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Neurophysiological signals as potential translatable biomarkers for modulation of metabotropic glutamate 5 receptors

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ABSTRACT

The Group I metabotropic glutamate receptor subtype 5 (mGluR5) is widely distributed in the brain with dense expression in the cerebral cortex, hippocampus, and basal ganglia. These receptors have been implicated in psychiatric and neurological disorders such as schizophrenia, Fragile X syndrome, addiction, anxiety/depression, Parkinson's disease and neuropathic pain. The present study evaluated the effects of the mGluR5 negative allosteric modulators (NAMs) 4-difluoromethoxy-3-(pyridine-2-ylethynyl)phenyl)5*H*-pyrrolo[3,4-b]pyridine-6(7*H*)-yl methanone (GRN-529) and methyl (3a*R*,4*S*,7a*R*)-4-hydroxy-4-[(3-methylphenyl)ethynyl]octahydro-1*H*-indole-1-carboxylate (AFQ056) on polysomnographic (PSG) and quantitative electroencephalographic (qEEG) measures in freely moving rats. Furthermore, the anxiolytic profile of GRN-529 was characterized in anesthetized rats by measuring stimulation-induced hippocampal theta oscillation. The present findings demonstrate that inhibition of mGluR5 via its allosteric site profoundly modulates high-level neuronal network activities as indicated by changes in sleep-wake activity and power distribution of qEEG. Both GRN-529 and AFQ056 reduced the total time spent in rapid-eye movement with AFQ056 producing a significant increase in wakefulness at the highest dose tested. Additionally, qEEG revealed significant compound-induced increases in delta power concomitant with more subtle decreases in theta and alpha band power. Receptor occupancy (RO) studies revealed that GRN-529 and AFQ056 at all doses resulted in over 45% mGluR5 occupancy. Furthermore, GRN-529 dose-dependently decreased elicited hippocampal theta frequency, consistent with previous findings using clinically active anxiolytic compounds. The described changes in neurophysiological signals identified in freely moving rats may be considered suitable translational biomarkers for the clinical evaluation of mGluR5 NAMs.

1. Introduction

Glutamate is the most prominent excitatory neurotransmitter in the human brain, acting on a variety of ionotropic and metabotropic glutamate receptors (Siegel, 2006). G protein-coupled metabotropic glutamate receptors (mGluR) are expressed both pre- and post-synaptically, and are known to regulate glutamate release, modulate neuronal excitability and contribute to synaptic transmission via interaction with presynaptic receptors modulating neurotransmitter release. The mGluR subtype 5 (mGluR5) is expressed postsynaptically in several brain regions, particularly in the limbic circuitry. Since mGluR5 functionally interact with *N*-methyl-D-aspartate (NMDA) receptors, their activity can modulate synaptic plasticity (Simonyi et al., 2005). Due to its ubiquitous cerebral distribution, mGluR5 is presumed to play a role in a variety of

Abbreviations: σ_{emg}, standard deviation of the EMG amplitude; AFQ056, methyl (3a*R*,4*S*,7a*R*)-4-hydroxy-4-[(3-methylphenyl)ethynyl]octahydro-1*H*-indole-1-carboxylate; AP, Anterior Posterior; DV, Dorsal Ventral; EEG, electroencephalogram; EMG, electromyogram; FFT, Fast-Fourier transform; GRN-529, 4-difluoromethoxy-3-(pyridine-2-ylethynyl)phenyl)5*H*-pyrrolo[3,4-b]pyridine-6(7*H*)-yl methanone; JVC, jugular vein-cannulated; LLOQ, lower limit of quantification; mGluR5, metabotropic glutamate receptor subtype 5; NAM, negative allosteric modulator; NMDA, *N*-Methyl-D-aspartate; qEEG, quantitative electroencephalogram; nPO, nucleus Pontis Oralis; PET, positron emission tomography; PSG, polysomnography; REM, rapid eye movement; RO, receptor occupancy; S-R, simulation response; NREM, non REM sleep; USP, United States Pharmacopeia.

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neurophysiological processes and its dysfunction is implicated in a multitude of central nervous system-related disorders (Ribeiro et al., 2010) including Fragile X syndrome (Bear et al., 2004), treatment-resistant depression (Pilc et al., 2008), schizophrenia (Krystal et al., 1994), epilepsy (Ure et al., 2006), Huntington's disease (Ribeiro et al., 2011), Parkinson's disease (Pisani et al., 2003), Alzheimer's disease (Sokol et al., 2011) and attention-deficit/hyperactivity disorder (Elia et al., 2010). Therefore, mGluR5 has attracted considerable interest as a potential drug target for treating this spectrum of neurological and psychiatric illnesses. Recently, mGluR5 agonists and antagonists, as well as positive (PAMs) and negative allosteric modulators (NAMs), have been developed and characterized pharmacologically in numerous preclinical models and assays (Gregoire et al., 2011; Rodriguez et al., 2010). Anxiolytic and antidepressant effects of mGluR5 NAMs have been reported in preclinical and clinical studies (Palucha and Pilc, 2007). Additionally, mGluR5 NAMs have demonstrated efficacy in preclinical models of drug addiction (Gass et al., 2009), and both preclinical and clinical activity for Fragile X syndrome (Jacquemont et al., 2011; Bear et al., 2004) and Parkinson's disease L-DOPA-induced dyskinesia (Gregoire et al., 2011; Berg et al., 2011). Given the limitation of the predictive validity of our animal models, exploratory clinical studies in patient populations will confirm their therapeutic benefits.

To facilitate drug development, mGluR5-specific positron emission tomography (PET) ligands have been developed, including the highly selective allosteric antagonists [^{11}C]ABP688 (Ametamey et al., 2006) and [^{18}F]F-PEB (Kuwabara et al., 2011). These tools allow for the calculation of ligand-receptor binding and receptor distribution, with the latter identifying mGluR5-rich regions in human brain (Ametamey et al., 2007; Kuwabara et al., 2011; Treyer et al., 2007). Although such PET studies quantify drug–receptor interaction, they do not reveal functional activity (Javitt et al., 2011). Ideally, translational biomarkers, discussed herein as neurophysiologic measures, demonstrate similar or identical interspecies exposure–pharmacodynamic relationships and thereby provide evidence for functional modulation of the pharmacologic target. Collectively, such PET and electrophysiological data provide crucial clinical evidence that a specific degree of target engagement is associated with a characteristic effect on neuronal processing.

In the present study, the selective mGluR5 NAMs 4-difluoromethoxy-3-(pyridine-2-ylethynyl)phenyl5*H*-pyrrolo[3,4-b]pyridine-6(7*H*)-yl methanone (GRN-529) (Hughes et al., 2013) and methyl (3a*R*,4*S*,7a*R*)-4-hydroxy-4-[(3-methylphenyl)ethynyl] octahydro-1*H*-indole-1-carboxylate (AFQ056) (Gasparini et al., 2003) (Fig. 1) were studied individually in rats for their effects on neurophysiologic signals, including polysomnography (PSG) and quantitative electroencephalography (qEEG). Previous studies have indicated that alterations in sleep physiology following drug treatment in rodents translate well to humans, particularly changes in rapid eye movement (REM) sleep parameters (Steiger and Kimura, 2010). Although less explored, drug-induced alterations in particular EEG oscillations (i.e. alpha and beta) have been shown to also translate from rats to humans (Coenen and van Luijtelaar, 1991; Krijzer et al., 1993; Saletu et al., 1989; van Lier et al., 2004). Since attenuation of glutamate neurotransmission alleviates symptoms of anxiety (Ballard et al., 2005), the effect of GRN-529 was also studied on nucleus pontis oralis (nPO) stimulation-induced hippocampal oscillation, a potential screening assay for anxiolytic drugs (McNaughton et al., 2007; Yeung et al., 2012). Receptor occupancy (RO) levels were also determined for GRN-529 and AFQ056 to allow for comparison of effects across experiments, as well as helping us to better understand these functional effects in the context of human efficacy measurements where RO levels were also ascertained.

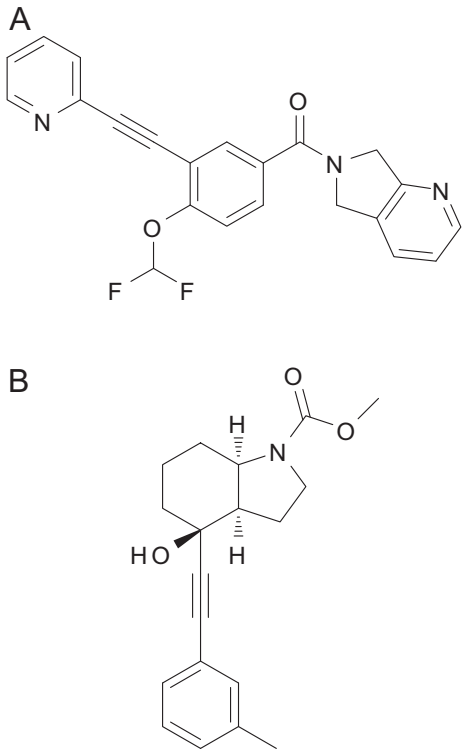

Fig. 1. Chemical structures. (A) 4-difluoromethoxy-3-(pyridine-2-ylethynyl)phenyl)5*H*-pyrrolo[3,4-b]pyridine-6(7*H*)-yl methanone (GRN-529) and (B) methyl (3a*R*,4*S*,7a*R*)-4-hydroxy-4-[(3-methylphenyl)ethynyl]octahydro-1*H*-indole-1-carboxylate (AFQ056).

2. Materials and methods

2.1. General methods

2.1.1. Chemicals and reagents

GRN-529 and AFQ056 (Fig. 1) were synthesized and fully characterized (>99% chemical purity, >99% enantiomeric excess) by Neuroscience Chemistry at Pfizer Worldwide Research and Development (WRD, Groton, CT). Chemicals and solvents of HPLC grade were supplied by Aldrich Fine Chemical Co. (Milwaukee, WI), Fisher Scientific (Pittsburgh, PA) or Thermo Fisher Scientific (Waltham, MA). Control Sprague-Dawley rat plasma was procured from Bioreclamation Inc. (Hicksville, NY). The in-life and bioanalytical portions of the rat pharmacokinetics studies were conducted at BioDuro, Pharmaceutical Product Development Inc. (Beijing, PRC). All matrix concentrations were determined by an internally characterized LC–MS/MS

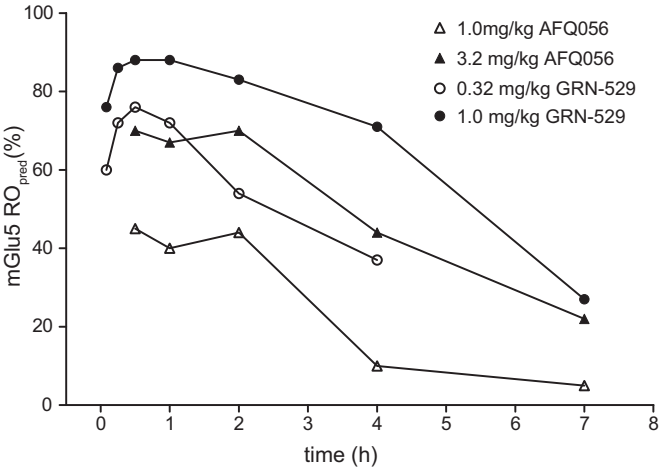

Fig. 2. Predicted mGlu5 receptor occupancy. Projected mGlu5 receptor occupancy (RO) versus time in rats ($n = 2$) following subcutaneous administration of GRN-529 (0.32 ○ or 1.0 ● mg/kg) or AFQ056 (1 Δ and 3.2 ▲ mg/kg).