Translational Pharmacology of the Metabotropic Glutamate 2 Receptor–Preferring Agonist LY2812223 in the Animal and Human Brain

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ABSTRACT

LY2812223 [(1R,2S,4R,5R,6R)-2-amino-4-(1H-1,2,4-triazol-3-ylsulfanyl)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid] was identified via structure-activity studies arising from the potent metabotropic glutamate mGlu2/3 receptor agonist LY354740 [(+)2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid] as an mGlu2-preferring agonist. This pharmacology was determined using stably transfected cells containing either the human mGlu2 or mGlu3 receptor. We extended the pharmacological evaluation of LY2812223 to native brain tissues derived from relevant species used for preclinical drug development as well as human postmortem brain tissue. This analysis was conducted to ensure pharmacological translation from animals to human subjects in subsequent clinical studies. A guanosine 5’-O-[3-[(197)35S]thio)triphosphate (GTPγS) functional binding assay, a method for measuring G-protein-coupled signaling that is inherent to the group 2 mGlu receptors, was used to evaluate LY2812223 pharmacology of native mGlu receptors in mouse, rat, nonhuman primate, and human cortical brain tissue samples. In native tissue membranes, LY2812223 unexpectedly acted as a partial agonist across all species tested. Activity of LY2812223 was lost in cortical membranes collected from mGlu2 knockout mice, but not those from mGlu3 knockout mice, providing additional support for mGlu2-preferring activity. Other signal transduction assays were used for comparison with the GTP binding assay (cAMP, calcium mobilization, and dynamic mass redistribution). In ectopic cell line–based assays, LY2812223 displayed near maximal agonist responses at the mGlu2 receptor across all assay formats, while it showed no functional agonist activity at the mGlu3 receptor except in the cAMP assay. In native brain slices or membranes that express both mGlu2 and mGlu3 receptors, LY2812223 displayed unexpected partial agonist activity, which may suggest a functional interplay between these receptor subtypes in the brain.

Introduction

Glutamate is a ubiquitous neurotransmitter that binds to and activates the glutamate receptor family of receptors made up of ion channels (Glu1–Glu4 receptors) and metabotropic G protein–coupled receptors (mGlu1–mGlu8 receptors) (O’Neil et al., 2010). The metabotropic glutamate (mGlu) receptors belong to the superfamily of G protein–coupled receptors (GPCRs) that are characterized by their distinct single-peptide structure that passes through the plasma membrane in a serpentine seven-transmembrane orientation. mGlu receptors belong to the class C GPCRs, which contrast in overall structure from the class A rhodopsin-like GPCRs and the class B peptide binding GPCRs (mGlu1 and mGlu5; group 2, mGlu2 and mGlu3; and group 3, mGlu4 and mGlu6–mGlu8). More recently, mGlu2 and mGlu3 were characterized pharmacologically and physiologically using selective pharmacological ligands and transgenic knockout (KO) mice. Therapeutic interest in the group 2 receptors has derived from their distribution in key brain regions and behavioral activity in rodent models suggesting utility in pain, anxiety, depression, and psychosis (De Filippis et al., 2015; Li et al., 2015).

LY2812223 [(1R,2S,4R,5R,6R)-2-amino-4-(1H-1,2,4-triazol-3-ylsulfanyl)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid], an mGlu2-preferring agonist, provides a unique selective orthosteric ligand for target validation and preclinical studies (Monn et al., 2015). The pharmacology of this ligand was initially evaluated using extracellular glutamate binding domain. mGlu receptors fall into three groups based on sequence similarity: group 1, mGlu1 and mGlu5; group 2, mGlu2 and mGlu3; and group 3, mGlu4 and mGlu6–mGlu8.

Abbreviations: FLIPR, fluorescence imaging plate reader; GPCR, G protein–coupled receptor; GTPγS, guanosine 5’-O-[3-[(197)35S]thio)triphosphate; KO, knockout; LY2812223, (1R,2S,4R,5R,6R)-2-amino-4-(1H-1,2,4-triazol-3-ylsulfanyl)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY300168, 10-((4-aminophenyl)-N,13-dimethyl-4,6-dioxo-11,12-diazatricyclo[7.5.0.0; {3, 7}]tetradeca-1(9),2,7,10-tetraene-12-carboxamide; LY341495, (2S)-(−)-5,5-dimethyl-2-morpholineacetic acid; VB, ventrobasal complex.

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recombinant receptors expressed in mammalian cell lines, where it exhibited maximally efficacious human mGlur2 receptor-mediated responses in both cAMP and Ca$^{2+}$ fluorescence imaging plate reader (FLIPR) formats but only partial agonist (cAMP) or no agonist (Ca$^{2+}$ FLIPR) responses in cells expressing human mGlu3 receptors. Moreover, behavioral efficacy was observed in rodents, indicating central mGlu2 engagement. However, the pharmacology was not assessed across several signaling assays in different animal species typically used in the path toward clinical development, including nonhuman primate and human brain tissue. The cell lines were engineered to optimize ligand discovery and development and may misreport the pharmacology measured in native tissue assays. For example, the cell lines used in the drug screening campaign and ligand optimization involved coexpression of each of the mGlu family members along with rat glutamate transporters (to reduce extracellular glutamate) and promiscuous G protein $G_{15}$ (to allow signaling through calcium mobilization). These highly engineered cell lines overexpress the receptor proteins, may not express brain cell–related protein partners, and may lack contextually relevant signaling proteins. Here, we report the pharmacological profiling of LY2812223 in several assays tested and thus show reasonable fidelity of translational pharmacology in animals and in human brain tissue.

**Materials and Methods**

**Materials.** Guanosine 5′-O-([35S]thio)triphosphate (GTP-$\gamma$S) was purchased from PerkinElmer (Melville, NY). LY2812223 ([1R,2S,4R,5R,6R]-2-amino-4-(1H-1,2,4-triazol-3-ylsulfanyl)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid), LY379268 ([1R,4R,5S,6R]-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid), LY404039 ([1R,4S,5S,6S]-4-amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic acid 2,2-dioxide), and LY341495 ([2S]-2-amino-2-[(1S,2S)-2-carboxyethylprop-1-yl]-3-(xanth-9-yl) propanoic acid), LY354740 ([1S,2S,5R,6S]-2-amino-4-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid), and LY459477 ([1R,2S,4R,5R,6R]-2-amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid) were synthesized by Eli Lilly and Company (Indianapolis, IN). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). CellKey 96-well plates (Molecular Devices) were coated with poly(D-lysine) and incubated in medium containing 250 mM (mGlur2) or 125 mM (mGlu3) L-glutamine. Compounds were tested in 10-point concentration response curves using 3× serial dilution. Intracellular calcium levels were monitored before and after the addition of compounds using Fluo-3 AM dye (Thermo Fisher Scientific, Waltham, MA) in a FLIPR instrument (Molecular Devices, Sunnyvale, CA). The maximal response (EC$_{max}$) was defined using 100 μM glutamate. The compound effect was measured as maximum minus minimum peak heights in relative fluorescent units corrected for basal fluorescence in the absence of glutamate. Agonist effects were quantified as the percentage of stimulation induced by compound alone relative to the maximal glutamate response. All data were calculated as relative EC$_{50}$ values using a four-parameter logistic curve fitting program (GraphPad Prism, version 6.05; GraphPad Software Inc., La Jolla, CA).

**cAMP Assays.** Twenty-four hours before assay, the human mGlur2-AV2 and mGlu3-AV2 cell lines were plated at a density of 8000–10,000 cells/well (mGlur2) or 6000–8000 cells/well (mGlu3) in tissue culture–treated, 96-well, half-area black plates and were incubated in medium containing 250 mM (mGlur2) or 125 mM (mGlu3) L-glutamine. Compounds were tested in 10-point concentration response curves using 3× serial dilution. The final reaction mixture contained 1 μM forskolin (F6886; Sigma-Aldrich, St. Louis, MO) and up to 25 μM compound. Reactions were incubated at 37°C for 20 minutes. DCG-IV ([2S,2'R,3'R]-2-(2',3'-Dicarboxycyclopropyl) glycin) was added as a positive control. Cell lysates were assayed using a cAMP cell-based assay kit (Cisbio Assays, Codolet, France) for signal visualization at room temperature for 1 hour. The homogeneous time-resolved fluorescence signal (ratio of fluorescence at 665 to 620 nM) was detected with an EnVision plate reader (PerkinElmer). Raw data were converted to picomoles per well of cAMP with a cAMP standard curve generated for each experiment. Relative EC$_{50}$ values were calculated from the top-bottom range of the concentration response curve using a four-parameter logistic curve fitting program (GraphPad Prism, version 6.05; GraphPad Software Inc.).

**Impedance Assay.** Human mGlur2-AV2 and mGlu3-AV2 cells were grown in the appropriate growth media prior to plating and were harvested when between 50% and 80% confluent. Prior to plating, CellKey 96-well plates (Molecular Devices) were coated with poly(D-lysine) (10 mg/ml for 2 hours). Cells were plated at 200,000 cells/well in 150 μl growth media in the afternoon. After plating, cells were allowed to attach for 20 minutes at room temperature and were then placed in a 37°C incubator and grown overnight. The next morning, each plate

stored at 4°C. The assay was initiated with the addition of 100 μl diluted membrane (5 μg/well final concentration) with GDP (1 μM) into a 96-well plate. Subsequently, 50 μl of 400 μM L-glutamic acid solution or buffer solution was added to each well and mixed. Then 50 μl GTPyS was added to reach a final concentration of 500 pM and allowed to incubate for 120 minutes at room temperature. Twenty microliters of 3% NP40 was added, and the solution was mixed well and incubated for 30 minutes at room temperature. Twenty microliters of anti-G$_{15}$ antibody was then added and mixed well and left to incubate for 60 minutes at room temperature, followed by 50 μl anti–rabbit SPA (scintillation proximity assay) beads (final incubation volume is 290 μl). The plate was covered with sealing tape, vortexed for 10–15 seconds, and incubated at room temperature for 3 hours before centrifugation for 10 minutes at 184g. The amount of radioactive GTP bound to the membrane was measured using a Wallac MicroBeta system (35S, 1 minute/well; PerkinElmer).

**Calcium Mobilization Assays.** AV12 cells stably expressing the rat glutamate transporter (excitatory amino acid transporter 1), G$_{15}$ subunit, and either the human mGlur2 or mGlu3 receptors (mGlur2-AV2 and mGlu3-AV2) were created as previously described (Schoepp et al., 1997). Go15 expression allows these G$_{i}$-coupled receptors to signal through the phospholipase C pathway, resulting in the ability to measure receptor activation via the calcium flux assay. Twenty-four hours before assay, cells were plated at 85,000 (mGlur2) or 115,000 (mGlu3) cells/well into 96-well, black-walled, poly(0-lysine)–coated plates and incubated in medium containing 250 μM (mGlur2) or 125 μM (mGlus) L-glutamine. Compounds were tested in 10-point concentration response curves using 3× serial dilution. Intracellular calcium levels were monitored before and after the addition of compounds using Fluo-3 AM dye (Thermo Fisher Scientific, Waltham, MA) in a FLIPR instrument (Molecular Devices, Sunnyvale, CA). The maximal response (EC$_{max}$) was defined using 100 μM glutamate. The compound effect was measured as maximum minus minimum peak heights in relative fluorescent units corrected for basal fluorescence in the absence of glutamate. Agonist effects were quantified as the percentage of stimulation induced by compound alone relative to the maximal glutamate response. All data were calculated as relative EC$_{50}$ values using a four-parameter logistic curve fitting program (GraphPad Prism, version 6.05; GraphPad Software Inc., La Jolla, CA).
was placed on the CellKey instrument and washed three times with assay buffer (Dulbecco’s modified Eagle’s medium/F12 media containing 20 mM HEPES) and then incubated in a 37°C incubator for 1 hour. After this incubation, the cell plate and the compound plate were placed back into the CellKey instrument for compound addition. Data were analyzed by GraphPad software.

**Rat Cortical Synaptosomes cAMP Assay.** Rat cortical synaptosomes were prepared essentially as described by Dunkley et al. (1988). Ten-week-old Sprague-Dawley rats were euthanized by decapitation, the brains were surgically removed, and the cortex was collected and rinsed several times in ice-cold homogenizing buffer (320 mM sucrose, 1 mM EDTA, and 5 mM Tris, pH 7.4). The tissue was cut into small pieces, homogenized using 10 strokes in a Dounce glass homogenizer, and centrifuged at 1600g for 10 minutes at 4°C. The supernatant was layered over the top of a three-step Percoll gradient (3%, 15%, and 23%) and spun at 48,000g for 30 minutes at 4°C. The pellet was resuspended in 35-40 ml washing buffer (140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1.2 mM NaH2PO4, 5 mM NaHCO3, 10 mM glucose, and 10 mM HEPES, pH 7.4), pelleted at 27,000g for 10 minutes at 4°C, and resuspended in 1 to 2 ml washing buffer. Protein concentration was determined using the Pierce Coomassie Plus assay. Synaptosomes (Pierce, Rockford, IL) were diluted to 200 μg/ml in washing buffer and stored on ice. Compounds were solubilized in 0.1 N NaOH as 10-mM stocks, stored at −20°C, and serially diluted in assay buffer supplemented with 20 mM HEPES buffer at the start of each experiment. The reversal of forskolin-stimulated cAMP production was conducted using the same homogenized time-resolved fluorescence methodology as described for recombinant cells. In this case, each well contained 5 μg purified synaptosomes, the reaction was conducted for 1 hour at 37°C, and the incubation with cAMP-βγ2 conjugate and anti-cAMP cryptate conjugate was conducted for 2 hours prior to signal detection.

**Electrophysiology.** The methods for these experiments were described in detail previously (Rorick-Kehn et al., 2007). Briefly, coronal brain slices 350 μm in depth containing the reticular thalamic nucleus (RTN) and ventrobasal complex (VB) nuclei of the thalamus were prepared from 12- to 16-day-old male Sprague-Dawley rats. Slices were placed into a submersion-type recording chamber mounted on an upright microscope and were continuously superfused (2 to 3 ml/min) with oxygenated extracellular recording solution containing 124 mM NaCl, 26 mM NaHCO3, 3 mM KCl, 10 mM glucose, 2.5 mM CaCl2, 1.3 mM MgCl2, and 0.4% ascorbic acid, pH 7.3, with osmolarity of 300 mOsmliter). The whole-cell variant of the patch-clamp technique was used for recording current signals from VB thalamic neurons. Patch pipettes were filled with a solution containing 130 mM CsMeSO4, 4 mM CaCl2, 5 mM MgCl2, 10 mM EGTA, 5 mM Na2ATP, and 1 mM Na2GTP, pH adjusted to 7.3 with CsOH, with osmolarity of 300 mOsml. Current signals were amplified, low-pass filtered at 5 kHz, digitized with an analog-to-digital converter, and collected onto a computer hard drive. Series resistance (10–30 MΩ) compensation was monitored, and recordings displaying >30% change in resistance were not included in subsequent analyses. Inhibitory postsynaptic currents were evoked from VB neurons (holding potential, −20 mV) using a stimulator triggering a constant current unit to deliver single stimulation pulses (100 μs, 200–2000 μA) to bipolar stimulating electrodes positioned proximal (50–200 μm) to the recording electrode. For all experiments, the GABA_A receptor–mediated responses were isolated from glutamatergic γ-aminobutyric-5-methyl-4-isoxazole propionic acid and N-methyl-D-aspartate receptor–dependent responses by addition to the extracellular solution of LY306168 (10-5 M) and DL-2-amino-5-phosphonopentanoic acid (50 μM), which are selective antagonists of these receptors, respectively. In addition, possible confounding effects of GABA_A receptor–mediated responses were eliminated by including the selective GABA_A receptor antagonist, SCH50911 [(2S)-(+)-5,5-dimethyl-2-morpholinooctanoic acid] (10 μM), in the extracellular recording solution.

**Results**

The in vitro pharmacology of the novel mGlu2-prefering agonist, LY2812223, was investigated along with the control pharmacologically balanced mGlu2/3 agonist, LY379268, in cell lines stably expressing the human mGlu2 and mGlu3 subtypes across several signaling assays (Fig. 1; Table 1). GTPγS binding assays directed G protein activation by the mGlu subtypes, an event proximal to receptor stimulation. The FLIPR assay measures downstream intracellular calcium mobilization coupled through an artificially expressed calcium-coupling G protein, G15. The cAMP assay measures G1-mediated decreases in cyclic nucleotide formation after activation of the signaling enzyme, adenylate cyclase. The CellKey assay measures impedance changes through plate sensors, which are general cellular activation signals.

As previously observed in the mGlu2 receptor–expressing cell line, LY2812223 and the control pharmacologically balanced, maximally efficacious mGlu2/3 agonist LY379268 generated agonist concentration response curves with efficacies in the range of 80%–100% (with 100% being defined as a saturating glutamate response). In contrast, LY2812223 was not active at the cell line expressing mGlu3 receptors except in the highly amplified cAMP assay, where it displayed a partial agonist response. Under identical conditions, LY379268 elicited full agonist responses.

A chemogenomics approach was used to further validate the selectivity of LY2812223 (Fig. 2; Table 2). LY2812223 was active as a partial agonist in membranes derived from the brain cortex from wild-type and mGlu3-KO mice but not in mGlu2-KO membranes employing GTPγS binding as the functional readout. In contrast, the dual mGlu2/3 agonist LY379268 was active in both KO systems (68%–76% efficacy).

Furthermore, the pharmacology was assessed subsequently in rat brain cortex membrane preparations (Fig. 3; Table 3). Both LY2812223 and LY379268 fully displaced the mGlu2/3 agonist radioligand, [3H]LY459477 (2-amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid), with Ki values of 110.8 nM and 14.8 nM, respectively, indicating that each was binding to the same glutamate orthosteric domain with high affinity (Fig. 3; Table 3). In rat cortical brain membrane preparations, LY2812223 demonstrated partial agonist (37% efficacy) compared with the full agonist response of LY379268 in the GTPγS binding assay (93% efficacy). This phenomenon was similarly observed in the rat cortical synaptosome preparation measuring changes in cAMP production, where LY2812223 behaved as a partial agonist (63% efficacy) compared with the full agonist response of LY379268 in the KO systems (90% efficacy). LY2812223 also displayed a modest suppression of GABAergic synaptic transmission in the VB thalamus, consistent with a partial agonist effect on mGlu2/3 receptors.

LY2812223 and LY379268 were finally assessed by the GTPγS binding assay in cortical membranes derived from both nonhuman primates and human postmortem brain samples (Fig. 4; Table 4). As was observed in rodent brain tissue preparations, LY2812223 elicited partial and LY379268 elicited maximal agonist responses in both nonhuman primate and human brain membranes, respectively.
LY2812223 is a recently described novel, potent, and maximally efficacious mGlu2-preferring agonist (Monn et al., 2015). The pharmacological selectivity and efficacy was initially defined primarily in recombinant cell lines stably expressing the mGlu family of receptors. However, the pharmacology of this drug candidate was not evaluated previously in native brain tissue preparations derived from species typically used to test compounds for toxicological, pharmacokinetic, biomarker, and behavioral studies. A functional GTP\(_{\gamma}\)S binding assay that has been optimized to Fig. 1. Comparison of agonist-stimulated functional responses between different in vitro assay methodologies. The human mGlu2 (left) and mGlu3 (right) receptors were stably expressed in AV12 cells. (A–D) Agonist compounds used were LY2812223 (A and B) and the mGlu2/3 full agonist LY379268 (C and D). Responses are expressed as the percentage of maximal stimulation activity in presence of 100 \(\mu\)M of the orthosteric agonist glutamate. Stimulation curves were analyzed by nonlinear regression analysis and are the results of independent experiments performed in duplicate. Symbols and error bars are expressed as means ± S.E.M., respectively. hmGlu, human mGlu.

Table 1

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Format</th>
<th>Human mGlu2</th>
<th>Human mGlu3</th>
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<td>EC(_{50})</td>
<td>(E_{\text{max}})</td>
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<td>cAMP</td>
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<td>Ca(^{2+})</td>
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N/D, No data because experiment performed.
measure GPCR activation or inhibition in brain membranes derived from multiple species was employed (DeLapp et al., 1999; Porter et al., 2002; Overk et al., 2010). Comparisons to other signaling pathways were also assessed using mechanistically diverse signal transduction assays.

Pharmacological selectivity across species has been observed for other GPCR ligands, creating unique challenges for these targets (Chan et al., 2008). Generally, GPCR drug screening is performed against the human recombinant receptor stably expressed at high levels in a clonal mammalian or insect cell line. The hits are then counter-screened against other family member subtypes and a broad range of common GPCRs, ion channels, kinases, and so forth to determine the selectivity profile. As physiologic assessment is done primarily in rodents, the hits are also counter-screened against the rodent homolog of the target receptor. Due to cost and need for efficiency, rarely are hits also evaluated in functional assays across species used in subsequent drug development studies, unless issues arise. Here we have developed a facile functional GTP binding assay using brain membranes that provides pharmacological assessments across a variety of species, reducing downstream drug development risk. Our results revealed an unexpected partial agonist-like pharmacology for LY2812223 in cortical tissues, which were tightly conserved across species, such as those involving behavioral efficacy, pharmacokinetic parameters, biomarker discovery, and toxicology, would not be confounded based on pharmacologic disparities across species.

Drug discovery is currently experiencing a paradigm shift to include direct assessments of target engagement and subsequent activity within the target tissue, in this case cortical membranes, to properly interpret pharmacodynamic effects and efficacy both preclinically and clinically (Durham and Blanco, 2015). By doing so, data linking target engagement to pharmacodynamic effects and preclinical efficacy should enhance the clinical experiment outcome. To bridge this gap, we relied on the GTP binding assay to study the functional in vitro pharmacology of LY2812223 in native cortical membranes from several species. However, because native brain cortical tissue expresses both mGlu2 and mGlu3 subtypes, mGlu2- and mGlu3-KO mice were used to assess selectivity. Consistent with the mGlu2-prefering agonist activity of LY2812223 in heterologous cell systems, GTP binding signal was lost in the mGlu2-KO mice but not in the mGlu3-KO mice, indicating that LY2812223 prefers the mGlu2 receptor subtype as measured by the GTP binding assay. In a recent autoradiographic study using a highly selective group 2 mGlu radioligand, [3H]LY459477, as well as mGlu2-, mGlu3-, and mGlu23- double KO mice, it was shown that mGlu2 and mGlu3 have a significant overlap in expression, particularly in cortical and hippocampal regions (Wright et al., 2013). We found that both LY379268 and LY2812223 completely displaced LY459477 binding in native rat cortical membranes; these observations are unsurprising, given that these molecules exhibit similar affinity for both mGlu2 and mGlu3 subtypes (Monn et al., 1999, 2015). The observation of maximal agonist efficacy for LY379268 (a full agonist at both mGlu2 and mGlu3 receptors) but partial agonist responses for

<table>
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<tr>
<th>Mouse</th>
<th>GTP(\gamma)S Binding</th>
<th>Ca(^{2+}) Oscillations</th>
<th>GTP(\gamma)S Binding</th>
<th>Ca(^{2+}) Oscillations</th>
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<tr>
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<td>20.6 ± 2.0</td>
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N/D, No data because analysis not able to determine a value.
LY2812223 (a full agonist at mGlu2 but a low-efficacy agonist at mGlu3) in tissues derived from wild-type mice, rats, monkeys, and humans is curious and may point to a functional interplay between mGlu2 and mGlu3 receptors in the cortex. Although it is speculative, it is possible that this functional interaction arises from a physical mGlu2-mGlu3 heterodimer. Hetero- and homodimerization of mGlu receptors has been well documented, with mGlu2-mGlu3 heterodimers being a preferred pairing (Doumazane et al., 2011). Partial agonist responses in native tissues where both of these receptors are expressed could conceivably be consistent with results from studies showing that heterodimerization can modify specificity and efficiency of signaling compared with homodimerized receptors through intersubunit interactions and rearrangements (Brock et al., 2007; Kammermeier, 2012). If an mGlu2-mGlu3 heterodimer exists in the cortex, LY379268 would be expected to simultaneously bind to and activate both subunit proteins, whereas LY2812223 would be expected to bind to both subunits but activate only one-half of the dimeric complex. Possible effects of LY2812223 on native mGlu3

Fig. 3. Comparison between LY2812223 and LY379268 in the rat brain. (A and B) Radioligand binding from rat cortical membranes is shown in (A) and functional GTP-\(\gamma\)-S binding is shown in (B). (C and D) Rat synaptosomes are depicted in (C) and the effects of LY404039 and LY2812223 on mGlu3-mediated modulation of GABAergic synaptic inputs from rat brain slices are shown in (D). GTP-\(\gamma\)-[\(\beta\)S], GTP-\(\gamma\); IPSC, inhibitory postsynaptic current.

### TABLE 3
Summary of pharmacology in rat cortical tissue

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<tr>
<th>Agonist</th>
<th>(^{3}H)LY459477 Binding</th>
<th>GTP-(\gamma)-S Binding</th>
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<td>(K_T (n = 4))</td>
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<tr>
<td>LY2812223</td>
<td>110.8 ± 17.4</td>
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<td>LY379268</td>
<td>14.7 ± 0.5</td>
<td>7.6 ± 1.9</td>
<td>92.8 ± 4.4</td>
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TABLE 4
Summary of GTPγS binding agonist response data for LY2812223 and LY379268 in monkey and human cortical membranes

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<th>Species</th>
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<td>$EC_{50}$</td>
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<td>Monkey</td>
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</tbody>
</table>

Fig. 4. Agonist functional GTPγS binding to native tissue cortical membranes derived from nonhuman primates and humans. Tissues were collected from the postmortem cortex of humans (open triangles) and nonhuman primates (filled triangles). (A and B) Results for agonists LY2812223 (A) and LY379268 (B) are shown. Data shown are the result of two to four independent experiments. Symbols and error bars are expressed as means ± S.E.M., respectively. GTP-γ-[35S], GTPγS, NHP, non-human primate.

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References


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