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In vitro pharmacological and rat pharmacokinetic characterization of LY3020371, a potent and selective $mGlu_{2/3}$ receptor antagonist☆

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ABSTRACT

Metabotropic glutamate $_{2/3}$ ($mGlu_{2/3}$) receptors are of considerable interest owing to their role in modulating glutamate transmission via presynaptic, postsynaptic and glial mechanisms. As part of our ongoing efforts to identify novel ligands for these receptors, we have discovered (1S,2R,3S,4S,5R,6R)-2-amino-3-[(3,4-difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; (LY3020371), a potent and selective orthosteric $mGlu_{2/3}$ receptor antagonist. In this account, we characterize the effects of LY3020371 in membranes and cells expressing human recombinant mGlu receptor subtypes as well as in native rodent and human brain tissue preparations, providing important translational information for this molecule. In membranes from cells expressing recombinant human $mGlu_2$ and $mGlu_3$ receptor subtypes, LY3020371.HCl competitively displaced binding of the $mGlu_{2/3}$ agonist ligand [^{3}H]-459477 with high affinity (hmGlu_2 K_i = 5.26 nM; hmGlu_3 Ki = 2.50 nM). In cells expressing hmGlu_2 receptors, LY3020371.HCl potently blocked $mGlu_{2/3}$ agonist (DCG-IV)-inhibited, forskolin-stimulated cAMP formation (IC_{50} = 16.2 nM), an effect that was similarly observed in hmGlu_3-expressing cells (IC_{50} = 6.21 nM). Evaluation of LY3020371 in cells expressing the other human mGlu receptor subtypes revealed high $mGlu_{2/3}$ receptor selectivity. In rat native tissue assays, LY3020371 demonstrated effective displacement of [^{3}H]-459477 from frontal cortical membranes (K_i = 33 nM), and functional antagonist activity in cortical synaptosomes measuring both the reversal of agonist-suppressed second messenger production (IC_{50} = 29 nM) and agonist-inhibited, K^+-evoked glutamate release (IC_{50} = 86 nM). Antagonism was fully recapitulated in both primary cultured cortical neurons where LY3020371 blocked agonist-suppressed spontaneous Ca^{2+} oscillations (IC_{50} = 34 nM) and in an intact hippocampal slice preparation (IC_{50} = 46 nM). Functional antagonist activity was similarly demonstrated in synaptosomes prepared from epileptic human cortical or hippocampal tissues, suggesting a translation of the $mGlu_{2/3}$ antagonist pharmacology from rat to human. Intravenous dosing of LY3020371 in rats led to cerebrospinal fluid drug levels that are expected to effectively block $mGlu_{2/3}$ receptors in vivo. Taken together, these results establish LY3020371 as an important new pharmacological tool for studying $mGlu_{2/3}$ receptors in vitro and in vivo.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DCG-IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine; (S)-3,4-DCPG, (*S*)-3,4-dicarboxyphenylglycine; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; mGlu, metabotropic glutamate; LY354740.H_2O, bicyclo[3.1.0]hexane-2,6-dicarboxylic acid, 2-amino-, (1S,2S,5R,6S)-(9CI).H_2O; LY379268, 2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid, 4-amino-, (1R,4R,5S,6R)- (9CI); LY459477, (1*R*,2*S*,4*R*,5*R*,6*R*)-2-amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY3020371, (1S,2R,3S,4S,5R,6R)-2-amino-3-[(3,4-difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; MGS0039, 2-amino-3-[(3,4-dichlorophenyl) methoxy]-6-fluoro-, (1R,2R,3R,5R,6R)-(9CI)-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo- (9CI)-benzo[f]quinoxaline-7-sulfonamide; NMDA, N-methyl-D-aspartate.

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1. Introduction

Glutamate is the principal excitatory neurotransmitter utilized by the mammalian nervous system. Cell-surface protein targets relevant to modulation of glutamatergic transmission include receptors that form ion channels and mediate fast synaptic transmission (ionotropic glutamate receptors, iGluRs), receptors coupled to G-proteins which modulate intracellular second messenger levels (metabotropic glutamate receptors, mGluRs) and uptake carriers (EAAT1-3) which function to remove glutamate from synapses following release (Jensen et al., 2015). iGluRs have been pharmacologically sub-classified into four groups (AMPA, Kainate, NMDA and delta) each of which is comprised of individual protein subunits (AMPA: GluA1-4; Kainate: GluK1-5; NMDA: GluN1, GluN2A-D, GluN3A,B; delta: GluD1,2) (Traynelis et al., 2010), while mGluRs are comprised of eight members ($mGlu_{1\text{-}8}$) (Pin and Duvoisin, 1995). Multiple mechanisms that target the modulation of glutamatergic transmission are under investigation as potential therapeutic agents (Hashimoto et al., 2013) and some, such as the AMPA receptor antagonist perampanel (Fycompa™) for epilepsy (Shvarts and Chung, 2013), and the NMDA antagonist memantine (Namenda™) for moderate to severe dementia in Alzheimer's disease (Reisberg et al., 2003) have been approved as drugs for human use. Despite extensive research focused on the identification of agents targeting mGluRs and positive clinical efficacy being demonstrated in certain instances (Dunayevich et al., 2008; Patil et al., 2007; Stocchi et al., 2013), no marketed drugs acting directly on mGlu receptors are currently approved.

Metabotropic $Glu_{2/3}$ receptors show prominent distribution in cortico-limbic brain structures (Petralia et al., 1996; Neki et al., 1996; Richards et al., 2005; Gu et al., 2008; Wright et al., 2013) and exhibit presynaptic, postsynaptic and glial localization. Presynaptic $mGlu_{2/3}$ receptors are found outside the active synaptic release zone and, when activated by either endogenous extrasynaptic glutamate or synthetic agonist ligands, inhibit glutamate release. Orthosteric agonists acting at these targets have demonstrated efficacy in preclinical models driven by excessive glutamate transmission, including stress/anxiety (Monn et al., 1997; Helton et al., 1998; Kłodzińska et al., 1999; Schoepp et al., 2003; Swanson et al., 2005; Rorick-Kehn et al., 2007), pain (Neugebauer et al., 2000; Simmons et al., 2002; Jones et al., 2005; Du et al., 2008; Kumar et al., 2010), and psychosis (Moghaddam and Adams, 1998; Cartmell et al., 1999, 2000; Nakazato et al., 2000; Takamori et al., 2003; Rorick-Kehn et al., 2007; Jones et al., 2011; Fabricius et al., 2011; Ago et al., 2012), and have produced clinical efficacy in both generalized anxiety disorder (Dunayevich et al., 2008) and schizophrenia patients (Patil et al., 2007). While the latter proof of concept trial findings were not replicated in a larger study (Downing et al., 2014), post-hoc analyses suggest responsive patient subgroups that might benefit from this mechanism of action (Kinon et al., 2015).

Alternatively, pharmacologic blockade of $mGlu_{2/3}$ receptors has been identified as a novel approach to enhance glutamate transmission in the brain, having potential therapeutic benefit in CNS disorders for which diminished glutamate transmission is suggested (Witkin and Eiler, 2006; Witkin et al., 2007). In this regard, $mGlu_{2/3}$ antagonists elicit robust antidepressant-like behaviors (Chaki et al., 2004, 2013; Yoshimizu et al., 2006; Pałucha-Poniewiera et al., 2010; Ago et al., 2013), show cognitive-enhancing effects (Woltering et al., 2010; Goeldner et al., 2013; Kim et al., 2014) and promote wakefulness (Feinberg et al., 2005) in rodents. Notably, the observed antidepressant effects have been shown to be both AMPA-receptor dependent (Karasawa et al., 2005; Koike and Chaki, 2014) suggesting enhanced glutamate signaling and to involve a post-synaptic signaling pathway (Alt et al., 2006; Li et al., 2010; Dwyer et al., 2012) which overlaps with that activated by ketamine (Duman et al., 2012), an NMDA receptor antagonist that has demonstrated rapid and prolonged efficacy in depressed patients who do not respond to approved antidepressant treatments (Berman et al., 2000; Zarate et al., 2006). Thus, the $mGlu_{2/3}$ antagonist mechanism may represent an opportunity to demonstrate ketamine-like antidepressant efficacy while avoiding the serious side effects associated with this use-dependent NMDA receptor antagonist (e.g. abuse liability, cognitive impairment, psychotomimetic effects; Iadarola et al., 2015). At least two $mGlu_{2/3}$ antagonist molecules (Fig. 1) have entered clinical development, an orthosteric antagonist oral prodrug BCI-838 (Yasuhara et al., 2006; Nakamura et al., 2006) that delivers active component BCI-632, previously known as MGS0039 (Nakazato et al., 2004; Yoshimizu et al., 2006) and a negative allosteric modulator (NAM), RO4995819, RG1538, Decoglurant (Gatti et al., 2006; clinicaltrials.gov, study NCT01457677). A preliminary report of a double-blind, placebo-controlled study of the latter molecule indicated a lack of efficacy on inventories of depression, cognition and physical functioning when dosed in conjunction with either selective serotonin uptake or mixed serotonin/norepinephrine uptake inhibitors in non-responding depressed patients (Umbricht et al., 2015), while preliminary data for BCI-632 have established safety and tolerability for this molecule as well as effects on qEEG (Gadient et al., 2012) that could be useful as a maker of central target engagement.

We have previously described the discovery (Ornstein et al., 1998a,b) and preclinical pharmacology (Kingston et al., 1998) of LY341495 (Fig. 1), the first known highly potent, systemically active orthosteric $mGlu_{2/3}$ receptor antagonist. This compound has been widely used to enable both in vitro and vivo preclinical target validation studies. More recently, we have worked to identify additional pharmacological agents possessing $mGlu_{2/3}$ receptor antagonist pharmacology, and through a directed medicinal chemistry effort (Chappell et al., 2014), have identified a series of novel bicyclic amino acids as potent and selective orthosteric antagonists of $mGlu_{2/3}$ receptors. In this account, we detail the in vitro pharmacological attributes of an exemplary member of this new series, LY3020371 (Fig. 1), which we now establish as a highly potent and selective $mGlu_{2/3}$ receptor antagonist both in cells expressing recombinant human receptor subtypes and in rodent and human native tissue assays. Importantly, we also show that following intravenous dosing in rats, pharmacologically relevant cerebrospinal fluid levels of LY3020371 are achieved.

2. Materials and methods

2.1. $mGlu_2$ and $mGlu_3$ receptor cAMP antagonist assays

Assays were conducted in recombinant AV12 cells stably expressing human $mGlu_2$ or $mGlu_3$ and the rat excitatory amino acid transporter 1 (EAAT1). The cell lines were maintained by culturing in Dulbecco's modified eagle's medium (DMEM) with high glucose and pyridoxine hydrochloride (Invitrogen) supplemented with 5% dialyzed fetal bovine serum (FBS), 1 mM sodium pyruvate, 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 1 mM L-glutamine; geneticin, and hygromycin B were used as selection antibiotics. Confluent cultures were grown at 37 °C in an atmosphere containing 6.5% CO_2, and passaged biweekly. Cells were harvested using 0.25% trypsin, suspended in freeze media (FBS with 10% DMSO) at 10^7 cells/mL, and aliquots were stored in liquid nitrogen. Twenty-four hours before the assay, cells were plated at a density of 8000–10,000 cells per well in a