Published in final edited form as:

Neurosci Lett. 2008 September 19; 442(3): 239–243. doi:10.1016/j.neulet.2008.06.083.

Activation of metabotropic glutamate5 (mGlu5) receptors induces spontaneous excitatory synaptic currents in layer V pyramidal cells of the rat prefrontal cortex

Gerard J. Marek and Ce Zhang¹

Lilly Research Laboratories, Psychiatric Disorders Discovery Biology, Lilly Corporate Center, Mail Drop 0510, Eli Lilly and Company, Indianapolis, IN 46285

Abstract

Serotonin and norepinephrine, in addition to direct postsynaptic excitatory effects, also enhances glutamate release onto layer V pyramidal cells of the prefrontal cortex/neocortex via Gq/11-coupled 5-hydroxytryptamine_{2A} (5-HT_{2A}) and α_1 -adrenergic receptors, respectively. Therefore, the present study was designed to test whether a metabotropic glutamate (mGlu) receptor subtype also coupled to Gq/11-proteins, the mGlu5 receptor, also induces EPSCs when recording from layer V cortical pyramidal cells of the rat medial prefrontal cortex (mPFC). The mGlu1/5 receptor agonist (S)-3,5-dihydroxyphenylglycine (DHPG) induces EPSCs at a similar frequency as a near-maximally effective 5-HT concentration. The mGlu5 receptor negative allosteric modulator 2-methyl-6-(phenylethynyl)pyridine (MPEP; 300 nM) potently blocked DHPG-induced EPSCs. Previous work has suggested that activation of 5-HT_{2A} and OX2 receptors induces glutamate release, while mGlu2, mGlu4, and mGlu8 receptor activation suppress transmitter release from thalamocortical terminals. Taken together with past results, these findings suggest that mGlu2, mGlu4, mGlu5 and mGlu8 may all act to modulate glutamate release from afferents impinging on layer V pyramidal cells of the mPFC. These findings further suggest that monoamines, neuropeptides and glutamate itself all enhance the excitability of prefrontal cortical output cells indirectly via modulation of glutamate release.

Keywords

DHPG; MPEP; hallucinogens; excitatory post-synaptic currents; medial prefrontal cortex; glutamate; pyramidal cells; mGlu5 receptors

Activation of 5-HT_{2A} receptors induces psychotomimetic effects in healthy volunteers with a profile similar to the positive symptoms of schizophrenia [1,2]. Conversely, blockade of 5-HT_{2A} receptors by M100907 or eplivanserin was found to significantly improve psychotic symptoms of schizophrenic patients compared to placebo in several large randomized clinical trials [3,4]. The effect of the 5-HT_{2A} receptor antagonists were only approximately half that of the positive comparator haloperidol in these same trials, however. At a cellular level,

Corresponding author: Gerard J. Marek, M.D., Ph.D., Eli Lilly and Company, Lilly Research Laboratories, Lilly Corporate Center, Mail Drop 0510, Indianapolis, IN 46285.

¹Current address: Dr. Ce Zhang, Department of Neurobiology, Shanxi Medical University, 56# Zinjian South Road, Taiyuan, Shanxi 03001, Peoples' Republic of China

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activation of 5-HT_{2A} receptors, in addition to direct excitatory effects on cortical pyramidal cells in the rat prefrontal cortex [5], also appears to induce glutamate release from thalamocortical afferents [6,7]. The glutamate released subsequently activates AMPA receptors in both the proximal and apical dendrites of layer V pyramidal cells. Both μ -opioid heteroreceptors and group II and group III metabotropic glutamate (mGlu) receptors, demonstrating heteroreceptor and autoreceptor function (mGlu2; mGlu4 and mGlu8), respectively, suppress glutamate release and excitatory postsynaptic currents (EPSCs) induced by 5-HT [8–11]. This induction of excitatory synaptic currents in the primary output cells of the prefrontal cortex is associated with in vivo functional effects ranging from induction of BDNF and immediate early genes to behavioral effects such as DOI-induced head shakes [8, 12–16].

Activation of a number of other neurotransmitter receptors similarly induce excitatory synaptic currents in layer V pyramidal cells. The pharmacology for the modulation of synaptic currents induced by norepinephrine at α_1 -adrenergic receptors is similar to that of 5-HT_{2A} receptors with respect to presumed mGlu2 autoreceptors and μ -opioid heteroreceptors and postsynaptic AMPA receptors [17]. Furthermore, activation of orexin₂ (OX2) receptors and nicotinic $\alpha 4\beta 2$ receptors also appears to induce excitatory synaptic currents in layer V pyramidal cells with the source of glutamate originating from thalamocortical afferents [18,19]. While the $\alpha 4\beta 2$ nicotinic receptors are part of a cationic channel, the OX2 receptors are coupled to Gq₁₁ proteins as are 5-HT_{2A} and α_1 -adrenergic receptors. Given that previous preliminary studies suggested activation of mGlu5 receptors may also mediate similar synaptic currents, we sought first to characterize the effects of a mGlu1/5 agonist ((S)-3,5-dihydroxyphenylglycine, DHPG) and a negative allosteric modulator of the mGlu5 receptor (2-methyl-6-(phenylethynyl) pyridine, MPEP) on the function of prefrontal cortical layer V pyramidal cells [20,21]. The results suggest that the effects of mGlu5 receptor activation involve the induction of EPSCs when recording from layer V prefrontal cortical pyramidal cells.

Brain slices were prepared from male Sprague-Dawley rats (Harlan, Indianapolis, IN; 120–200 g) as described previously [22]. Briefly, rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated. Coronal slices (500 μ m) were cut with a oscillating-blade tissue slicer at a level corresponding to approximately 2.5 mm anterior to bregma [23]. A slice containing the medial prefrontal cortex (mPFC) was then transferred to the stage of a fluid-gas interface chamber which had a constant flow of humidified 95% O₂, 5% CO₂. The slices were perfused in a chamber heated to 34° C with normal ACSF which consisted of (in mM) NaCl 126; KCl 3; CaCl₂ 2; MgSO₄ 2; NaHCO₃ 26; NaH₂PO₄ 1.25; D-glucose 10. Intracellular recording and single-electrode voltage clamping were conducted in layer V pyramidal cells using an Axoclamp-2A (Axon Inst., Burlingame, CA) as previously described [6]. Stubby electrodes (~8 mm, shank to tip) with relatively low capacitance and resistance (30–60 M Ω) were filled with 1 M potassium acetate. The cells were voltage-clamped at –70 mV. The voltage-clamp signals were low-pass filtered (1,000 kHz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments Inc.). EPSC frequencies were obtained from 10 successive episodes (one second duration) during the baseline and drug treatment periods.

EPSC frequency and amplitude were determined with a “Mini Analysis Program” (Synaptosoft, Inc., www.synaptosoft.com); using thresholds of 5 pA and an area of ~150 fCs^{–1} for synaptic currents. Statistical comparisons of within-cell responses were made using two-tailed paired t-tests requiring p<0.05 for statistical significance. The determination of EC₅₀ values for the suppression of 5-HT-induced increases in EPSC frequency or of evoked EPSPs were calculated by non-linear curve fitting (Delta Graph).

The drugs used in this study were obtained from the following suppliers: Sigma Aldrich (St. Louis, MO, USA: 5-HT creatine sulfate; norepinephrine bitartrate); Alamone Labs (Jerusalem, Israel, tetrodotoxin (TTX); and Tocris (www.Tocris.com: (S)-3,5-dihydroxyphenylglycine, DHPG; 2-methyl-6-(phenylethynyl) pyridine hydrochloride, MPEP).

Layer V pyramidal cells of the mPFC (predominantly from the prelimbic area; Cg3) were recorded in a zone *ca* 1/2-2/3 the distance between the pial surface and the subcortical white matter. All of the cells in the present series (n=22) had the previously reported characteristics of regularly spiking pyramidal cells [24,25].

The frequency of EPSCs induced in layer V pyramidal cells of the mPFC by bath application of DHPG, 5-HT and NE was examined within the same set of cells (n=5, Fig. 1). The group I mGlu receptor agonist DHPG (50 μ M) induced ~ an 8-fold increase in EPSC frequency, similar to frequency of 5-HT-induced EPSCs. There was a highly significant correlation between the frequency of 5-HT- and DHPG-induced EPSCs ($r=0.997$). In contrast, NE induced ~ a 2-fold increase in EPSC frequency. The difference between the frequency of 5-HT- and NE-induced EPSCs was similar to a previous within-cell comparison [17]. In preliminary experiments, we found that the DHPG-induced EPSCs were modulated similarly to 5-HT-induced EPSCs. Thus, the mGlu2/3 receptor agonist LY354740 and the mGlu2/3 receptor antagonist LY341495 suppressed and enhanced, respectively, DHPG-induced EPSCs. Preliminary experiments also found that the μ -opioid receptor agonist DAMGO and the GluA1-4/GluK5 receptor antagonist LY293558 suppressed the DHPG-induced EPSCs (n=2).

The negative allosteric mGlu5 receptor modulator MPEP (300 nM) potently blocked DHPG-induced EPSCs recorded from a layer V mPFC pyramidal cells in a manner consistent with non-competitive inhibition. A between-cell comparison found that the maximal DHPG response (300 μ M) was 73.9% of the 100 μ M 5-HT response in the absence of MPEP (n=4) while the maximal DHPG response (1,000 μ M) was 41.9% of the 100 μ M 5-HT response in the presence of MPEP (n=5; Figure 2). The EC₅₀'s for the DHPG-induced EPSCs were 35.3 μ M (29.8–41.7 μ M 95% confidence interval) and 439 μ M (284–678 μ M 95% confidence interval) in the absence and presence of MPEP, respectively. The frequency of EPSCs for 100 μ M 5-HT-induced EPSCs in these same cells was 19.9 ± 2.8 and 25.2 ± 2.95 (N/s; mean $\pm$ SEM) without and with MPEP pretreatment, respectively.

TTX was applied to six cells. DHPG did not induce spontaneous EPSCs in any of the six cells following application of TTX which blocked impulse flow in each cell (not shown). The effects of DHPG were somewhat inconsistent with three cells showing outward currents and three shows demonstrating outward currents (Fig. 3). In general, only one of the six cells showed an effect consistent with blocking an inward current induced by activation of mGlu5 receptors when comparing the DHPG-induced currents before and after MPEP (300 nM). One reason for inconsistent effects of DHPG could be that DHPG engages GABA-A receptor mediated activity in some of the cells. However, it should be noted that following TTX under these conditions, the “noise” in the voltage tracing induced by synaptic currents is lost (not shown). This also argues against the notion that DHPG-induced synaptic currents could actually be GABA-A receptor mediated synaptic currents. However, not all sodium currents are equally sensitive to TTX. Nevertheless, there is no simple relationship between postsynaptic actions of mGlu5 receptor activation and the DHPG-induced EPSCs recorded from layer V pyramidal cells.

The present preliminary pharmacological studies demonstrate that activation of mGlu5 receptors induces spontaneous excitatory synaptic currents when recording from layer V pyramidal cells of the rat mPFC. The potency of MPEP in blocking the DHPG-induced EPSCs is in line with the potency (IC₅₀=36 nM at human mGlu5) and selectivity for mGlu5 over

human mGlu1b receptors (no agonist or antagonist activity up to 30 μ M; [20]). Previously, we and others have found evidence for inhibitory modulation of 5-HT-induced EPSCs from thalamocortical afferents by mGlu2, mGlu4, and mGlu8 receptors. The mGlu5 receptor mRNA is present throughout the intralaminar and midline thalamic nuclei of the rat [26]. While mGlu5 receptor protein in the rat may be predominantly located at postsynaptic sites on neurons or in glia throughout the rat prefrontal cortex, electron microscopic evidence for presynaptic mGlu5 receptors has been described in the neocortex [27], though not all studies are in agreement with this conclusion. In the primate prefrontal cortex, a greater percentage of mGlu1 α than mGlu5 receptors are located on spines and dendrites while a greater percentage of mGlu5 than mGlu1 α receptors are located on unmyelinated axons, terminals and glia [28]. Evidence suggesting that activation of cortical mGlu5 receptors induces release of excitatory amino acids has also been obtained with *in vitro* slice preparations or a cerebrocortical synaptosomal preparation [29, 30].

A portion of the DHPG-induced synaptic currents could reflect activity mediated by activation of GABA-A receptors. The EPSCs recorded under these conditions do not appear to be contaminated by reversed IPSCs mediated by GABA-A receptor activation for the following reasons. A small fraction of 5-HT-induced EPSCs (~15%) are blocked by bicuculline during intracellular recordings using KCl-containing electrodes suggesting the presence of some reverse IPSPs. In contrast, the 5-HT-induced EPSCs recorded with non-chloride containing electrodes (i.e., potassium acetate or gluconate) at holding potentials near E_{Cl} , are completely blocked by the AMPA/kainate antagonist LY293558 [6]. In preliminary experiments, the AMPA/kainate receptor antagonist LY293558 completely suppressed DHPG-induced synaptic currents.

The present studies do not rule out a role for mGlu1 receptors in these effects. Further experiments examining the effect of agonists/antagonists for group II and group III mGlu receptors on DHPG-induced EPSCs as improved pharmacological tools become available will be an important step towards better understanding the relationship of multiple mGlu receptor subtypes on the axons and axon terminals of thalamocortical afferents. The present studies also do not demonstrate whether mGlu receptor subtypes from all three groups are in the same axons/terminals. Nevertheless, the presence of mGlu receptor subtypes from all three major groups of mGlu receptors previously has been described such as the example of the suprachiasmatic nucleus [31].

Recently, it was suggested that there is a molecular complex between postsynaptic 5-HT_{2A} and mGlu2 receptors in the somatosensory cortex [32]. However, the conclusion that localization of 5-HT_{2A} receptors is solely at a postsynaptic site [33] may be flawed given the transgenic rescue strategies employed. The interpretation that the rescue of 5-HT_{2A} receptors in the neocortex was responsible for recovery of function was confounded by the simultaneous rescue by the same promoter of 5-HT_{2A} receptors in the claustrum, an additional source of glutamatergic afferents to layer V neocortical neurons. Furthermore, the attempted recovery of function by rescue of thalamic 5-HT_{2A} receptors using a promoter that is most heavily, if not entirely, expressed in primary sensory thalamic nuclei (which have a different laminar projection in the cortex, a different functional role in integrating the thalamocortical transmission, and a different complement of GPCRs) rather than the midline and intralaminar thalamic nuclei would not have been predicted to restore the effects of activating 5-HT_{2A} receptors in the neocortex.

The apparent presence of mGlu5 receptors inducing glutamate release from the same presumed terminals as contain 5-HT_{2A} and mGlu2 receptors raises an interesting question whether mGlu5 and mGlu2 receptors form a molecular complex. This same question could be asked of α 1-adrenergic and OX2 receptors as well. Analogously, the question could be asked if a number

of G_i/G_o -coupled receptors such as mGlu4, mGlu8, μ -opioid, and adenosine A1 receptors are similarly coupled in a molecular complex with this group of $G_{q/11}$ -coupled receptors (see Fig. 4).

While further lesion, tract tracing and other physiological experiments will be required to confirm the presynaptic localization of mGlu5 receptors to the same terminals as 5-HT_{2A} and α_1 -adrenergic receptors, the present preliminary experiments may shed some light on why the prodrug of the mGlu2/3 receptor agonist LY404039 appears to be more efficacious than would be expected for a 5-HT_{2A} receptor antagonist in the treatment of schizophrenia. Namely, there are other neurotransmitters capable of inducing spontaneous excitatory synaptic currents onto layer V pyramidal cells other than 5-HT_{2A} receptors (e.g., norepinephrine, orexin, glutamate, acetylcholine). In fact, atypical antipsychotics such as clozapine which also block both 5-HT_{2A} and α_1 -adrenergic receptors act similarly to the mGlu2/3 receptor agonist LY354740 in suppressing 5-HT-induced EPSCs at when recording from layer V pyramidal cells [34]. At face value, these thalamocortical synapses may be highly salient to behavioral effects relevant to psychiatric indications as the intralaminar and midline thalamic nuclei are involved in attention and arousal, are the sole thalamic projection to the striatum, and also significant projects to the subiculum and hippocampal complex, amygdala and throughout the association cortex. However, there is also significant potential for dissociations of the biological roles played by mGlu2/3 receptors compared to the range of $G_{q/11}$ -coupled and $G_{i/o}$ -coupled receptors implicated in thalamocortical synapses based on differences in the neuroanatomical localization for many of these receptors in a range of important limbic-related brain regions in which mGlu2/3 receptors are also found such as the dorsal and ventral striatum, thalamus, hippocampus and the amygdala.

Acknowledgments

The electrophysiological experiments were conducted in the Department of Psychiatry at the Yale University School of Medicine (Ribicoff Research Facilities of the Connecticut Mental Health Center). These studies were supported by PHS Grants K08 MH01551, R01 MH62186, a NARSAD Young Investigator Award, and the State of Connecticut (GJM).

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Figure 1.

The mGlu1/5 receptor agonist DHPG induces EPSCs in layer V pyramidal cells of the mPFC. DHPG (50 μ M), 5-HT (100 μ M), and NE (100 μ M) induces EPSCs as shown by an example (top) where several one sec traces were provided under basal conditions and following a one minute application of each drug. The summary graph (n=5) is shown below where all three Gq-coupled receptor agonists were assessed in each cell.

Figure 2.

The mGlu5 receptor antagonist MPEP blocks DHPG-induced EPSCs recorded from layer V pyramidal cells of the mPFC. DHPG (3–300 μ M, $n=4$) induced EPSCs in a concentration-dependent fashion (squares) while the apparent maximal effect of DHPG (3–1000 μ M) was reduced in an independent sample of cells in the presence of bath application MPEP (300 nM, $n=5$, circles) for 20 min prior to beginning the concentration-response relationship. All responses are plotted as the raw data without normalizing to the maximal 5-HT response (100 μ M) in each individual cell.

DHPG-induced current after TTX

Figure 3.

Post-synaptic effects of DHPG in layer V pyramidal cells following TTX treatment. Shown in the figure are the currents induced by DHPG after TTX treatment to which completely suppressed neuron firing and also in the absence or presence of MPEP (300 nM) for six different cells.

Figure 4.

The mGlu5 receptor acts as one of a number of $G_{q/11}$ -coupled receptors to induce glutamate release from glutamatergic terminals arising, in part, from the intralaminar and midline thalamic nuclei. Thalamic lesion studies have resulted in ~50% loss of 5-HT- or orexin agonist-induced EPSCs and have also resulted in 20–40% decreases in mGlu2 receptor and μ -opioid receptor binding in layers I and Va of the prefrontal cortex. Other potential sources of excitatory afferents to layer V pyramidal cells include the claustrum, an area involved in multi-modal sensory processing in the prefrontal cortex and the neocortex. Both 5-HT_{2A} and OX2 receptor activation could contribute to this non-thalamic input to layer V pyramidal cells. The inhibitory G_i/G_o -coupled receptors suppressing glutamate release from this non-thalamic source remain to be determined.