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The metabotropic glutamate 2/3 receptor agonist LY379268 induces anxiety-like behavior at the highest dose tested in two rat models of anxiety

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Abstract

The activation of Group II metabotropic glutamate 2/3 (mGlu_{2/3}) receptors reduces the excessive glutamate release that is hypothesized to be associated with neurodegenerative and psychiatric disorders. LY379268 is a highly potent mGlu_{2/3} receptor agonist that has shown efficacy in several animal models of stroke, epilepsy, drug abuse, schizophrenia, and pain. The present study investigated the effects of LY379268 on anxiety-like behavior in rats assessed in the light/dark and open field tests. The effects of LY379268 on motility in a locomotor activity chamber were also investigated in rats. Administration of the two lower doses of LY379268 used (0.3 and 1 mg/kg) did not influence rats' performance either in the light/dark or in the open field test. Importantly, the administration of a higher LY379268 dose (3 mg/kg) induced decreases in the number of transitions between the light and dark chambers and time spent in the light chamber compared to the vehicle-treated animals in the light/dark test. In the open field test, rats that received 3 mg/kg LY379268 made fewer entries and spent less time in the central zone of the apparatus, exhibited a decrease of rearing episodes, but displayed higher grooming activity compared to controls. Nevertheless, the 3 mg/kg dose did not alter locomotor activity compared with vehicle-treated rats in a motility test. The present results indicate that the highest LY379268 dose used in this study induced an anxiety-like effect in the light/dark and open field tests that cannot be attributed to changes in locomotor activity, while lower doses had not effect.

Keywords

mGlu_{2/3} receptor; LY379268; light/dark test; open field test; anxiety; rat

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Conflict of Interest Statement

The other authors report no financial conflict of interests.

1. Introduction

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) that acts on both ionotropic and metabotropic glutamate (mGlu) receptors. The mGlu receptor family consists of eight receptor subtypes divided into three groups based on sequence homology, pharmacological profile and signal transduction pathways (Conn and Pin, 1997). Experimental evidence suggests that ligands for specific mGlu receptor subtypes have potential for the treatment of several central nervous system (CNS) disorders, including depression, anxiety, schizophrenia chronic pain and epilepsy (Marek, 2004; Nicoletti et al., 2011; Schoepp and Marek, 2002).

Group II mGlu receptors include the mGlu2 and mGlu3 receptors. These receptors are localized primarily presynaptically in the cortex, thalamus, striatum, amygdala and hippocampus (Ohishi et al., 1993a, 1993b; Petralia et al., 1996; Shigemoto et al., 1997). These areas of the brain are thought to play a critical role in anxiety (Linden et al., 2004; Nicoletti et al., 2011; Swanson et al., 2005; Walker and Davies, 2002). Hyperactivity of glutamatergic transmission in these structures is hypothesized to be associated with the pathogenesis of anxiety (Linden et al., 2004; Swanson et al., 2005; Walker and Davies, 2002).

The activation of mGlu_{2/3} receptors provides a negative feedback mechanism to prevent excessive presynaptic glutamate release in limbic regions implicated in the pathophysiology of affective disorders (Chavez-Noriega et al., 2002; Schoepp and Marek, 2002). The mGlu_{2/3} receptor agonist LY354740 has been reported to have anxiolytic-like effects in different animal models of anxiety, including fear-potentiated startle, the elevated plus-maze and the conflict drinking test (Helton et al., 1998; Klodzinska et al., 1999; Linden et al., 2004).

LY379268 is a selective agonist for Group II mGlu_{2/3} receptors with higher affinity for these receptors compared with LY354740 (Monn et al., 1999). The behavioral actions of LY379268 have not yet been fully clarified, and both anxiogenic and anxiolytic effects of LY379268 have been reported (Imre et al., 2006; Satow et al., 2008; Takahashi et al., 2009; Wieronska et al., 2012). Thus, additional research is required to determine the effects of LY379268 on anxiety-like behavior.

The aim of the present study was to further investigate the effects of LY379268 on anxiety-like-behavior in the rat using different experimental approaches; the light/dark box and the open field tests. The light/dark box test is a procedure that is based on the innate aversion of rodents to brightly illuminated areas and the conflicting tendency of rodents to explore novel environments (Crawley and Goodwin, 1980). The open field test involves encounter with a novel environment and give rise to behavioral and physiological reactions related to anxiety (Prut and Belzung, 2003). Finally, locomotor activity was also assessed as an independent measure of the potential motoric effects of the compound that could influence rats' performance in the light/dark box and the open field tests.

2. Materials and methods

2.1. Animals

Male 3-month-old albino Wistar rats (Hellenic Pasteur Institute, Athens, Greece) that weighed 250–300 g were used in this study. The animals were housed in Makrolon cages (47.5 cm length × 20.5 cm height × 27 cm width) three per cage, in a climate-regulated environment (21 ± 1°C; 50–55% relative humidity) under a 12 h/12 h (lights on at 7:00 AM) light/dark cycle with free access to food and water.

The procedures that involved animals and their care were conducted in accordance with international guidelines and national and international laws and policies (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *NIH Guide for Care and Use of Laboratory Animals*, NIH publication no. 85-23, 1985).

2.2. Chemicals

LY-379268 ([–]-2-oxa-4-aminobicyclo[3.1.0.]hexane-4,6-di-carboxylic acid) was custom-synthesized and purchased from ANAWA (Wangen, Switzerland). LY379268 was dissolved in saline (0.9%, NaCl). To improve the solubility of the compound, 1 µl of 5 M sodium hydroxide (NaOH) per milligram of LY379268 was added to the solution. The solution was sonicated for 5 min, and the pH was adjusted to 7.4 using 1 M NaOH (Jones et al., 2011). LY379268 doses (0.3, 1 and 3 mg/kg) were selected based on the results of a previous study in which the same dose range was chosen to investigate the role of this compound on anxiety-like behavior (Imre et al., 2006). Control animals received isovolumetric amounts of the vehicle solution (i.e., saline that contained the same amount of NaOH). The compounds were administered intraperitoneally (i.p.) in a volume of 1 ml/kg.

2.3. Light/dark test

The light-dark box apparatus consisted of a wooden box (48 cm length × 24 cm height × 27 cm width) divided into two equal-size compartments by a barrier that contained a doorway (10 cm height × 10 cm width). One of the compartments was painted black and was covered with a lid and the other compartment was painted white and illuminated with a 60 W light bulb positioned 40 cm above the box. The test was performed as described previously (Pitsikas et al., 2008). On the test day, the rats were transported to the darkened test room and left in their home cages for 2 h. Then the animals were placed in the middle of the lit compartment, facing away from the dark chamber. The rats were allowed to freely explore the apparatus for 5 min. The latency to enter (with all four paws) the dark compartment, number of transitions and time spent in the light and dark compartments were recorded. A between-subjects design was used for the factor dose: thus, each rat was tested only once. That is, for testing the effects of LY379268 on rats' performance in the light/dark test, naive rats were randomly divided into four experimental groups (10 rats per group): vehicle, and 0.3, 1 and 3 mg/kg LY379268 (i.p., 30 min pretreatment).

2.4. Open field test

The test apparatus consisted of a dark open box made of Plexiglas (70 cm length × 50 cm height × 70 cm width). The open field arena was divided-by black lines-into 16 squares of 17.5 × 17.5 cm. The central 4 squares were defined as the central zone, in which animals' activity was regarded as a measure of anxiety (Prut and Belzung, 2003). Testing was done under normal light conditions. On the test day, the rats were transported to the testing room and left in their home cages for 2 h. Each animal was then placed in the same corner of the open field arena and its behavior was recorded for 5 min. The variables observed were: (a) the first latency to enter the central zone of the open field arena (b) the number of entries in the central zone of the open field arena (c) the amount of the time spent in the central zone as defined by all forepaws being in the central 4 squares of the apparatus, (d) the number of squares crossed (i.e., horizontal activity), (e) the number of rearing behaviors (i.e., vertical activity, defined as raising both forepaws above the floor while balancing on hind limbs), and (f) the duration of grooming events.

A between-subjects design was used for the factor dose: thus, each rat was tested only once. That is, for testing the effects of LY379268 on rats' performance in the open field test, naive rats were randomly divided into four experimental groups (8 rats per group): vehicle, and 0.3, 1 and 3 mg/kg LY379268 (i.p., 30 min pretreatment).

2.5. Locomotor activity test

Spontaneous locomotor activity was assessed in an activity cage (catalog number 7420, Ugo Basile, Varese, Italy). The apparatus consisted of a box made of Plexiglas (41 cm length $\times$ 33 cm height $\times$ 41 cm width). Every movement of the animal produced a signal caused by variations in the inductance and capacitance of resonance circuitry of the apparatus. The signals were then automatically converted into numbers that reflected horizontal activity counts. Changes in activity counts represent a standard behavioral assay for testing the motoric effects of drugs. On the test day, naive rats were transported to the darkened test room and left in their home cages for 2 h. Thereafter, each animal was placed into the locomotor activity arena and spontaneous locomotion was recorded for 5 min. Separate cohorts of naive rats were used for the locomotor activity experiment and the light/dark box experiment. A between-subjects design was used for the factor dose. Thus, each rat was tested only once. That is, for testing the effects of LY379268 on locomotor activity, naive rats were randomly divided into four experimental groups (8 rats per group): vehicle, and 0.3, 1 and 3 mg/kg LY379268 (i.p., 30 min pretreatment).

Experiments were conducted between 9:00 AM and 3:30 PM during the light phase of the light/dark cycle. To avoid the presence of olfactory cues, all the apparatus (light/dark box, open field arena and motor activity cage) were thoroughly cleaned with 20% ethanol and then wiped with dry paper after each trial.

Behavior in the light/dark and open field tests was video-recorded. Data evaluation was subsequently performed by two observers who were unaware of the pharmacological treatment of each subject.

2.6. Statistical analysis

Data are expressed as mean $\pm$ S.E.M. and were evaluated by one-way analysis of variance (ANOVA). *Post-hoc* comparisons were made with the Tukey's *post-hoc* test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Light/dark test

The effects of LY379268 on animals' performance in the light/dark test are depicted in Figure 1. LY379268 did not affect the latency to enter the dark compartment (Fig. 1A). Importantly, however, LY379268 significantly decreased the number of transitions between compartments (Fig. 1B) as revealed by a statistically significant main effect of *Treatment* [$F(3,39)=5.685$, $P < 0.01$]. The *post-hoc* comparisons showed that the rats that received 3 mg/kg LY379268 made fewer transitions compared with the vehicle- and the 0.3 mg/kg LY379268-treated animals ($P < 0.05$). Further, treatment with LY379268 significantly decreased the total time spent in the light compartment [main effect of *Treatment* [$F(3,39)=3.462$, $P < 0.05$]. The *post-hoc* analysis showed that rats treated with 3 mg/kg LY379268 spent less time in the light compartment compared with the vehicle-treated animals ($P < 0.05$, Fig. 1C).

3.2. Open field test

LY379268 did not modify the first latency to enter the central zone of the open field arena (Fig. 2A). LY379268 significantly decreased the number of entries in the central area of the apparatus as revealed by a statistically significant main effect of *Treatment* [$F(3,31)=3.068$, $P < 0.05$]. The *post-hoc* comparisons showed that the rats that received 3 mg/kg LY379268 made fewer visits compared with the vehicle-treated animals ($P < 0.05$, Fig. 2B). Moreover, treatment with LY379268 significantly decreased the total time spent in the central zone of

the apparatus [main effect of *Treatment* [$F(3,31)=3.786$, $P<0.05$]. The *post-hoc* analysis showed that rats treated with 3 mg/kg LY379268 spent less time in the central area of the open field compared with the vehicle- and 1 mg/kg LY379268-treated animals ($P<0.05$, Fig. 2C).

In addition, LY379268 did not affect rats' motor abilities in this test (Fig. 3A) but significantly influenced rearing episodes [$F(3,31)=3.423$, $P<0.05$]. The *post-hoc* comparisons showed that rats treated with 3 mg/kg LY379268 had fewer rearing episodes compared to all the other treatment groups ($P<0.05$, Fig. 3B). Finally, treatment with this mGlu_{2/3} receptor agonist significantly affected animals' grooming activity [$F(3,31)=3.113$, $P<0.05$]. The *post-hoc* comparisons showed that grooming activity of rats treated with 3 mg/kg LY379268 was significantly higher respect to all the other treatment groups ($P<0.05$, Fig. 3C).

3.3. Locomotor activity test

The statistical analysis of the locomotor activity data did not reveal a significant main effect of *Treatment* [$F(3,31)=0.85$, not significant, Fig. 4].

4. Discussion

The light/dark test has been shown to reliably predict the anxiolytic- and anxiogenic-like effects of drugs in rodents (Crawley and Goodwin, 1980). This test has the advantages of being quick and easy to use without prior training of the animals, and neither food nor water deprivation are required (Bourin and Hascoet, 2003). Transitions in this test are considered an index of activity/exploration because habituation over time is seen with this measure, whereas the time spent in each compartment reflects aversion/attraction (Belzung et al., 1987).

Administration of 3 mg/kg, but not 0.3 or 1 mg/kg, of LY379268 decreased the time spent in the light compartment and significantly reduced the number of transitions between compartments. LY379268 did not alter the latency of the first entry into the dark box at any of the doses tested. This pattern of results reflects an anxiogenic-like effect of this mGlu_{2/3} receptor agonist at the highest dose tested in the light/dark test.

The open field test is a standard neophobic test of anxiety. In this test, rodents naturally tend to avoid open spaces. Thus, the number of entries and the time spent in the central area of an open field arena is a measure of anxiety state (Prut and Belzung, 2003). LY379268 (3, but not 0.3 or 1, mg/kg) induced an anxiogenic-like effect in the open field test, as evidenced by significant reduction of the number of entries and the time spent in the central area of the open field arena, whereas the number of square crossed was not changed. In addition, this highest dose of LY379268 (3 mg/kg) significantly decreased rearing episodes providing another indication of a decrease in exploratory behavior. LY379268, at any dose tested, did not alter the latency of the first entry into the open field arena and did not affect animals' motor activity in the open field test.

When animals are exposed to mild threat or stressful environment, self-grooming behavior can occur (Gispén and Isaacson, 1981; Spruijt et al., 1992). Anxiogenic stimuli can increase self-grooming while anxiolytic drugs can reduce self-grooming which it can be considered an index of anxiety. In our study, animals were tested in an unfamiliar open field arena, and thus, the novelty stress could contribute to the increased grooming behavior. Our results indicate that LY379268 (3, but not 0.3 or 1, mg/kg) significantly increased self-grooming in rats without influencing their general motility levels further supporting the anxiogenic profile of this dose of LY379268.

Drugs that affect general motor function may affect performance in anxiety tests because of changes in motoric activity that are unrelated to any anxiogenic- or anxiolytic-like effects of the compound. Thus, the assessment of motor activity in rodents after administration of a test compound is needed to evaluate the possibility of nonspecific motoric effect that may confound the interpretation of the results from the anxiety tests.

The present locomotor activity results indicate that the dose of LY379268 (3 mg/kg) that produced an anxiogenic-like effect in the light/dark and in the open field test did not induce any changes in locomotor activity in a different cohort of rats the activity of which was assessed in a traditional locomotor activity chamber. Thus, the anxiety-like responses of LY379268 at the highest dose tested cannot be attributed to changes in locomotor activity.

The effects of LY379268 on anxiety-like behavior are still a matter of debate. Previous studies reported both anxiolytic- and anxiogenic-like responses after LY379268 administration in rodents. LY379268 induced anxiolytic-like effects reflected by decreased ultrasonic vocalizations in rat pups (Satow et al., 2008), and increased hypothermia in the stress-induced hypothermia test in mice (Satow et al., 2008; Wieronska et al., 2012), but no effects of LY379268 were observed in the elevated plus maze test in mice (Satow et al., 2008).

Furthermore, administration of 3 mg/kg LY379268 in rats, reduced rearing episodes, evoked freezing behavior and increased the startle reflex, behaviors that are considered to reflect increased anxiety (Imre et al., 2006). In the same study, a lower dose of LY379268 (1 mg/kg) had no such anxiogenic-like effects, although the animals exhibited a reduction in exploratory behavior, reflected in decrease number of rearing episodes, in the open field test (Imre et al., 2006). Nevertheless, the possibility that this effect may be a consequence of the locomotor suppressant effects of this LY379268 dose cannot be excluded (Imre et al., 2006). The latter interpretation is consistent with the possibility that high doses of mGlu_{2/3} receptor agonists have locomotor activity-suppressant effects (Henry et al., 2002), although such effects were not seen in the present study. Altogether, the available data suggest a dual effect of LY379268 on anxiety-like states.

Importantly, the previously reported anxiolytic-like effects of LY379268 were observed in unconditioned non-exploration-driven anxiety-related tests (Bouwknicht and Paylor, 2008), such as ultrasonic vocalization (Satow et al., 2008) and stress-induced hypothermia (Satow et al., 2008; Wieronska et al., 2012). Conversely, LY379268 did not induce anxiolytic-like effects and even induced anxiogenic-like effects in unconditioned exploration-driven anxiety-related procedures that are primarily based on the conflict between the desire to explore and avoidance of novel environments (Bouwknicht and Paylor, 2008), such as in the elevated plus maze (Satow et al., 2008) the light/dark box and the open field test (present results).

Specifically, LY354740, a less potent analog of LY379268, has anxiolytic-like effects in both animals and humans (for review, see Swanson et al., 2005). LY379268 has considerably higher selectivity at the mGlu₃ receptor than LY354740, whereas LY354740 has equal affinity for both mGlu₂ and mGlu₃ receptors (for review, see Imre, 2007). Furthermore, LY379268 can be clearly differentiated from LY354740 because it has increased agonist potency in cells that express the mGlu₆ receptor subtype (Laurie et al., 1997). We cannot exclude the possibility that these differences in potency and affinity may represent a plausible explanation for the different profiles displayed by these mGlu_{2/3} receptor agonists in anxiety-like behavior.

5. Conclusions

In summary, the present results suggest an anxiogenic-like, rather than anxiolytic-like, action of the mGlu_{2/3} receptor agonist LY379268 in the light/dark box and the open field test in rats.

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Figure 1.

Vehicle and LY379268 were injected intraperitoneally 30 min before testing. The histogram shows the means $\pm$ S.E.M. of 10 rats per treatment group. (A) Latency to enter the dark chamber. (B) Number of transitions. * $P < 0.05$ compared with the vehicle- and 0.3 mg/kg LY379268-treated groups. (C) Time spent in the light chamber. * $P < 0.05$ compared with the vehicle-treated group.

Figure 2.

Vehicle and LY379268 were injected intraperitoneally 30 min before testing. The histogram shows the means $\pm$ S.E.M. of 8 rats per treatment group. (A) Latency to enter the central zone. (B) Number of entries in the central zone. * $P < 0.05$ compared with the vehicle-treated group. (C) Time spent in the central zone. * $P < 0.05$ compared with the vehicle- and 1 mg/kg LY379268-treated groups.

Figure 3.

Vehicle and LY379268 were injected intraperitoneally 30 min before testing. The histogram shows the means $\pm$ S.E.M. of 8 rats per treatment group. (A) Number of squares crosses. (B) Number of rearings. * $P < 0.05$ compared with all the other groups. (C) Grooming duration. * $P < 0.05$ compared with all the other groups.

Figure 4.

Vehicle and LY379268 were injected intraperitoneally 30 min before testing. The histogram shows the means $\pm$ S.E.M. of 8 rats per treatment group. (A) Locomotor activity counts.