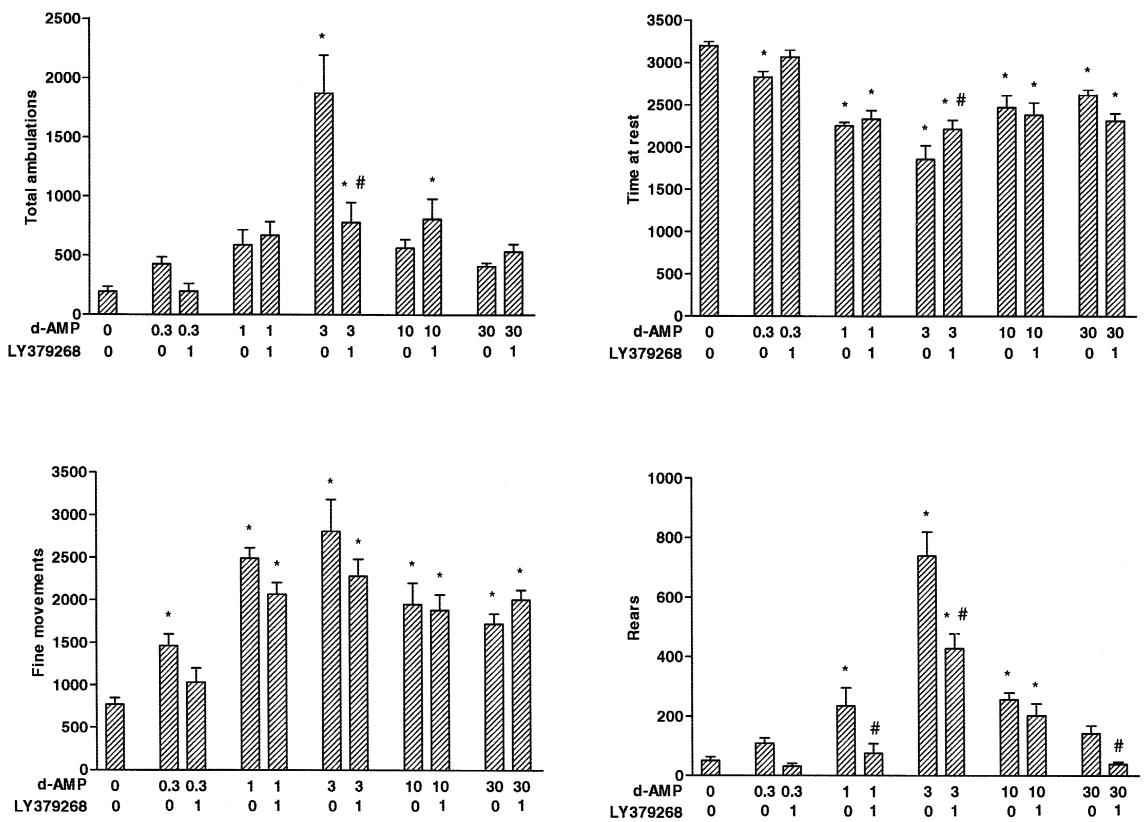

Fig. 1. Effect of LY379268 (1 mg/kg) on D-amphetamine (D-AMP; 0.3 to 30 mg/kg)-evoked behaviors as measured in an automated motor behavior monitor. LY379268 or sterile water were administered (s.c.) 30 min prior to D-amphetamine or sterile water s.c. injection. Behaviors were monitored over a 60-min time period immediately following injection of D-amphetamine or sterile water. Data (mean + S.E.M.) are presented as the total number of behaviors expressed during 60 min; $n = 4$ to 6 rats. $^{*}P < 0.05$, when compared with the vehicle/vehicle control group. $^{\#}P < 0.05$, when compared with the corresponding D-amphetamine/vehicle control group.

2.2. Drugs

D-Amphetamine sulfate was obtained from Sigma (St. Louis, MO, USA). LY379268 was synthesized as described in Monn et al. (1999). LY341495 was provided by Dr. Paul L. Ornstein (Lilly Research Laboratories, Indianapolis, IN, USA).

2.3. Statistical analysis

Statistical analyses of behaviors were carried out using the GraphPad Prism graphics program. Data were analyzed by a one-way analysis of variance (ANOVA), and then post hoc comparisons were made using the Neuman–Keuls test. Differences were considered significant at $P < 0.05$.

3. Results

Fig. 1 shows that only one dose of D-amphetamine (3 mg/kg) produced a significant increase in ambulations (970% of basal) and this was significantly inhibited by 1 mg/kg LY379268 (65% inhibition). However, all doses from 0.3 to 30 mg/kg D-amphetamine significantly increased the expression of fine motor movements with a maximal effect 364% of basal at 3 mg/kg D-amphetamine. Interestingly, there was no effect of 1 mg/kg LY379268 on fine movements produced by any of these doses of D-amphetamine (Fig. 1). The number of rears was also significantly increased by 1, 3 and 10 mg/kg D-amphetamine (461%, 1449% and 504% of basal, respectively) and the responses to 1 and 3 mg/kg D-amphetamine were significantly attenuated by 1 mg/kg LY379268 (87% and 45% inhibition, respectively) (Fig. 1). Although there was no statistically significant augmentation in rears by 30 mg/kg D-amphetamine, the slightly increased response was significantly reduced by 1 mg/kg LY379268 to below the response of the vehicle/vehicle controls. In support of the increases in fine motor movements and rears observed across the dose range there was a significant reduction in the time spent at rest at all doses of D-amphetamine tested and LY379268 (1 mg/kg) significantly reversed the reduced time at rest evoked by 3 mg/kg D-amphetamine (27%).

In order to determine if the inhibitory effects of LY379268 on D-amphetamine-produced ambulations and rears were mediated via a $mGlu_{2/3}$ receptor-dependent mechanism, the effects of the selective $mGlu_{2/3}$ receptor antagonist LY341495 (3 mg/kg) were examined. LY341495 (3 mg/kg) was without significant effect on ambulations and rears following administration of vehicle or D-amphetamine (3 mg/kg) per se (Fig. 2). LY379268 (3 mg/kg) inhibited D-amphetamine-evoked ambulations and rears (but not time at rest or fine motor movements; data not shown) by 58% and 83%, respectively. When LY341495 (both 1 and 3 mg/kg) was co-administered with LY379268, the inhibition of D-amphetamine-elicited ambulations was no longer significant and the attenuation of rearing was blocked in a dose-dependent manner (Fig. 2).

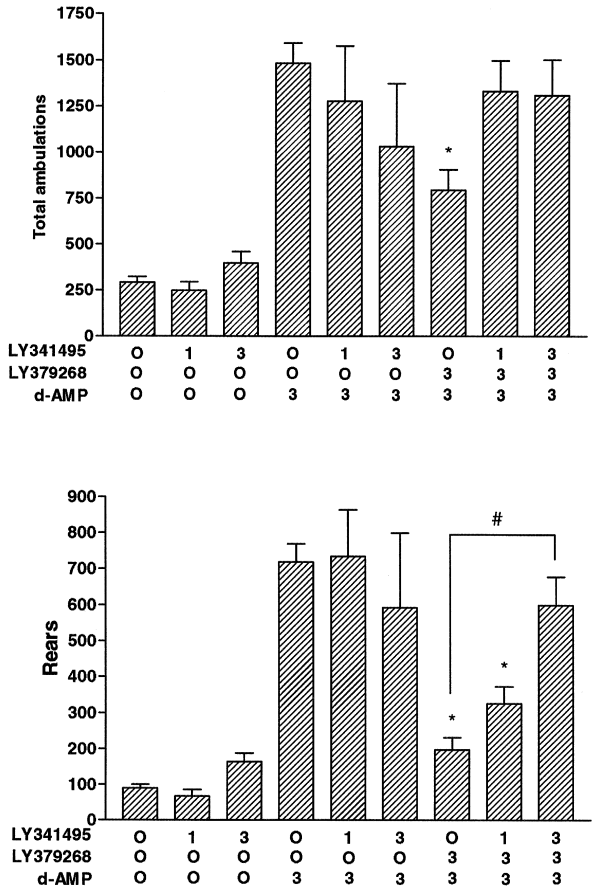

Fig. 2. Effect of the $mGlu_{2/3}$ antagonist LY341495 on LY379268 inhibitions of amphetamine-induced ambulations and rears as measured in an automated motor behavior monitor. LY379268 (3 mg/kg) or sterile water, and/or LY341495 (1 or 3 mg/kg) were administered (s.c.) 30 min prior to D-amphetamine or sterile water s.c. injection. Behaviors were monitored over a 60-min time period immediately following injection of D-amphetamine or sterile water. Data (mean + S.E.M.) are presented as the total number of behaviors expressed during 60 min; $n = 5–14$ rats. $^{*}P < 0.05$, when compared with the corresponding D-amphetamine/vehicle control group. $^{\#}P < 0.05$, when compared with the corresponding control (no LY341495) group.

4. Discussion

These data confirm previous reports showing that D-amphetamine produces dose-dependent effects on motor activities (Randrup and Munkvad, 1974; Arnt, 1995; Antoniou et al., 1998). Furthermore, the bell-shaped dose–response to increasing doses of D-amphetamine, with maximal responses of all behaviors observed at 3 mg/kg, is in agreement with the results of our former study (Cartmell et al., 1999). Also in agreement with our earlier work and

that of others, we show here that higher doses of D-amphetamine (10 and 30 mg/kg) appear to be associated more with the expression of fine motor movements (which include stereotypies) than with ambulatory and rearing behaviors (see Antoniou et al., 1998). Ambulations, fine motor movements and rears increased up to a maximal effect at 3 mg/kg, and then at doses of 10 mg/kg and greater, ambulations and rearing declined. The maximal ambulatory and rearing responses, as well as the maximal reduction in time spent at rest (all at 3 mg/kg D-amphetamine), were all reversed by 1 mg/kg LY379268. In the present study, 1 mg/kg LY379268 reduced ambulations by 65% and rears were inhibited by 45%. Although these effects of LY379268 were also observed in our previous experiments, the extent of inhibition of ambulations by this dose of LY379268 was somewhat smaller in our earlier report (39% inhibition; Cartmell et al., 1999). However, the attenuation in rearing activity by 1 mg/kg LY379268 was equivalent in both studies (approximately 40%; Cartmell et al., 1999). The increase in rears produced by D-amphetamine at doses of 1, 3 and 30 mg/kg (but interestingly, not by 10 mg/kg) was also significantly reduced by 1 mg/kg LY379268. The maximal reduction in time at rest, evoked by 3 mg/kg, was also significantly reversed by LY379268, probably reflecting the marked effects observed on ambulations and/or rears.

Given the comparable dose–response profiles for D-amphetamine-evoked ambulations and rears, it is tempting to speculate that these behaviors might be linked, possibly implicating similar brain circuitry and/or neurotransmitter systems. It may be that increased locomotion and rearing might be reflective of exploratory activities, which are then superceded by stereotypic behaviors with greater doses of D-amphetamine. We show here that D-amphetamine-evoked fine motor movements appear to be comparatively resistant to group II mGlu receptor modulation. This finding is of particular interest given that LY379268 attenuated PCP-evoked fine motor movements with equivalent potency to that observed for the inhibition of PCP-evoked ambulations (Cartmell et al., 1999). Obviously, PCP and D-amphetamine have very different neurochemical actions and elicit distinct behavior profiles. In addition to increased ambulatory movement, animals treated with PCP show a characteristic response of head weaving, falling, turning and intense backward walking which are all attenuated by LY379268 (Cartmell et al., 2000). On the other hand, the fine motor movements produced by D-amphetamine appear to have different underlying mechanisms as LY379268 was without effect on these behaviors. It may be that certain D-amphetamine-elicited behaviors are mediated via brain areas with limited expression of group II mGlu receptors. However, as the inhibition of D-amphetamine-evoked stereotypies can be predictive of the occurrence of extra-pyramidal side effects in the clinic, the lack of effect of LY379268 on the increase in fine motor movements produced by D-amphetamine in this model is of potential therapeutic interest.

It is clear that the actions of LY379268 on PCP or certain D-amphetamine behaviors are $mGlu_{2/3}$ receptor related as they have in common blockade by the $mGlu_{2/3}$ receptor antagonist LY341495. Regardless of the other brain mechanisms involved, the inhibition of ambulations and rears, coupled with a lack of effect of LY379268 on D-amphetamine-evoked fine motor movements shown in this study suggest possible antipsychotic actions of LY379268 in the absence of extra-pyramidal side effects. Nevertheless, as schizophrenia is a heterogeneous human disorder that others and us are attempting to model in animals, ultimately clinical studies are required to determine whether $mGlu_{2/3}$ receptor agonists have therapeutic benefits/side effects in humans.

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