Subchronic administration of LY354740 does not modify ketamine-evoked behaviour and neuronal activity in rats

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ABSTRACT

Acute treatment with LY354740 \{1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate\}, a potent and selective agonist for group II metabotropic glutamate receptors (mGlu2/3), has previously been shown to block some schizophrenia-like effects of N-methyl-D-aspartate (NMDA) receptor antagonists, suggesting a novel therapeutic strategy for schizophrenia. The present study examined the effects of subchronic pretreatment with LY354740 (0.3, 3 and 10 mg/kg i.p.) on ketamine-evoked (12 mg/kg s.c.) prepulse inhibition deficits, hyperlocomotion and c-fos expression. At all doses, LY354740 failed to reverse both behavioural and neuronal effects of the ketamine. These results therefore do not support the putative antipsychotic role of LY354740.
**INTRODUCTION**

LY354740 is a potent and selective agonist at the metabotropic glutamate 2/3 receptors (mGlu2/3) without significant affinity for the other mGlu receptors or ionotropic glutamate receptors (Schoepp and Marek, 2002). Numerous electrophysiological and microdialysis studies have demonstrated that LY354740 is able to suppress glutamatergic transmission in limbic synapses (Anwyl, 1999). Behaviourally, it exhibits potent anxiolytic-like effects without sedative effects or motor impairments (Helton et al., 1998). Furthermore, systemic LY354740 has been shown to be active in the N-methyl-D-aspartate (NMDA) receptor hypofunction model of schizophrenia. Exposure to non-competitive NMDA receptor antagonists like phencyclidine (PCP), or its analogue ketamine, produces behavioural syndromes in healthy individuals that resemble both positive and negative symptoms of schizophrenia (Krystal et al., 1994). In rodents, these symptoms manifest as impaired prepulse inhibition, cognitive deficits and augmented locomotion (Jentsch and Roth, 1999). The behavioural effects of these drugs have been attributed primarily to the blockade of NMDA receptors located on γ-aminobutyric acid (GABA) interneurons, which in turn leads to disinhibition of neuronal activity in limbic structures (Moghaddam et al., 1997). Accordingly, microdialysis studies in conscious rats have indicated PCP- and ketamine-evoked increases in glutamate and dopamine outflow in the prefrontal cortex and limbic striatal regions (Lorrain et al., 2003a; Moghaddam et al., 1997).

Moghaddam and Adams (1998) showed that LY354740 attenuated the disruptive effect of PCP on working memory, stereotypy, locomotion and cortical glutamate efflux. Several lines of evidence extended this study showing that both LY354740, and the more potent mGlu2/3 receptor agonist LY379268, effectively block PCP- and ketamine-evoked motor impairments (Cartmell et al., 1999a; Lorrain et al., 2003b; Spooren et al., 2000a). These data indicate that
the activation of mGlu2/3 receptors may represent a novel non-dopaminergic therapeutic strategy for certain schizophrenia-related symptoms (Schoepp and Marek, 2002). Recent studies have, however, cast doubt on this hypothesis, as LY354740 failed to restore NMDA receptor antagonist-induced deficits in prepulse inhibition and cognitive function (Henry et al., 2002; Ossowska et al., 2000; Schreiber et al., 2000).

The aim of this study was to assess the proposed antipsychotic properties of LY354740. Since acute treatment with LY354740 gave ambiguous results in the NMDA receptor hypofunction model of schizophrenia, we used a subchronic treatment regime (8 days), with a dose range of 0.3-10 mg/kg (i.p.). Unlike PCP, ketamine is available for use in human giving the possibility to replicate animal studies in human. To this end, we investigated the effects of LY354740 on ketamine-evoked prepulse inhibition deficits and hyperlocomotion. In order to establish the putative cerebral site of action of LY354740, c-fos expression was analyzed in brain regions where we previously found ketamine to alter c-fos pattern.

**MATERIALS AND METHODS**

**ANIMALS**

Forty-six male Wistar rats (n=6/group) weighing 248±8 g at the start of the experiments were individually housed with a 12/12 h light/dark cycle (lights on at 7:00 a.m.). Food and water were available ad libitum. All animals were handled and weighed daily to minimise stress during the experiment. The Animal Ethics Committee of the University of Groningen approved the protocols (FDC: 2935).

**DRUGS**

Ketamine (Sigma, Germany) and LY354740 {1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate}(Eli Lilly and Company, Indianapolis) were dissolved in saline. Animals were injected with either saline or one of the three different doses of LY354740 (0.3, 3 and 10 mg/kg) intraperitoneally (i.p.) for eight consecutive days. On the experimental days, 30 min after the daily injection, either saline or 12 mg/kg ketamine were applied subcutaneously (s.c.). The behavioural testing and the immunohistochemistry procedures were the same as those used in previous experiments.
BEHAVIOURAL TESTS

Prepulse inhibition: studies were performed on the 6th day of the treatment using a TSE Startle Response Measuring System (Bad Homburg, Germany). Following subcutaneous injection, the rats were placed in the startle box for a 5-minute acclimatization period with a 70 dB background noise. This noise continued throughout the session. The test session consisted of four trial types: (1) rats were exposed to 120 dB, 40-ms startle pulse; (2) a 85 dB 20-ms prepulse preceded the startle pulse by 100 ms; (3) prepulse alone; (4) no-stimulus. One session consisted of 35 trials with 3 consecutive startle pulse alone trials in the beginning and 32 subsequent trials (each of them 8 times) presented in a random order, with range 10-20 s intertrial interval. Percentage prepulse inhibition was calculated as follows: 100-[100×(mean amplitude on prepulse trials/mean amplitude on startle pulse alone trials)].

Open field: the rats were tested in an open field (circular black arena with a diameter of 1 m) on the 7th day of the treatment. Rats were placed in the centre of the field at certain time points i.e. at 0, 20 and 40 min post subcutaneous injection and observed for a period of 8 min. Behaviour was recorded with a videotracking system (Etho Vision, 3.0, Wageningen, The Netherlands). The distance moved within the arena was analysed.

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On the 8th day of the treatment the rats were terminated two hours after the subcutaneous injection, with an overdose of isoflurane preceding a transcardial perfusion with 4% paraformaldehyde solution dissolved in 1.0 M sodium phosphate buffer (pH 7.4). C-fos staining was performed on free-floating sections (40 µm) under continuous agitation. C-fos positive cells in the area of interest were blindly quantified in a single focus plane, using a computerised image analysis system (Lecia Qwin version 2.3). The average of immunoreactive cells was expressed as number of positive nuclei/0.1 mm².

STATISTICAL ANALYSIS

Statistical analyses were done with SPSS (version 12.0), and P<0.05 was considered significant. Locomotor activity was analyzed with repeated measures analysis of variance (ANOVA), with time (3 trials) being specified as within subject factor, pretreatment and treatment as between subject factor. Sphericity assumed modelling, with Huynh-Feldt adjustment, was applied. C-fos and prepulse inhibition were analyzed with two-way ANOVA with pretreatment treatment as between subject factors. An LSD post hoc test was used for the pairwise comparisons.

RESULTS

PREPULSE INHIBITION

Ketamine reduced prepulse inhibition (decrease of ~70%, Fig. 1A) in the saline pretreated group (F (1, 39) =39.086, P<0.001) and the startle reflex magnitude (F (1, 39) =13.089, P<0.001). Subchronic pretreatment with different dosages of LY354740 failed to restore this ketamine-evoked prepulse inhibition deficits (pretreatment×treatment interaction; F (3, 39) =1.601, P<0.205) and reduction in startle magnitude (F (3, 39) = 0.298, P<0.827). LY354740 on its own
had no main effect neither on prepulse inhibition (F (3, 39) = 0.560, P<0.644) nor on startle magnitude (F (3, 39) = 0.673, P<0.574).

Figure 1. Effect of pretreatment with mGlu2/3 receptor agonist LY354740 (0.3-10 mg/kg i.p.) in the prepulse inhibition paradigm (A) and the open field test (B). LY354740 did not restore the ketamine-evoked reduction in prepulse inhibition and startle magnitude. Similarly, LY354740 could not prevent the ketamine-induced hyperlocomotion represented as total distance moved (cm). Pretreatment with LY354740 alone had no effect on prepulse inhibition and baseline locomotor activity. The * indicates P<0.05 treatment effect versus control.
**Open Field**

Overall ANOVA revealed a main effect of treatment (F (1, 39) = 77.921, P<0.001) and treatment×time interaction (F (2, 32) = 32.874, P<0.001). All groups receiving ketamine showed hyperlocomotion (increase of ~ 100% in the baseline activity) in the first (t=0, P<0.001) and second trial (t=20, P<0.05), which returned to control level 40 min after the injection (Fig. 1B). There was no main effect of pretreatment (F (3, 39) = 1.707, P<0.181) and pretreatment×treatment interaction (F (3, 39) = 1.118, P<0.353). LY354740 had no effect on both baseline and ketamine-evoked locomotion.

**Immunohistochemistry**

Table 1 provides an overview of the data obtained from c-fos analysis of various regions. In general, pretreatment with LY354740 alone did not alter the c-fos expression compared to control. In the dentate gyrus and the CA1 subregion of the hippocampus however, the numbers of c-fos positive cells were significantly lower in the LY354740- treated groups (dentate gyrus; F (3, 37) = 2.882, P<0.049 and CA1; F (3, 37) = 3.800, P<0.018).

With the exception the caudoputamen and the dorsomedial nucleus of the hypothalamus, two-way ANOVA revealed main effect of ketamine on the c-fos expression: in the cortical areas, the amygdala, the nucleus accumbens, CA1-CA3 subregions of hippocampus, ventral tegmental area and the paraventricular nucleus of the hypothalamus significantly higher number of c-fos labeled cells were found, whereas in the dentate gyrus ketamine decreased the c-fos expression (F (1, 37) = 22.640, P<0.001) compared to control.

Subchronic administration of LY354740 could not prevent the ketamine-induced c-fos expression. Although there was no significant pretreatment×treatment interaction, in the CA1-CA3 subregion of hippocampus as well as in the nucleus accumbens core the ketamine-induced c-fos expression was no longer significant at the dose of 3 and 10 mg/kg LY354740.
Chapter 3

Similarly, under the conditions of the present experiment, LY354740 pretreatment with LY354740 prevents the prepulse inhibition-disruptive effects of ketamine. Insofar as we did not observe that subchronic treatment. Mean number of c-fos positive cell per 0.1 mm² ± SEM.

\(^{a}\)P<0.05; \(^{b}\)P<0.01; \(^{c}\)P<0.001; significant changes in c-fos expression compared to control (s+s). Two-way ANOVA followed by LSD post hoc test for pairwise comparison.

### Table 1. Regional c-fos expression following LY354740 pretreatment and ketamine/saline treatment.

<table>
<thead>
<tr>
<th>Treatment (s.c.)</th>
<th>Saline (0.3mg/kg)</th>
<th>3mg/kg</th>
<th>10mg/kg</th>
<th>Ketamine (12mg/kg)</th>
<th>Saline (0.3mg/kg)</th>
<th>3mg/kg</th>
<th>10mg/kg</th>
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<tr>
<td>Cingulate cortex</td>
<td>11±2</td>
<td>22±6</td>
<td>7±2</td>
<td>12±2</td>
<td>52±5</td>
<td>48±4</td>
<td>38±4</td>
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<tr>
<td>Infalimbic cortex</td>
<td>24±3</td>
<td>32±3</td>
<td>24±3</td>
<td>26±3</td>
<td>54±7</td>
<td>48±4</td>
<td>45±4</td>
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<td>Prelimbic cortex</td>
<td>19±2</td>
<td>23±4</td>
<td>17±3</td>
<td>16±3</td>
<td>44±5</td>
<td>49±4</td>
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<td>Retrosplenial cortex</td>
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<td>7±1</td>
<td>12±2</td>
<td>72±5</td>
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<td>Entorhinal cortex</td>
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<td>5±1</td>
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<td>CA1</td>
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<td>43±7a</td>
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### Discussion

The primary interest of this study was to evaluate the proposed antipsychotic action of the mGlu2/3 receptor agonist LY354740 in terms of the ketamine model of schizophrenia. Our study is in agreement with previous reports (Ossowska et al., 2000; Schreiber et al., 2000) insofar as we did not observe that subchronic pretreatment with LY354740 prevents the prepulse inhibition-disruptive effects of ketamine. Similarly, under the conditions of the present experiment, LY354740...
also failed to restore ketamine-evoked hyperlocomotion and c-fos induction in many brain regions investigated. The overall increase in c-fos expression (or decrease, in the case of dentate gyrus) shown in this study and previous microdialysis studies indicate that systemic ketamine can affect multiple brain sites involving several neurotransmitter systems. The resulting disturbances eventually culminate in the marked behavioural effects of ketamine. For instance, ketamine and other NMDA receptor antagonists selectively activate dopamine release in the nucleus accumbens and prefrontal cortex of rats which is associated with hyperlocomotion and cognitive deficits (Jentsch and Roth, 1999). These effects could be prevented by administration of AMPA/Kainate receptor antagonists systemically or locally into the ventral tegmental area, primarily source of dopaminergic afferents to the prefrontal cortex and nucleus accumbens (Moghaddam et al., 1997; Mathe et al., 1998). Thus, dopamine release is thought to be secondary to increased glutamatergic neurotransmission at non-NMDA receptors. This concept is not, however, supported by a recent studies (Moghaddam and Adams 1998; Lorrain et al., 2003a) in which systemic administration of a mGlu2/3 receptor agonists was shown to prevent the NMDA receptor antagonists-induced augmentation in cortical glutamate release without blocking the increase in cortical dopamine efflux. Ketamine may also have direct agonistic effects on dopamine D₂ receptor (Kapur and Seeman, 2002). Collectively, these data indicate that ketamine and other NMDA receptor antagonists can have an impact on the dopamine system that may be independent from glutamatergic mechanism, therefore it cannot be prevented by activation of mGlu2/3 receptors.

In addition to influencing glutamate and dopamine neurotransmission, ketamine and other NMDA receptor antagonists can increase serotonin and noradrenaline release in brain regions that mediate prepulse inhibition, such as prefrontal cortex, nucleus accumbens and hippocampus (Moghaddam et al., 1997; Lindenfors et al., 1997; Lorrain et al., 2003b). Accordingly, atypical
antipsychotics having antagonist actions at multiple receptors (dopamine \(D_1, D_2, D_4\), noradrenergic \(\alpha_1\), serotonin 5-HT\(_{2A}\), 5-HT\(_{2C}\) and muscarinic \(M_1\)), such as clozapine, are effective in preventing NMDA receptor antagonists induced prepulse inhibition deficits (Geyer et al., 2001). In particular, antagonist action on 5-HT\(_{2}\) and \(\alpha_1\)-adrenergic receptors contributes to the efficiency of these drugs, since the highly selective 5-HT\(_{2A}\) receptor antagonist M100907 and \(\alpha_1\)-adrenergic receptor antagonist prazosin, but not the \(D_2\) receptor antagonist haloperidol, is effective in this model (Geyer et al., 2001). This may help to explain the ineffectiveness of the highly selective mGlu2/3 receptor agonist LY354740 in the present study: the drug may not have effectively countered all the wide-ranging neuropharmacological effects of ketamine. On the other hand, we cannot rule out the possibility that tolerance developed to the ability of LY354740 to counter ketamine-induced deficits (Galici et al., 2005).

In the present experiment, LY354740 had no measurable effect on animals’ spontaneous activity and it did not alter c-fos expression in most of the area investigated. This result is in line with the hypothesis that, under non-pathological neuropharmacological conditions, mGlu2/3 receptors play no role in modulation of neurotransmission (Scanziani et al., 1997). In the dentate gyrus and CA1, however, we observed reduced basal c-fos expression following LY354740 pretreatment. Group II mGlu receptors have been found with the highest densities in the terminal fields of the perforant path projection from the entorhinal cortex to CA1 stratum lacunosum moleculare and the mid-molecular layer of the dentate gyrus (Petralia et al., 1996; Shigemoto et al., 1997). Therefore, it is conceivable that these areas are sensitive for the pretreatment with LY354740. In fact, the anxiolytic effects of this compound have been associated with suppressed hippocampal neurotransmission (Linden et al., 2004; Tatarczynska et al., 2001). Under normal conditions, LY354740 also produces cognitive impairment by its inhibitory effect on hippocampal synaptic transmission (Higgins et al., 2004). On the other hand, LY354740 has been shown to improve
PCP-evoked working memory deficit by blocking increased glutamate release in the prefrontal cortex (Moghadam and Adams, 1998). Although our results do not support the antipsychotic properties of LY354740, in future studies it will be important to examine the possible relationship between the effects of LY354740 on hippocampus and cognitive deficits-evoked by ketamine and other NMDA receptor antagonist.