Neuropharmacology 66 (2013) 202–214

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Negative allosteric modulation of metabotropic glutamate receptor 5 results in broad spectrum activity relevant to treatment resistant depression

Z.A. Hughes*, S.J. Neal, D.L. Smith, S.J. Sukoff Rizzo, C.M. Pulicicchio, S. Lotarski, S. Lu, J.M. Dwyer, J. Brennan, M. Olsen, C.N. Bender, E. Kouranova, T.H. Andree, J.E. Harrison, G.T. Whiteside, D. Springer, S.V. O'Neil, S.K. Leonard, L.E. Schechter, J. Dunlop, S. Rosenzweig-Lipson, R.H. Ring

Neuroscience Research Unit, Pfizer Global Research, 700 Main Street, Cambridge, MA 02139, USA

ARTICLE INFO

Article history:
Received 17 December 2011
Received in revised form
23 March 2012
Accepted 5 April 2012

Keywords:
Glutamate
Antidepressant
Anxiolytic
Analgesic
Ketamine
STAR*D
Receptor occupancy

ABSTRACT

Evidence suggests that 30–50% of patients suffering from major depressive disorder (MDD) are classified as suffering from treatment resistant depression (TRD) as they have an inadequate response to standard antidepressants. A key feature of this patient population is the increased incidence of co-morbid symptoms like anxiety and pain. Recognizing that current standards of care are largely focused on monoaminergic mechanisms of action (MOAs), innovative approaches to drug discovery for TRD are targeting glutamate hyperfunction.

Here we describe the in vitro and in vivo profile of GRN-529, a novel negative allosteric modulator (NAM) of metabotropic glutamate receptor 5 (mGluR5). In cell based pharmacology assays, GRN-529 is a high affinity (Ki 5.4 nM), potent (IC50 3.1 nM) and selective (>1000-fold selective vs mGluR1) mGluR5 NAM. Acute administration of GRN-529 (0.1–30 mg/kg p.o.) had dose-dependent efficacy across a therapeutically relevant battery of animal models, comprising depression (decreased immobility time in tail suspension and forced swim tests) and 2 of the co-morbid symptoms overrepresented in TRD, namely anxiety (attenuation of stress-induced hyperthermia, and increased punished crossings in the four plate test) and pain (reversal of hyperalgesia due to sciatic nerve ligation or inflammation). The potential side effect liability of GRN-529 was also assessed using preclinical models: GRN-529 had no effect on rat sexual behavior or motor co-ordination (rotarod), however it impaired cognition in mice (social odor recognition). Efficacy and side effects of GRN-529 were compared to standard of care agents (antidepressant, anxiolytic or analgesics) and the tool mGluR5 NAM, MTEP. To assess the relationship between target occupancy and efficacy, ex vivo receptor occupancy was measured in parallel with efficacy testing. This revealed a strong correlation between target engagement, exposure and efficacy across behavioral endpoints, which supports the potential translational value of PET imaging to dose selection in patients. Collectively this broad spectrum profile of efficacy of GRN-529 supports our hypothesis that negative allosteric modulation of mGluR5 could represent an innovative therapeutic approach to the treatment of TRD.

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

1. Introduction

Depression is the leading cause of disability in established market economies around the world. In the primary care setting, only 40% of patients respond to first-line antidepressant medication, and only a portion of these responders achieves remission (Schulberg et al., 1998). Patients typically enter second-line therapy due to lack of efficacy (40%) or side effects (32%). In an effort to better understand the effectiveness of treatments for major depressive disorder (MDD) the NIMH funded study, Sequenced Treatment Alternatives to Relieve Depression (STAR*D), was

Abbreviations: MOA, mechanism of action; NAM, negative allosteric modulator; PPS, painful physical symptoms; MDD, major depressive disorder; STAR*D, sequenced treatment alternatives to treat depression; PWT, paw withdrawal threshold.

* Corresponding author. Tel.: +1 860 715 6556, +1 860 326 8401 (mobile); fax: +1 860 686 6659.
E-mail address: Zoe.Hughes@Pfizer.com (Z.A. Hughes).

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2012.04.007

undertaken to investigate various treatment options for patients who did not reach remission after treatment with the prototypical SSRI, citalopram (Rush et al., 2004). The STAR*D has been the largest study to date that examined variables correlated with treatment response in depression. A key finding to emerge from the analysis of the STAR*D data was the observation treatment refractory patients had an increased incidence of psychiatric and neurological co-morbidities. In particular, it was noted that patients suffering from 'anxious depression' were both less likely to reach remission and had an increased latency to achieve remission (Fava et al., 2008). Moreover, it was observed that the presence of co-morbid painful physical symptoms (PPS) also correlated significantly with lower remission rates and latency to remit (Leuchter et al., 2010). Although it is unclear whether these co-morbid conditions participate in the biology underlying resistance to monoaminergic standards of care in depression, it is reasonable to hypothesize that agents with unique mechanisms of action (MOAs) that expand the efficacy profile of current standards of care to incorporate effects on these co-morbid symptom domains, may represent innovative approaches to novel antidepressant drug discovery. It is reasonable to suggest that patients who are refractory to these monoaminergic approaches may have a distinct underlying pathophysiology or unique intolerance to side effects associated with these mechanisms, and may only benefit from agents that engage different neurotransmitter systems.

Support for a glutamate hypothesis of depression can be found in the numerous clinical and preclinical observations implicating glutamatergic dysregulation. For instance, depressed patients exhibit elevated glutamate both in plasma and the limbic brain areas involved in the neural circuitry for the regulation of mood disorders (Sanacora et al., 2004). Collectively the data indicate that hyperfunction of the glutamatergic system is significantly correlated with depression, and suggest that agents which negatively regulate glutamatergic neurotransmission may hold promise in treating depression. In support of this, NMDA glutamate receptor antagonists such as ketamine and MK-801 have antidepressant effects in preclinical models of antidepressant activity (Papp and Moryl, 1994; Trullas and Skolnick, 1990). Interestingly, these findings have translated to small clinical trials demonstrating robust efficacy of ketamine in TRD (Berman et al., 2000; Zarate et al., 2006). Positive modulation of AMPA receptors also reduces glutamatergic transmission and preclinical data indicates that AMPA positive modulators are efficacious in models of depression (Li et al., 2001). Targeting metabotropic glutamate receptors (mGluRs) represents an alternate method of negatively regulating glutamatergic function. Selective antagonists of mGluR2/3, like MGS0039 and LY341495, have antidepressant effects (Chaki et al., 2004) in preclinical models.

In terms of available preclinical evidence, mGluR5 provides a compelling therapeutic approach for TRD. The prototypic mGluR5 negative allosteric modulators (NAMs) MPEP and MTEP are efficacious in preclinical antidepressant- and anxiolytic models (Brodkin et al., 2002; Busse et al., 2004; Palucha et al., 2005; Tatarczynska et al., 2001). A positive proof of concept was achieved for the mGluR5 NAM, fenobam, as a treatment for anxiety in human subjects (Pecknold et al., 1982; Porter et al., 2005). mGluR5 NAMs are also effective analgesics in preclinical models of neuropathic and inflammatory pain (Kumar et al., 2010; Montana et al., 2009). Together with the findings of the STAR*D study that TRD patients have a higher incidence of co-morbidities such as anxiety and PPS, the broad spectrum of preclinical efficacy of compounds of this mechanism suggests that selective mGluR5 NAM compounds may have efficacy in TRD.

To more thoroughly explore this hypothesis preclinically, we have established a battery of behavioral assays that encompasses the spectrum of key symptoms of TRD. Using this approach and a novel mGluR5 NAM, GRN-529, we demonstrate a broad spectrum of efficacy for this mechanism of action. Moreover, we have established a firm understanding of the relationship between target engagement and efficacy across these preclinical models. Together this provides preclinical support for pursuing negative allosteric modulation of mGluR5 as a therapeutic approach for TRD.

2. Materials and methods

2.1. Animals

All animal studies were performed in accordance to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Pub. 85–23, revised 1996) and under the approval of either the Princeton or Groton Institutional Animal Care and Use Committees (IACUC). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, where available.

All animals were received from Charles River Laboratories and group housed (except mice for Social Odor Recognition which were singly housed) in an AAALAC-accredited facility in a climate-controlled room on a 12-h light/dark cycle. Food and water were available ad libitum.

2.2. Drugs and reagents

GRN-529 ((4-difluoromethoxy-3-(pyridine-2-ylethynyl)phenyl)5H-pyrrolo [3,4-b]pyridine-6(7H)-yl methanone) was synthesized by Wyeth Medicinal Chemistry, Princeton, NJ. MPEP (2-methyl-6-(phenylethynyl)pyridine), MTEP (3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine), fluoxetine and celecoxib were purchased from Toronto Research Chemicals (Toronto, Canada), and gabapentin from Organix (Woburn, MA). [^{3}H]MPEP (60 Ci/mmol), [^{3}H]MPEPy (80 Ci/mmol) were purchased from American Radiolabeled Chemicals (ARC). [^{3}H]ABP688 (84.9 Ci/mmol) was supplied by Wyeth Radiosynthesis/Chemical Development Group. Tris hydrochloride (pH 7.4), Hanks' balanced salts (pH 7.4) and Hepes were purchased from Invitrogen (CA). Glutamic pyruvic transaminase was purchased from Calzyme Labs (San Luis Obispo, CA). The Calcium 3 assay kit was obtained through Molecular Devices (Sunnyvale, CA). L-Glutamate, polyethyleneimine, pyruvic acid, and probenecid and all other compounds and reagents were purchased from Sigma-Aldrich (St Louis, MO).

For systemic administration GRN-529 was dissolved in 0.5% methylcellulose/2% Tween 80/97.5% dH_2O, all other drugs were dissolved in sterile water. Mice were dosed at 10 ml/kg, and rats at 2 ml/kg. All doses were corrected for salt form.

2.3. Experimental procedures

2.3.1. Radioligand binding using rat mGluR5 receptor expressing Hek293 cell membranes

4 nM [^{3}H]MPEP, buffer (50 mM Tris–HCl, pH 7.4) and ~15–20 μg of rat mGluR5-HEK293 membrane protein were added to 96-well microtiter plates in a total volume of 250 μl. Non-specific binding was defined using 1 μM MPEP. Following a 60 min incubation, the reaction was terminated by vacuum filtration with ice-cold 50 mM Tris–HCl, pH 7.4 through a pre-soaked (0.5% polyethyleneimine) GF/B filter plate using a Packard FilterMate Harvester. Filters were dried and counted using Microscint 20 liquid scintillation cocktail in a Packard TopCount scintillation counter.

2.3.2. Functional inhibition (antagonism) of L-glutamate-induced intracellular calcium influx

Stable cell lines of rat mGluR5-HEK293 (NM_017012.1), human mGluR5-HEK293 (NM_000842) or human mGluR1-HEK293 (NM_000838) were plated at 80,000 cells/well in 96-well black walled clear-bottomed plates and incubated overnight in glutamate/glutamine-free medium at 37 °C/5% CO_2. Following overnight incubation, cell culture media was removed and cells were loaded with 160 μl calcium indicator dye (Calcium 3 dye) supplemented with 20 mM Hepes, 3 U/ml Glutamic pyruvic transaminase, 3 mM pyruvic acid, and 2.5 mM probenecid for 1 h at 37 °C/5% CO_2. Compound stock solutions were prepared in 100% DMSO, diluted and added to the assay plate to a final DMSO concentration of 0.3%–0.4%. 20 μl of compound was added to the cells and incubated for 5 min at 37 °C, followed by a 10 min acclimation to room temperature. Cells were then challenged with 20 μl of a supermaximal L-glutamate concentration (50 μM final) and changes in intracellular calcium measured. Functional activity at mGluR2 was measured similarly, using a recombinant CHO cell line co-expressing human mGluR2 (NM_000839) and a G_q/G_{i3} chimera, plated at a seeding density of 50,000 cells/well. Agonist activity was determined by adding the compound of interest (10 μM) to the well. Antagonist activity was assessed by pre-treating cells with the compound of interest (10 μM) for 5 min followed by a submaximal concentration (EC_{80}) of glutamate (50 μM). PAM activity was evaluated by performing a L-glutamate concentration response curve (3 nM–100 μM) in the absence or presence of 10 μM compound of interest. Calcium