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Differentiating the roles of mGlu2 and mGlu3 receptors using LY541850, an mGlu2 agonist/mGlu3 antagonist

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ABSTRACT

Despite the potential therapeutic relevance of group II metabotropic glutamate (mGlu) receptors, there has been a lack of pharmacological tools for separating the roles of mGlu2 and mGlu3 receptor subtypes. LY541850 was claimed from human mGlu receptors expressed in non-neuronal cells to be a selective orthosteric mGlu2 agonist and mGlu3 antagonist. We have verified this pharmacological profile of LY541850 in hippocampal slices. Field excitatory post-synaptic potentials (fEPSPs) evoked by stimulation of the temporo-ammonic path (TAP) input to CA1 stratum lacunosum moleculare (SLM) were inhibited by LY541850 in mGlu3−/− mice (EC_{50} 38 nM) and wild-type littermates (EC_{50} 42 nM) to a similar extent but were not significantly affected in mGlu2−/− mice. The group II agonist, DCG-IV, inhibited the fEPSP in all three genotypes. Co-application of DCG-IV and LY541850 in mGlu3−/− and wild-type littermates resulted in an additive effect, whereas in mGlu2−/− mice, LY541850 reversed the inhibitory action of DCG-IV. These results confirm the selective mGlu2 agonist and mGlu3 antagonist actions of LY541850. A similar profile of activity was seen in medial perforant path synapse to the dentate gyrus. Systemic administration of LY541850 to wild-type mice, reduced the increase in locomotor activity following both phencyclidine and amphetamine administration. These data support the hypothesis that mGlu2 receptors mediate the antipsychotic effects of mixed group II agonists.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

Ionotropic and metabotropic glutamate (mGlu) receptors mediate and regulate excitatory transmission throughout the central nervous system (CNS). The mGlu receptors are G-protein coupled receptors distributed on post- and pre- synaptic neuronal membranes and on glia. In the CNS, group II mGlu receptors, mGlu2 and mGlu3, are expressed on all three elements of the tripartite synapse, although, they are mainly located presynaptically (Petralia et al., 1996; Shigemoto et al., 1997; Schoepp, 2001) where, as putative autoreceptors, they inhibit glutamate release (Cartmell and Schoepp, 2000). Interestingly, mGlu3 receptors have also been shown to be expressed on astrocytes (Petralia et al., 1996; Tamaru et al., 2001) where they provide a neuroprotective role (Bruno et al., 2001). However, the relative lack of selective pharmacological tools has hindered progress in assigning particular functional roles to these two receptor subtypes.

The mGlu receptors are potential targets for psychiatric and neurological diseases (Niswender and Conn, 2010). Indeed, there is a plethora of evidence from both laboratory animal preclinical and human clinical studies, suggesting a therapeutic role for group II mGlu receptors (Moussawi and Kalivas, 2010; Spooren et al., 2010; Chaki and Hikichi, 2011; Chiechio and Nicoletti, 2011). This is based largely on ligands that act non-selectively on both mGlu2 and mGlu3 receptors. For example, such orthosteric agonists have provided positive data in clinical trials of anxiety and schizophrenia, examples of successful translation from animal experiments. Thus, LY354740, a prototypical mixed mGlu2/mGlu3 agonist has been shown to exert an anxiolytic effect in rats, as tested in the elevated plus maze and fear potentiated startle assays

Abbreviations: DCG-IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine; LY354740, (1S,2S,5R,6S)-(+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY379268, (1S,2R,5R,6R)-2-amino-4-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY341495, (2S,1′S,2′S)-2-(9-xanthylmethyl)-2-(2′-carboxycyclopropylglycine; LY541850, (1S,2S,4R,5R,6S)-2-amino-4-methylbicyclo[3.1.0]hexane2,6-dicarboxylic acid.

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(Monn et al., 1997; Helton et al., 1998), and in humans (Grillon et al., 2003; Dunayevich et al., 2008). In addition, multiple mGlu2/3 receptor agonists (e.g. LY354740, LY379268 and LY404039) have been shown to reduce the phencyclidine- and amphetamine-induced locomotor activity in rats (Moghaddam and Adams, 1998; Cartmell et al., 1999; Monn et al., 2007; Rorick-Kehn et al., 2007) and an oral prodrug form of LY404039 (LY2140023·H_2O) was effective in a clinical trial of schizophrenia (Patil et al., 2007). Data from transgenic mice (Spooren et al., 2000; Fell et al., 2008; Woolley et al., 2008) and mGlu2 selective positive allosteric modulators (Galici et al., 2005; Johnson et al., 2005) point to the importance of mGlu2 receptors in mediating acute antipsychotic-like effects in rodent. However, the lack of orthosteric agonists capable of differentiating between mGlu2 and mGlu3 receptors has hindered direct pharmacological experiments aimed at determining which of the two receptors underlies these effects in rodents without the use of transgenic animals. An orthosteric agonist with selectivity for one of the group II mGlu receptor subtypes would be the ideal tool for comparison with dual orthosteric agonists, e.g. LY379268.

Mixed mGlu2 and mGlu3 receptor agonists, such as DCG-IV (Hayashi et al., 1993) and LY354740 (Monn et al., 1997; Schoepp et al., 1999) and the non-selective competitive group II antagonist, LY341495 (Kingston et al., 1998) have helped identify but not separate between the roles of mGlu2 and mGlu3. It was therefore of interest that methyl substitution at C4 position of LY354740 produced the single enantiomer, (1S,2S,4R,5R,6S)-2-amino-4-methylbicyclo[3.1.0]hexane2,6-dicarboxylic acid (LY541850), which when tested on heterologously expressed recombinant human receptors, proved to be an mGlu2 agonist and an mGlu3 antagonist with good selectivity over other mGlu receptors (Dominguez et al., 2005). For convenient comparisons, the structures and biochemical data for LY541850 and relevant group II mGlu receptor agonists are provided in Table 1. We have previously shown that LY395756, the racemic form of LY541850, can distinguish between native mGlu2 and mGlu3 receptors (Ceolin et al., 2011) but this is the first study using the single enantiomer following its initial identification.

By studying its effect on synaptic transmission in two hippocampal pathways known to express group II mGlu receptors, we have verified that LY541850, selectively activates mGlu2 receptors in mGlu3−/− mice and antagonises mGlu3 receptors in mGlu2−/− mice. Utilising this unique pharmacology, we have used systemic administration of this drug to determine the role of mGlu2 receptors in two behavioural models of psychosis in wild-type mice. LY541850 reduced the enhanced locomotor activity induced by both phencyclidine and amphetamine implying that mGlu2 receptor activation alone is sufficient to provide antipsychotic activity.

Preliminary reports of this study have been published in poster form (Hanna et al., 2011).

2. Methods

2.1. Slice preparation

Experiments were performed using hippocampal slices prepared from wild-type CD-1 mice (5–6 or 10–16 weeks old) and separate mGlu2−/− and mGlu3−/− receptor CD-1 mice (10–16 weeks old) as generated by homologous recombination (Linden et al., 2005) at Eli Lilly & Co. Ltd., UK, and were bred at Charles River, UK. Animals were housed in a regulated environment (21 ± 1 °C) with food available *ad libitum* and maintained on a 12 h light/dark cycle. All experiments were carried out in the light phase and in accordance with the Animals (Scientific Procedures) Act 1986.

Animals were killed by cervical dislocation and the brain removed rapidly and placed into ice-cold oxygenated artificial cerebrospinal fluid (aCSF; see below). Coronal slices (400 μm) were prepared using a Vibroslicer (Campden Instruments Ltd, UK) in aCSF composed of (in mM): NaCl (124), $NaHCO_3$ (26), KCl (3), NaH_2PO_4 (1.4), $MgSO_4$ (1), $CaCl_2$ (2) and D-glucose (10), and saturated with 95% O_2 and 5% CO_2. Slices were placed in a Petri dish containing bubbled aCSF where the hippocampus was isolated from the remainder of the brain. For recordings in CA1-SLM, the CA3 region and part of the dentate gyrus (DG) was removed and a cut was made across stratum oriens and stratum radiatum between CA1 and the subiculum (Empson and Heinemann, 1995). Slices were then left to recover at room temperature in aCSF for at least 1 h. Slices were then transferred to the submerged recording chamber where they were maintained at 29–30 °C and perfused at 2 ml/min with oxygenated aCSF as above.

2.2. Stimulating and recording techniques

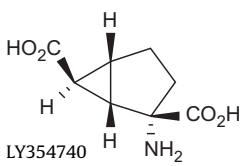
The bipolar stimulation electrodes, made from twisted insulated nickel-chromium wire (0.050 mm diameter) and insulated except for the tip, was placed in the SLM of the subicular region or in the medial and lateral perforant pathways to the dentate gyrus (DG). Single stimuli (or occasionally a pair with a 50 ms interval), comprising a square-wave pulse, 100 μs in duration and 10–25 V in amplitude, were delivered at 30 s intervals (0.033 Hz).

The glass extracellular recording electrode, filled with 3 M NaCl and with a resistance usually of 2–4 MΩ, was placed in CA1-SLM or in the mid-molecular layer of DG. Positions of the recording and stimulating electrodes were adjusted so as to give optimal field excitatory post-synaptic potentials (fEPSPs). Input-output curves were obtained, and, to obtain baselines, the stimulus strength was adjusted so as to generate fEPSPs of 70–80% of the maximal response.

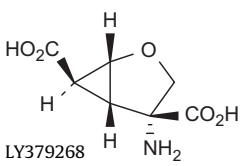
2.3. Data acquisition and analysis

The raw DC signal was band pass filtered at 0.1–3 kHz, digitized and collected for on-line analysis at a sampling rate of 20 kHz using WinLTP software (Anderson and Collingridge, 2007); (www.ltp-program.com). The fEPSPs were signal averaged every 2 min and the amplitudes were measured and plotted on-line. Data from single experiments were displayed as the signal averaged (4 sweeps), post-filtered fEPSPs. For the pooling of experiments, the fEPSP amplitude was normalised to a 30 min baseline and expressed as the mean ± SEM. Statistical significance was tested using paired Student's *t*-test, one-way or two-way ANOVA followed by

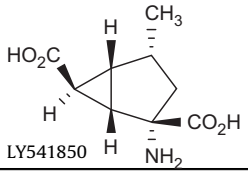
Table 1
Compound structures and activity on recombinant human receptors.



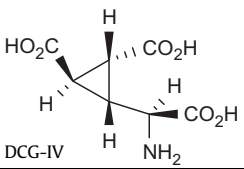
LY354740



LY379268



LY541850



DCG-IV

Compound	Displacement of ^{3}H-341495 binding to membranes expressing recombinant mGlu2 or mGlu3[a] Ki (μM)		Functional (cAMP) responses in mGlu receptor-expressing cells[b,c] EC_{50} (μM) or IC_{50} (μM)	
	mGlu2	mGlu3	mGlu2	mGlu3
LY354740[a]	0.074 ± 0.009	0.093 ± 0.003	0.010 ± 0.002	0.038 ± 0.003
LY541850[a]	0.165 ± 0.003	0.302 ± 0.048	0.161 ± 0.019	*1.05 ± 0.23*
LY379268[b]	0.014 ± 0.001	0.0058 ± 0.0006	0.00269 ± 0.00026	0.00458 ± 0.00004
DCG-IV[c]	0.34 ± 0.02	0.062 ± 0.01	0.35 ± 0.10	0.09 ± 0.03

[a] Data for LY354740/LY541850 are from Dominguez et al. (2005).
[b] Data for LY379268 are from Monn et al. (1999).
[c] Data for DCG-IV are for binding from Johnson et al. (1999) and for function from Brabet et al. (1998).