Unique Signaling Profiles of Positive Allosteric Modulators of Metabotropic Glutamate Receptor Subtype 5 Determine Differences in In Vivo Activity

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Background: Metabotropic glutamate receptor subtype 5 ($mGlu_5$) activators have emerged as a novel approach to the treatment of schizophrenia. Positive allosteric modulators (PAMs) of $mGlu_5$ have generated tremendous excitement and fueled major drug discovery efforts. Although $mGlu_5$ PAMs have robust efficacy in preclinical models of schizophrenia, preliminary reports suggest that these compounds may induce seizure activity. Prototypical $mGlu_5$ PAMs do not activate $mGlu_5$ directly but selectively potentiate activation of $mGlu_5$ by glutamate. This mechanism may be critical to maintaining normal activity-dependence of $mGlu_5$ activation and achieving optimal in vivo effects.

Methods: Using specially engineered $mGlu_5$ cell lines incorporating point mutations within the allosteric and orthosteric binding sites, as well as brain slice electrophysiology and in vivo electroencephalography and behavioral pharmacology, we found that some $mGlu_5$ PAMs have intrinsic allosteric agonist activity in the absence of glutamate.

Results: Both in vitro mutagenesis and in vivo pharmacology studies demonstrate that VU0422465 is an agonist PAM that induces epileptiform activity and behavioral convulsions in rodents. In contrast, VU0361747, an $mGlu_5$ PAMs optimized to eliminate allosteric agonist activity, has robust in vivo efficacy and does not induce adverse effects at doses that yield high brain concentrations.

Conclusions: Loss of the absolute dependence of $mGlu_5$ PAMs on glutamate release for their activity can lead to severe adverse effects. The finding that closely related $mGlu_5$ PAMs can differ in their intrinsic agonist activity provides critical new insights that is essential for advancing these molecules through clinical development for treatment of schizophrenia.

Key Words: Agonist, allosteric modulators, convulsions, glutamate, $mGlu_5$, schizophrenia, seizure

A large number of clinical and preclinical studies increasingly support the hypothesis that selective activators of a metabotropic glutamate receptor subtype 5 ($mGlu_5$) may provide a novel therapeutic approach for treatment of schizophrenia, cognitive disorders, and some genetic childhood developmental disorders. A major breakthrough that allowed $mGlu_5$ selective activators to emerge as a viable approach was discovery of highly selective positive allosteric modulators (PAMs) of this receptor. Discovery of selective allosteric modulators is providing fundamental advances in our understanding of G protein-coupled receptor (GPCR) signaling and modulation, causing a paradigm shift in studies of GPCR signaling and drug discovery (1). Unlike traditional agonists, GPCR PAMs do not activate the receptor directly but maintain dependence on normal signaling mechanisms, selectively increasing receptor responses to endogenous agonists. Theoretically, this unique property of PAMs could provide therapeutic advantages by maintaining the normal temporal and spatial requirements of receptor activation by natural agonists. However, at present, there has been no clear demonstration that PAMs of $mGlu_5$ or other GPCRs can elicit fundamentally different effects from traditional agonists in intact systems.

One of the most exciting aspects of targeting $mGlu_5$ for treatment of schizophrenia is that $mGlu_5$ PAMs have potential utility in treatment of all major symptom domains (positive, negative, and cognitive symptoms) (1–4). Animal studies reveal that $mGlu_5$ PAMs have robust efficacy in rodent models predictive of antipsychotic efficacy and enhance multiple aspects of cognitive function (5–10). However, recent preliminary reports suggest that $mGlu_5$ PAMs may also have severe target-dependent adverse effects, including induction of behavioral convulsions (11–14), potentially preventing $mGlu_5$ PAMs from advancing to clinical development. Interestingly, traditional $mGlu_5$ agonists can also induce seizure activity (15,16). This is especially important in light of recent studies suggesting that some $mGlu_5$ PAMs exhibit allosteric agonist activity when evaluated in overexpressing cell lines (11,17); however, this has only been observed with artificially high levels of $mGlu_5$ expression (9). It is not known whether $mGlu_5$ PAMs that have been evaluated in vivo elicit functionally relevant agonist activity and

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the potential impact of allosteric agonist activity in the central nervous system (CNS) has not been evaluated.

Given the recent findings of adverse effects of $mGlu_5$ PAMs and the potential impact of authentic allosteric agonist activity in $mGlu_5$ PAMs if it exists, we engineered an inducible cell line allowing assessment of novel $mGlu_5$ PAMs for allosteric agonist activity with either high or low levels of $mGlu_5$ expression. Interestingly, we found that closely related $mGlu_5$ PAMs can exhibit fundamentally different profiles in terms of $mGlu_5$ allosteric agonist activity. Most notably, the $mGlu_5$ PAM VU0424465 has robust allosteric agonist activity in a low-expressing cell-based system and in multiple native systems. Moreover, VU0424465 induces intense epileptiform activity in hippocampal slices and generalized seizure activity and behavioral convulsions in rats. In contrast, closely related compounds behave as pure $mGlu_5$ PAMs with no observable agonist activity in any cell line or native system. These compounds exhibit in vivo efficacy in animal models predictive of antipsychotic activity but do not induce seizure activity or observable adverse effects. Thus, subtle structural changes can lead to a molecular switch of pure $mGlu_5$ PAMs to robust $mGlu_5$ allosteric agonists, dramatically altering the effects of $mGlu_5$ PAMs CNS. This provides direct demonstration that maintaining activity-dependence of $mGlu_5$ activation with pure $mGlu_5$ PAMs can provide major advantages, avoiding adverse effect liabilities observed with $mGlu_5$ agonists. These critical new mechanistic insights reveal key advantages of $mGlu_5$ PAMs and the critical importance of chemically optimizing pure PAMs using native systems and cell lines that do not overexpress $mGlu_5$ to achieve efficacy without inducing adverse effects associated with direct receptor activation.

Methods and Materials

Materials

Dulbecco's Modified Eagle's Medium, fetal bovine serum, and antibiotics were purchased from Invitrogen (Carlsbad, California). Dihydroxyphenylglycine (DHPG) and LY341495 were purchased from Ascent Scientific (Bristol, United Kingdom) and Tocris (Ellisville, Missouri), respectively. VU0422465 (18), VU0361747 (10), 5-methyl-6-(phenylethynyl)-pyridine (5MPEP) (19), and 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP) (20) were synthesized as described previously. Unless otherwise stated, all other reagents were purchased from Sigma-Aldrich (St. Louis, Missouri) and were either analytical or high-performance liquid chromatography grade.

Cell Culture and Mutagenesis

HEK293A cells lines stably expressing rat $mGlu_5$ were maintained (9), and point mutations were introduced into rat $mGlu_5$ and polyclonal stable cell lines generated (21), as described previously.

Fluorescence-Based Calcium Assays in Rat $mGlu_5$ Cells

Measurement of $mGlu_5$-mediated intracellular Ca^{2+} mobilization was performed using the Ca^{2+} sensitive dye, Fluo-4, and a Flexstation II, as described previously (9). Data were transformed and fitted using GraphPad Prism 5.0 (Graph-Pad Software, San Diego, California).

Brain Slice Electrophysiology

Animals were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were 5 to 6 weeks for long-term depression (LTD) and 24 to 30 days for epileptiform activity. LTD experiments were conducted as previously described (9). For epileptiform experiments, 400-μm transverse slices were made as described for LTD experiments, except that slices were transferred directly from cutting to room temperature artificial cerebrospinal fluid (ACSF; in mmol/L: 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 2 $CaCl_2$, 1.2 $MgCl_2$) for 1 hour. Slices were transferred to the recording chamber and perfused with 30°C to 32°C ACSF. Recording electrodes were placed in the cell body layer of CA3. Field potential recordings (spontaneous events) were acquired. $mGlu_5$ compounds or DHPG were applied using a perfusion system. Sampled data was analyzed offline using MiniAnalysis (Synaptosoft, Fort Lee, New Jersey) to determine the amplitude and interevent interval of the spontaneous events and normalized to the predrug period.

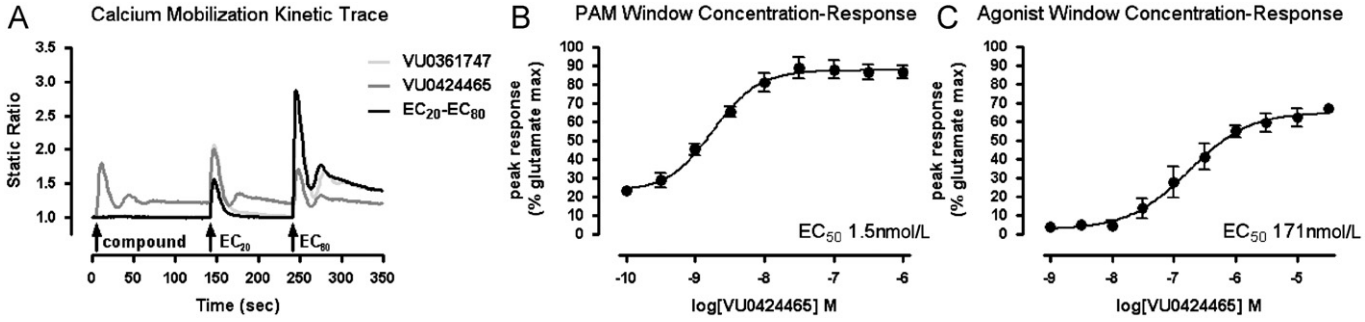

Figure 1. VU0424465, a metabotropic glutamate receptor subtype 5 ($mGlu_5$) positive allosteric modulator optimized for direct agonist activity, induces calcium mobilization in the absence of glutamate. **(A)** Representative raw calcium traces following the addition of 30 μmol/L VU0424465 (compound; 3–143 sec; agonist) and followed by an EC_{20} of glutamate (143–240 sec; positive allosteric modulator [PAM]) and EC_{80} of glutamate (240–350 sec); control trace represents the response to an EC_{20} and EC_{80} of glutamate following the addition of vehicle alone. Static ratio represents relative fluorescence units normalized to initial values. VU0424465, but not VU0361747, clearly induces a concentration-dependent release of calcium when added alone. Potencies were determined by adding a concentration-response curve of VU0424465 **(B)** followed by an EC_{20} of glutamate (PAM) or **(C)** in the absence of glutamate (agonist). VU0424465 potentiated the calcium mobilization evoked by glutamate in a concentration-dependence manner. The PAM EC_{50} of VU0424465 is 1.5 nmol/L, whereas the agonist potency is 171 nmol/L with a 65% maximum glutamate response. Data represent the mean ± SEM of five independent experiments performed in duplicate. For these studies, rat $mGlu_5$-expressing cell lines were used for full in vitro characterization of novel compounds to correlate in vitro data to in vivo studies in rats; similar potency/efficacy profiles were observed in human $mGlu_5$-expressing cell lines.