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Effects of the mGluR2/3 agonist LY379268 on ketamine-evoked behaviours and neurochemical changes in the dentate gyrus of the rat

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Abstract

One of the functions of group II metabotropic glutamate receptors (mGluR2/3) is to modulate glutamate release. Thus, targeting mGluR2/3s might be a novel treatment for several psychiatric disorders associated with inappropriate glutamatergic neurotransmission, such as schizophrenia. In an effort to evaluate the antipsychotic properties of LY379268, a potent and selective mGluR2/3 agonist, we examined its effect on ketamine-evoked hyperlocomotion and sensorimotor gating deficit (PPI) in rats, an animal model of schizophrenia. We also measured the ex vivo tissue level of glutamate (Glu), dopamine (DA) and serotonin (5-HT) as well as the DA metabolites DOPAC and the major 5-HT metabolite HIAA to determine the neurochemical effects of ketamine (12 mg/kg) and LY379268 (1 mg/kg) in the dentate gyrus (DG). While LY379268 (1–3 mg/kg) reduced ketamine-evoked hyperlocomotion (12 mg/kg), it could not restore ketamine-evoked PPI deficits (4–12 mg/kg). In the DG we found that ketamine decreased Glu and DA levels, as well as HIAA/5-HT turnover, and that LY379268 could prevent ketamine effects on Glu level but not on monoamine transmission. These results may indicate that the inability of LY379268 to reverse PPI deficits is attributable to its lack of effect on ketamine-induced changes in monoamine transmission, but that LY379268 can prevent ketamine-evoked changes in glutamate, which is sufficient to block hyperlocomotion. In addition to the partial effectiveness of LY379268 in the ketamine model of schizophrenia, we observed a dual effect of LY379268 on anxious states, whereby a low dose of this compound (1 mg/kg) produced anxiolytic effects, while a higher dose (3 mg/kg) appeared to be anxiogenic. Additional work is needed to address a possible role of LY379268 in schizophrenia and anxiety treatment.

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Keywords: PPI deficit; Hyperlocomotion; Tissue dissection; Neurotransmitter level; Schizophrenia; Anxiety

1. Introduction

Glutamate, as the major excitatory neurotransmitter in the mammalian central nervous system, plays a role in many brain processes including sensory and motor function, cognition and regulation of perception and emotion (Meldrum, 2000). Consequently, abnormal changes in glutamatergic neurotransmission

can lead to a neuronal dysfunction, resulting in a variety of neurological and psychiatric disorders, such as anxiety and schizophrenia (Chavez-Noriega et al., 2002). In the search of novel treatment for these disorders, the members of group II metabotropic glutamate receptors (mGluR2/3) have emerged as potential therapeutic targets. Activation of the mGluR2/3 receptors provides a negative feedback mechanism to prevent excessive presynaptic glutamate release in limbic regions that have been implicated in pathology of affective disorders (Chavez-Noriega et al., 2002; Schoepp and Marek, 2002). In particular, the potent, selective mGluR2/3 agonist, LY354740 has been shown to have anxiolytic-like activity in several 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;

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Linden et al., 2004). Furthermore, LY354740 and its more potent analogue, LY379268, have been shown to be active in the NMDA receptor hypofunction model of schizophrenia.

Non-competitive NMDA receptor antagonists, such as phencyclidine (PCP), or its analogue ketamine, administered to healthy individuals produce symptoms common in schizophrenia (Javitt and Zukin, 1991; Krystal et al., 1994) and have been used in rodents to model behavioural abnormalities associated with schizophrenia, including disruption in the prepulse inhibition of startle reflex (PPI), working memory deficits and augmented locomotion (Aultman and Moghaddam, 2001; Imre et al., 2006; Lorrain et al., 2003b; Mansbach and Geyer, 1991). The adverse behavioural effects of these drugs have been attributed primarily to blockade of NMDA receptors on inhibitory GABAergic neurons, which in turn leads to release of various neurotransmitters including glutamate (Glu), dopamine (DA), serotonin (5-HT) and noradrenaline (NE) in fore-brain regions (Lindfors et al., 1997; Lorrain et al., 2003a,b; Moghaddam et al., 1997). Among these responses, recent findings have highlighted the importance of glutamate neurotransmission: both LY354740 and LY379268 blocked NMDA antagonists-evoked glutamate release in the prefrontal cortex (PFC) and nucleus accumbens, attenuating the disruptive effects on locomotion and working memory (Moghaddam and Adams, 1998). Furthermore, LY379268 shares common neurochemical characteristics with clozapine-like atypical antipsychotics which are generally effective in this model due to their multiple antagonist properties (Geyer and Ellenbroek, 2003). Similar to clozapine and risperidone, LY379268 increased the turnover of dopamine and 5-HT in the PFC and striatum, as measured by in vivo microdialysis and ex vivo tissue destruction (Cartmell et al., 2000b,c, 2001). These studies imply that modulation of excitatory neurotransmission via mGluR2/3 may represent a novel approach to treat schizophrenia.

However, recent reports did not support this hypothesis, since LY354740 failed to reverse the disruption of prepulse inhibition induced by NMDA antagonists, nor did it restore cognitive dysfunction (Ossowska et al., 2000; Schreiber et al., 2000). Moreover, studies from this laboratory showed that subchronic pretreatment with LY354740 did not prevent ketamine-evoked hyperlocomotion and PPI deficits (Imre et al., in press).

Because of these contradictory results with LY354740, the primary aim of this study was to explore the potential of a related mGluR2/3 receptor agonist as a novel antipsychotic, using the ketamine model of schizophrenia. Therefore, we investigated the ability of LY379268 to suppress ketamine-evoked hyperlocomotion and PPI deficits. To our knowledge, this is the first study that reports on the effects of LY379268 on sensorimotor gating deficits.

We also investigated the neurochemical effects of ketamine and LY379268 treatment on neurotransmission in the dentate gyrus (DG). This subregion of the hippocampus has raised particular interest for three reasons. First, aside from the PFC and amygdala, the hippocampus has been shown to mediate the PPI disruptive actions of NMDA antagonists (Bakshi and Geyer, 1998). Second, both NMDA and mGluR2/3 receptors are abundant in the DG (Petrulia et al., 1994, 1996). Third, our c-fos data have

indicated that both ketamine and LY354740 decrease cell activity within this region and this reduction was more profound when the two drugs were administered together (Imre et al., in press). Such a synergistic effect was not observed in other brain areas investigated. To this end, we examined the ex vivo tissue levels of glutamate and monoamines in the DG following treatment with ketamine and LY379268. We also measured the level of the DA metabolite 3, 4-dihydroxyphenylacetic acid (DOPAC), as well as the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA), in order to calculate the tissue DOPAC:DA, and 5-HIAA:5-HT ratios, which are considered to be indications for drug-induced changes in neurotransmitter utilization across different brain regions.

2. Materials and methods

2.1. Animals

Fifty-four adult male Wistar rats weighing 200–250 g at the beginning of the experiments were individually housed and maintained on a 12 h light/dark cycle with the light cycle beginning at 7.00 am. Food and water were available ad libitum (standard rat chow). All animals were handled and weighed daily to minimise stress during the experiments. The Animals Ethics Committee of the University of Groningen approved all protocols (DEC 2935).

2.2. Drugs

Ketamine (Sigma, Germany) and LY379268 (–)-2-oxa-4-aminobicyclo [3.1.0] heptane-4, 6-dicarboxylic acid (Eli Lilly and Company, Indianapolis) were dissolved in saline. In order to perfectly dissolve LY379268 in saline, 1 µl of 5 N NaOH was added for every mg of LY379268 (pH ~6) and placed in a bath sonicator for a short time. All solutions were freshly prepared before injection. Animals were injected with LY379268 (1–3 mg/kg) intraperitoneally (i.p.) 30 min prior to test, while ketamine (4–12 mg/kg) was applied subcutaneously (s.c.) immediately before the start of behavioural tests. A 7-day interval was allowed between each experiment. Following each behavioural test the animals were divided into groups ($n=6$ /group) in a way that each new group was balanced, containing 1 animal from every former group.

2.3. Open field test (OF)

The behavioural effects of the drug treatments were tested in an open field which consisted of a circular black arena with a diameter of 1 m bordered by 40-cm high walls. The testing room was illuminated with 3 25-W bulbs placed around the open field. Immediately following the second injection (12 mg/kg ketamine or saline), rats were placed in the centre of the open field (trial 1; $t=0$) for a period of 5 min and this test was repeated 20 ($t=20$), 40 ($t=40$), 60 ($t=60$) and 90 ($t=90$) min after the second injection. The test was performed in the light period between 0800 and 1200 h. Locomotor behaviour was recorded with a video tracking system (Etho Vision 3.0. Noldus Information

Technology, Wageningen, The Netherlands). Rearing behaviour was manually recorded. Locomotor and exploratory behaviour were expressed as total distance moved and rearing duration respectively.

2.4. Prepulse inhibition (PPI)

One week after the OF tests, the PPI test was performed using a TSE Startle Response Measuring System. The rats were locked in a small cage (270 × 100 × 125 mm) restricting major movements and exploratory behaviour and were placed on a transducer platform. Acoustic stimuli were generated by means of high-quality high-linearity speakers situated on both sides of the cage. The complete set-up was operated in a sound-attenuating isolation chamber equipped with a ventilation fan and house light.

We tested the effect of different doses of LY379268 (1 and 3 mg/kg) alone or in combination with 12 mg/kg ketamine. Since this dose of ketamine was two-fold higher than a dose necessary to induce PPI deficits (Imre et al., 2006), we also examined the effect of 1 mg/kg LY379268 on PPI deficits evoked by low doses of ketamine (4 and 8 mg/kg). Immediately after the second (ketamine or saline) injection, the rats were placed in the startle box for a 5 min acclimatisation period with a 70 dB background noise. This noise continued throughout the session.

The sessions consisted of four types of trials and were given 8 times in a random order: (1) prepulse (PP) alone: 85 dB sound for 20 ms; (2) startle pulse (SP) alone: 120 dB sound for 40 ms; (3) prepulse+startle pulse (PP+SP): 85 dB prepulse for 20 ms followed 100 ms later by 120 dB sound; (4) nothing: 70 dB background noise. One session consisted of 35 trials and lasted ~9 min, with 3 consecutive SP alone trials in the beginning and 32 subsequent trials (each of them 8 times) presented in a random order, with 15-s average (range 10–20 s) intertrial interval.

PPI was defined as the percentage reduction in mean startle response magnitude (SRM) in the presence of the prepulse compared to mean SRM in the absence of the prepulse: $100 - [100 \times (\text{mean SRM PP+SP trials} / \text{mean SRM SP alone trials})]$. The first three SP alone trials, which were presented at the beginning of the test session, were not included in the calculation of the PPI values. Since these first pulses are necessary to achieve a relatively stable level of startle reactivity for the remainder of the session (based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus, Geyer et al., 1990).

2.5. Biochemistry

One week after the PPI tests, 24 non-naïve rats were terminated to assess neurotransmitter level using HPLC analysis. In addition, an extra group of drug-naïve rats ($n=6$, same age and weight as non-naïve rats) were included in order to examine the possible carry-over effects of the drug treatments. These “blank” controls did not receive any injection while the non-naïve rats underwent the same injection procedure as for the behavioural tests. Thirty min after the last injection (12 mg/kg ketamine

or saline), rats were anaesthetised with 5% isoflurane and decapitated; brains were quickly removed and frozen in -80°C and trunk blood was collected for corticosterone radio immunoassay (RIA).

Serial 300 μm coronal sections were made with a cryostat microtome (-15°C) and kept frozen on dry ice. Tissue samples were dissected from the dentate gyrus (DG: bregma -2.45 to -3.70 ; Swanson, 1992) by using a needle punch technique (16GA11/2; 1.6×40 , inner diameter: 1 mm, BD Microlance). Four punches per animal were taken (one punch $\sim 0.23 \text{ mm}^3$) and diluted in 100 μl 0.1 M HClO_4 . Brain tissue was pulverised and then centrifuged (13,500 rpm for 10 min), the supernatant was analysed by HPLC with electrochemical and fluorescence detection.

Analysis of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), hydroxyindoleacetic acid (HIAA), serotonin (5-HT) and noradrenaline (NE) was performed by a Shimadzu LC-10 AD high performance liquid chromatograph equipped with a 15-cm reversed phase column (supelcosil 3 μm , C18, $150 \times 4.60 \text{ mm}$, Bester, Amstelveen, The Netherlands) and an electrochemical detector (ESA, Chelmsford, MA, USA) at a potential setting of 300 mV. The mobile phase consisted of 10% MeOH, 4.2 g Na Acetate/l, 150 mg OSA (octane sulphonic acid)/l adjusted to pH 4.10. The injection volume was 20 μl and the flow rate 1 ml/min.

Analysis of Glutamate was performed after derivatisation with OPA (*ortho*-phthaldehyde) by a Shimadzu LC-10 AD high performance liquid chromatograph equipped with a 15-cm reversed phase column (supelcosil 3 μm , C18, $150 \times 4.60 \text{ mm}$, Bester, Amstelveen, The Netherlands) and a fluorescence detector (Waters 470, fluorescence detection, Waters, Milford, Massachusetts, USA) with λ_{ex} set at 350 nm and λ_{em} set at 450 nm. The mobile phase consisted of 26% MeOH, 10 g/l disodiumphosphate (Na_2HPO_4), 150 mg/l EDTA, 2.19 ml/l THF (tetrahydrofuran) adjusted to pH 5.27. The injection volume was 20 μl and the flow rate 1 ml/min.

Fig. 1. Effect of LY379268 per se (dose range 1–3 mg/kg) on locomotion represented as total distance moved. All doses of LY379268 decreased locomotion compared to the control in all five trials but this effect was significant at the dose of 2 and 3 mg/kg LY379268. A–C: characteristic walking pattern of control (A), 1 mg/kg LY379268 (B) and 2–3 mg/kg LY379268 (C) treated rats in the open field arena during the first trial ($t=0$). Points represent the mean $\pm$ S.E.M. for $n=6$ rats/trial. The * indicates $P < 0.05$ at dose of 2 and 3 mg/kg LY379268 versus control.

Fig. 2. Effect of LY379268 (1–3 mg/kg) on ketamine-evoked hyperlocomotion (12 mg/kg) represented as total distance moved. A–C: characteristic walking pattern in the open field arena during the first trial ($t=0$). Consistent with a short-lasting effect, ketamine significantly increased locomotion (A) in the first two trials. Pretreatment with 1 (B) and 3 mg/kg (C) of LY379268 was able to prevent this effect of ketamine. Points represent the mean $\pm$ S.E.M. for $n=6$ rats/trial. The * indicates $P<0.001$ for ketamine versus control.

2.6. Corticosterone radio immuno-assay

The corticosterone fraction was extracted from the plasma (10 μ l) with Chromosorb (Alltech) and 30% dichloromethane (Rathburn 1001). Before the extraction a trace amount of ^3H labeled (200 Bq) corticosterone (TRK-406 Amersham) was added to the samples to determine recovery which was 20%. A standard curve was determined by using non-labeled corticosterone, which was treated in the same way as the extracted plasma samples.

For the quantification, samples were mixed with ^3H (500 Bq) labeled corticosterone and polyclonal antibody raised against corticosterone (rabbit nr 568 UMCG). The tubes were incubated at 60 $^{\circ}\text{C}$ for 30 min and the equilibrium reaction was established in a water/ice bath for 1 h. The reaction was stopped by adding

Fig. 3. Effect of pretreatment with LY379268 (1 and 3 mg/kg) on exploratory behaviour represented as time spent rearing. Ketamine caused a significant decrease in rearing duration in the first three trials ($t=0$ and $t=20$, $P<0.001$; $t=40$, $P<0.05$). LY379268 (1 mg/kg) was not able to prevent this ketamine effect. When administered alone, LY379268 dose-dependently decreased rearing behaviour. Data expressed as mean $\pm$ S.E.M. for $n=6$ rats/trial.

Fig. 4. Effect of pretreatment with LY379268 (1 and 3 mg/kg) on prepulse inhibition (PPI) and startle reflex magnitude (SRM). LY379268 on its own did not alter PPI. Administration of 12 mg/kg ketamine significantly disrupted PPI, resulting in prepulse facilitation (PPF). Pretreatment with LY379268 did not prevent this ketamine effect but did tend to exacerbate it dose-dependently. Treatment with ketamine (12 mg/kg) caused a slight tendency towards a decrease in SRM. Treatment with 1 mg/kg LY379268 has no effect on its own while 3 mg/kg LY379268 per se significantly increased SRM (Inset). Interestingly, the combination of the two drugs did not alter SRM. Data expressed as mean $\pm$ S.E.M. for $n=6$ rats. The * indicates $P<0.05$ vs control.

charcoal suspension and incubated for 15 min in the water/ice bath. The tubes were centrifuged for 15 min at 3000 g and 4 $^{\circ}\text{C}$ to spin down the charcoal bound with free corticosterone. The supernatant was poured into 1 ml of scintillation fluid (Ultimagold XR, Canberra Packard) and samples were counted in β -counter for 4 min or 4000 preset counts. The amount of corticosterone was calculated from the standard curve.

2.7. Statistical analysis

Data are presented as mean $\pm$ SEM. Statistical analyses were done with SPSS (version 12.0), and $P<0.05$ was considered significant. Locomotor and exploratory behaviour were analysed by repeated measures ANOVA, with time (5 trials) as a

Fig. 5. Effect of pretreatment with LY379268 (1 mg/kg) on PPI deficits evoked by different doses of ketamine. Irrespective of the dosage of ketamine, 1 mg/kg LY379268 could not prevent the disruptive effect of ketamine on PPI. Data expressed as mean $\pm$ S.E.M. for $n=6$ rats. The * indicates $P<0.05$ vs control.

Table 1
Effect of LY379268 (1 mg/kg) and ketamine (12 mg/kg) on plasma corticosterone level

	Control		Ketamine (12 mg/ kg)	LY379268 (1 mg/kg)	LY379268+ ketamine
	(Drug-naïve)	(Saline)			
Corticosterone (µg/100 ml)	1.3±0.3	4.2±1.2	40.7±2.1*	3.0±0.6	42.9±2.1*

Data are presented as mean±S.E.M.

* $P<0.001$ vs non-naïve control.

within subject factor and pretreatment and treatment as between subject factors. Sphericity assumed modelling, with Huynh–Feldt adjustment, was applied. The PPI test and the HPLC results were analysed with two-way ANOVA with pretreatment and treatment as a between subject factor. A LSD post hoc test was used for pair-wise comparisons.

3. Results

3.1. Open field test

As repeated measures ANOVA revealed, treatment with LY379268 per se had a main effect on locomotor activity ($F_{3,35}=39.040$, $P<0.001$) and rearing ($F_{3,35}=24.711$, $P<0.001$). At the doses of 2 and 3 mg/kg LY379268, animals displayed freezing behaviour resulting in decreased locomotion ($t=0$, 20, 40 and 60, $P<0.001$ and $t=90$, $P<0.05$; Fig. 1) and maximal reduction in time spent rearing in each trial ($P<0.001$; Fig. 3). Subjectively, some side effects were noted: rats urinated frequently and appeared to be hypersensitive to tactile stimuli. Animals treated with 1 mg/kg LY379268 revealed no gross abnormalities, although they appeared to be less active, as shown by the non-significant decrease in total distance moved (Fig. 1) and significantly decreased rearing behaviour ($P<0.05$; Fig. 3).

As expected, within two minutes after its administration, ketamine induced hyperlocomotion ($F_{1,35}=21.840$, $P<0.001$; Fig. 2) which remained significantly elevated in the first two trials ($t=0$, $P<0.001$ and $t=20$, $P=0.020$). In consequence of the ketamine-evoked hyperactivity, rats did not stand on their

hind legs leading to reduction in the rearing behaviour during the first three trials ($t=0$ and $t=20$, $P<0.001$; $t=40$, $P<0.05$; Fig. 3).

There was a main effect of time ($P<0.001$), time×treatment interaction ($P<0.001$) and time×pretreatment×treatment interaction ($P<0.001$). Although test of between subject effects showed no pretreatment×treatment interaction ($F_{2,35}=2.389$, $P=0.109$), administration of 1 and 3 mg/kg of LY379268 suppressed the ketamine-evoked hyperlocomotion (Fig. 2). It should be noted that animals treated with 3 mg/kg of LY379268 together with ketamine still displayed the above-mentioned side effects evoked by LY379268. The dose of 1 mg/kg LY379268 significantly reduced the ketamine-evoked hyperlocomotion ($t=0$ and $t=20$, $P<0.05$; Fig. 2) without any side effects. None of the doses of LY379268 could restore the ketamine effect on rearing behaviour (Fig. 3).

3.2. Prepulse inhibition

Univariate ANOVA showed a significant ketamine main effect on PPI ($F_{2,28}=3.935$, $P<0.05$). In fact, 12 mg/kg ketamine caused maximal reduction in prepulse inhibition resulting in manifestation of prepulse facilitation (PPF; the startle amplitude in PP+SP trials was larger than in SP alone trials) as the negative PPI value represented (Fig. 4). Pretreatment with LY379268 (1 and 3 mg/kg) was not able to restore this ketamine-evoked PPI deficit. Although there was no pretreatment×treatment interaction ($F_{2,28}=1.196$, $P=0.319$), the compound appeared to exacerbate dose-dependently this symptom of ketamine. None of the doses of LY379268 per se altered PPI compared to the control group ($F_{2,28}=2.2821$, $P=0.077$). However, administration of the highest dose (3 mg/kg) alone increased the startle reflex magnitude fivefold ($F_{2,28}=11.633$, $P<0.001$). Interestingly, this effect of 3 mg/kg LY379268 was reduced by ketamine (pretreatment×treatment interaction, $F_{2,28}=9.735$, $P<0.001$; Fig. 4).

Our preliminary dose-response studies demonstrated that ketamine is able to induce PPI deficits at the dose of 4 and 8 mg/kg (Imre et al., 2006). Pretreatment with 1 mg/kg LY379268 could not prevent the PPI deficit evoked by the lower doses of ketamine (Fig. 5).

Table 2
Ex vivo brain tissue concentration of neurotransmitters in the dentate gyrus: DA, DOPAC, 5-HT, HIAA, noradrenaline (ng/sample±S.E.M.) and glutamate (µg/sample±S.E.M.), DOPAC/DA ratio HIAA/5-HT ratio (mean±S.E.M.)

	Control		Ketamine (12 mg/kg)	LY379268 (1 mg/kg)	LY379268+ ketamine
	(Drug-naïve)	(Saline)			
Glutamate	603.1±53	585.6±58	365.8±71*	551.2±81	490.4±53
Dopamine	11.7±4	9.9±3	3.7±0.4*	5.4±0.9	4.2±0.6*
DOPAC	28.8±6	22.9±3	12.3±0.9	12.4±1	22.8±6
DOPAC/DA	2.59±1.01	3.73±1.30	3.37±0.24	2.55±0.57	4.11±1.04
5-HT	17.9±5	26.7±4	14.8±3	30.6±5	23.1±6
HIAA	40.2±10	51.3±8	20.6±4*	44.9±10	32.7±9
HIAA/5-HT	1.74±0.25	1.95±0.1	1.34±0.08*	1.54±0.2	1.42±0.11*
Noradrenaline	109.1±19	121.6±5	96.6±14	130.1±16	175.6±46

* $P<0.05$ vs non-naïve control.

3.3. Plasma corticosterone level

As Table 1 illustrates, there was no difference in plasma corticosterone level between drug-naïve and non-naïve control groups. Ketamine significantly increased the plasma corticosterone level (tenfold higher compared to control; $F_{1, 20} = 555.989$, $P < 0.001$), which was unaffected by LY379268 (pretreatment $\times$ treatment interaction, $F_{1, 20} = 1.104$, $P = 0.306$). Pretreatment with 1 mg/kg LY379268 had no effect on baseline corticosterone level ($F_{2, 28} = 0.79$, $P = 0.781$).

3.4. Neurochemical results

Table 2 provides an overview of the data obtained from HPLC analysis of dentate gyrus (DG). Comparison of drug-naïve animals (blank control) and non-naïve animals (saline-treated control) showed no differences in any neurotransmitter levels. Thus, for indication of drug treatment induced changes in the tissue content of neurotransmitters, saline-treated animals were used as control group.

Ketamine significantly decreased tissue glutamate levels ($F_{1, 20} = 5.535$, $P < 0.032$), which was restored by LY379268 (pretreatment $\times$ treatment interaction, $F_{1, 20} = 4.282$, $P < 0.05$). LY379268 alone had no effect on glutamate ($F_{1, 20} = 0.552$, $P < 0.446$). A significant reduction of dopamine concentration was observed following treatment with ketamine ($F_{1, 17} = 5.474$, $P < 0.035$). LY379268 could not prevent this ketamine effect (pretreatment $\times$ treatment interaction, $F_{1, 17} = 2.453$, $P < 0.140$) and it had no effect on dopamine on its own ($F_{1, 17} = 1.684$, $P < 0.215$).

There was a ketamine main effect on HIAA level ($F_{1, 17} = 7.572$, $P < 0.014$) and HIAA/5-HT turnover ($F_{1, 17} = 10.124$, $P < 0.005$). Pretreatment with LY379268 could not prevent these ketamine effects and it had no effect on its own.

No changes were found in the tissue levels of noradrenaline, 5-HT and DOPAC, as well as in DOPAC/dopamine ratio. However, it should be noted that 3 samples of LY379268+ketamine treated group were excluded from the DOPAC analysis due to a technical failure.

4. Discussion

The reversal effects of LY379268 on NMDA antagonist-related motor impairments have been well characterized (Cartmell et al., 1999a,b; Lorrain et al., 2003b) and our findings replicated these previous reports, as LY379268 (1–3 mg/kg) suppressed ketamine-evoked hyperlocomotion. However, administration of higher doses produced anxiogenic-like side effects. Animals treated with 2 and 3 mg/kg of LY379268, alone or in combination with ketamine, exhibited freezing behaviour that was accompanied by frequent defecation and urination. Furthermore, at the dose 3 mg/kg LY379268 a ~ 5 fold increase in the startle reflex magnitude was observed (Fig. 4), which is also considered to be a sign of anxiety (Koch, 1999). Similar anxiogenic-like effects have usually been observed at higher doses of LY379268 (10–100 mg/kg), resulting in ataxia to which tolerance developed following repeated administration, whereas the ability to block PCP-induced hyperlocomotion was retained (Cartmell et al., 2000a). Cartmell et al. also

reported that although 3 mg/kg LY379268 reduced basal levels of locomotion, it did not produce motor impairment as measured on the rotarod apparatus, but it blocked PCP-related motor behaviours. Similarly, in the present study 1 mg/kg of LY379268 suppressed ketamine-induced hyperlocomotion without producing side effects. When it was applied alone, animals were grossly normal, showing no changes in startle reflex and plasma corticosterone level, but showed reduced exploratory behaviours, as the decrease in locomotion and rearing behaviour indicated (Figs. 1 and 3). These results might be interpreted as a reduced response to novelty stress (i.e. open field) due to an anxiolytic-like action of LY379268 at a low dose. Certainly there is evidence to suggest that group II receptor activation might be anxiolytic. Wide range of preclinical (Helton et al., 1998; Klodzinska et al., 1999; Linden et al., 2004; Shekhar and Keim, 2000) and recent clinical findings (Grillon et al., 2003; Schoepp et al., 2003) have reported that LY354740, a close structural analogue of LY379268, could suppress anxiety-related behaviour, while LY379268 has been shown to prevent immobilization-stress evoked hyperlactinemia (Johnson and Chamberlain, 2002). However, further behavioural studies would be necessary to confirm possible anxiolytic properties of the LY379268 at a low dose range.

To date, all available studies have characterized the antipsychotic effects of LY379268 on NMDA antagonist evoked motor behaviours rather than PPI deficits. The loss of PPI, however, provides an operational measure for studying sensorimotor gating deficits across species and for predicting antipsychotic activity in novel compounds (for review see: Geyer et al., 2001). Only two studies have examined the role of mGluR2/3 in PPI deficits using the less potent agonist LY354740 which could not prevent PCP-induced PPI disruption (Ossowska et al., 2000; Schreiber et al., 2000). Moreover, our previous results from this laboratory have also shown that subchronic treatment with LY354740 was ineffective in restoring ketamine-evoked PPI deficits (Imre et al., in press). Consistent with these findings, we found here that LY379268 failed to oppose the PPI deficits evoked by ketamine at a dose range of 4–12 mg/kg. Taken together, these data indicate that the underlying mechanism for disruption of PPI by NMDA antagonists cannot be prevented by activation of mGluR2/3 receptors.

Recent in vivo microdialysis and ex vivo tissue destruction studies have indicated that LY379268 shares common biochemical effects with atypical antipsychotics, which are generally active in the NMDA hypofunction model. Similar to clozapine and risperidone, LY379268 increased the tissue turnover of DA and 5-HT in the PFC and in the striatum (Cartmell et al., 2000c). In contrast, in the present study, LY379268 (1 mg/kg) had no effect on DA turnover or 5-HT turnover in the dentate gyrus. It is likely that methodological differences, including dose ranges, time course and tissue dissection technique, underlie this apparent discrepancy.

Nevertheless, we demonstrated here that ketamine reduced the tissue level of glutamate, dopamine and HIAA, as well as 5-HT turnover. Pretreatment with LY379268 could only prevent the ketamine effect on glutamate without altering the basal glutamate concentration (Table 2). This is in line with in vivo microdialysis studies which have demonstrated that activation of group II mGlu receptors by LY379268 or LY354740 has no effect on basal

glutamate levels in the PFC or nucleus accumbens, even though these drugs can suppress NMDA antagonist-evoked glutamate (but not dopamine) release (Lorrain et al., 2003a; Moghaddam and Adams, 1998). Moghaddam and Adams (1998) also demonstrated that the reduction in PCP-induced glutamate efflux by LY354740 was accompanied with blockade of PCP-evoked hyperlocomotion and stereotypical behaviour. Accordingly, the present data showed that LY379268 prevents the effects of ketamine on locomotion and glutamate transmission in the DG, but not on PPI and monoamine levels. Therefore, it is conceivable that blockade of ketamine and other NMDA antagonist-induced changes in glutamate neurotransmission can reverse hyperlocomotion, but that it is neither sufficient nor capable in restoring PPI deficits. This latter symptom may rather be linked to changes in monoamine transmission. Previous reports do support such a relationship, as multireceptor-antagonist antipsychotics (mixed 5-HT₂/D₂ antagonists) like clozapine, chlorpromazine, and ziprasidone appeared to be effective in reversing NMDA antagonist-induced PPI deficits (Mansbach et al., 2001; Swerdlow et al., 1998). In particular, antagonist action on 5-HT₂ receptors contributes to the efficiency of these drugs, since the highly selective 5-HT_{2A} receptor antagonist M100907, but not the dopamine antagonist haloperidol, is effective in this model (Keith et al., 1991; Mansbach et al., 2001; Varty et al., 1999).

In summary, the potent and selective mGluR2/3 agonist LY379268 is partly effective in the NMDA hypofunction model of psychosis since it could block the hyperlocomotion but not the PPI deficits induced by ketamine. We also demonstrated that ketamine reduced glutamate, dopamine and 5-HT turnover in the DG and that LY379268 could only prevent the ketamine effect on glutamate transmission. Thus, it can be concluded that blockade of glutamate transmission by LY379268 is not sufficient to reverse PPI deficits evoked by ketamine. In future studies it will be important to examine the effect of LY379268 on cognitive symptoms in this model.

A final, not completely marginal, observation of this study was that in a narrow dose range, LY379268 showed a dual action in the anxious states, as 3 mg/kg evoked freezing behaviour and an increased startle reflex, while 1 mg/kg decreased exploratory behaviours interpreted as anxiolytic effects. Since its analogue LY354740 was found to be effective in several animal models of anxiety and in clinical studies, it is conceivable that LY379268 might have similar properties. Additional work should address the possible role of mGluR2/3 receptors and LY379268 in anxiety.

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