Physiological Antagonism between 5-Hydroxytryptamine<sub>2A</sub> and Group II Metabotropic Glutamate Receptors in Prefrontal Cortex<sup>1</sup>

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ABSTRACT

In prefrontal cortex, 5-hydroxytryptamine<sub>2A</sub> (5-HT<sub>2A</sub>) receptors have been linked to the action of hallucinogens and atypical antidepressant/antipsychotic drugs. Previously, we have shown in cortical layer V pyramidal cells that a nonselective metabotropic glutamate (mGlu) receptor agonist suppresses the induction of excitatory postsynaptic potentials/currents (EPSPs/EPSCs) via activation of 5-HT<sub>2A</sub> receptors. In this study, we tested the ability of the selective mGlu2/3 agonist (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740) and the selective mGlu2/3 antagonist 2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3(xanthy-9-yl)-3-propanoic acid (LY341495) to modulate serotonin(5-HT)-induced EPSPs and electrically evoked EPSPs by using intracellular recording from layer V pyramidal cells in medial prefrontal cortex. The mGlu2/3 antagonist LY341495 increased the frequency and amplitude of 5-HT-induced EPSCs, suggesting a role for mGlu2/3 receptors in mediating the action of endogenous glutamate on autoreceptors. Conversely, the mGlu2/3 agonist LY354740 was highly effective and potent (EC<sub>50</sub> = 89 nM) in suppressing glutamate release induced by 5-HT<sub>2A</sub> receptor activation in the medial prefrontal cortex, probably via a presynaptic mechanism. The mGlu2/3 antagonist LY341495 potently blocked the suppressant effect of LY354740 on 5-HT-induced EPSCs as well as electrically evoked early EPSPs. Autoradiography with the radioligands [3H]LY354740 and [3H]1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane shows a striking overlap of the laminar distribution of mGlu2/3 and 5-HT<sub>2A</sub> receptors in the medial prefrontal cortex that is not apparent in other cortical regions. These findings suggest a close coupling between mGlu2/3 and 5-HT<sub>2A</sub> receptors in the prefrontal cortex that may be relevant for novel therapeutic approaches in the treatment of neuropsychiatric syndromes such as depression and schizophrenia.

5-Hydroxytryptamine<sub>2A</sub> (5-HT<sub>2A</sub>) receptor antagonists block the psychotomimetic effects of hallucinogens in humans (Vollenweider et al., 1998) and are thought to contribute to the therapeutic effects of atypical antidepressant/antipsychotic drugs (Kroeze and Roth, 1998; Marek and Aghajanian, 1998b). Furthermore, increased glutamate release in the prefrontal cortex appears to be a common feature shared by both noncompetitive N-methyl-D-aspartate antagonists and hallucinogenic drugs (Aghajanian and Marek, 1999b), both of which mimic some of the symptoms of acute psychosis. 5-HT<sub>2A</sub> receptor activation increases the frequency of “spontaneous” (nonelectrically evoked) excitatory postsynaptic currents (EPSCs) in apical dendrites of neocortical layer V pyramidal cells in a novel manner, suggesting focal release of glutamate from discrete pathways (Aghajanian and Marek, 1997). This induction of an increase in the frequency of EPSCs by serotonin (5-HT) is completely blocked by the α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)/kainate antagonist LY293558 (Aghajanian and Marek, 1997). The ability of 5-HT<sub>2</sub> antagonists to block 5-HT-induced EPSCs is clearly mediated through 5-HT<sub>2A</sub>.

ABBREVIATIONS: 5-HT<sub>2A</sub>, 5-hydroxytryptamine<sub>2A</sub>; mGlu, metabotropic glutamate; EPSPs, excitatory postsynaptic potentials; EPSCs, excitatory postsynaptic currents; 5-HT, serotonin; L-SOP, L-serine-O-phosphate; LY354740, (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate; LY379268, (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate; DOI, (−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; TTX, tetrodotoxin; ACSF, artificial cerebrospinal fluid.
rather than 5-HT<sub>2C</sub> receptors (Aghajanian and Marek, 1997; Marek and Aghajanian, 1999).

The potent partial 5-HT<sub>2A/2C</sub> agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a hallucinogenic drug, induces an increase in the frequency of EPSCs, reaching only ~10% of the level for 5-HT itself. However, DOI induces an increase in the late component of electrically evoked EPSCs, an effect which is blocked by the selective 5-HT<sub>2A</sub> antagonist and putative antipsychotic drug M100,907 (Aghajanian and Marek, 1999a). We have previously suggested that a common mechanism of glutamate release may underlie both the increased frequency of 5-HT-induced EPSCs and electrically evoked late EPSPs occurring after DOI application (Aghajanian and Marek, 1999a). This common mechanism may involve the “asynchronous” pathway of glutamate release because addition of Sr<sup>2+</sup> to a Ca<sup>2+</sup>-free artificial cerebrospinal fluid (ACSF) supported 5-HT-induced EPSCs under conditions in which the “synchronous” pathway of glutamate release was blocked (Goda and Stevens, 1994; Aghajanian and Marek, 1999a). Furthermore, addition of Sr<sup>2+</sup> to a Ca<sup>2+</sup>-free ACSF also supported electrically evoked late EPSCs under conditions in which the evoked early EPSCs, via the synchronous pathway of glutamate release (Goda and Stevens, 1994), were blocked. The electrically evoked late EPSCs after DOI application appeared similar to the electrically evoked late EPSCs from the Sr<sup>2+</sup>-substitution experiments. Whether the electrically evoked late EPSCs represent asynchronous release of transmitter or conventional polysynaptic EPSCs, agents that suppress prefrontal glutamate release induced by activation of 5-HT<sub>2A</sub> receptors could provide a novel therapeutic approach to the treatment of depression and schizophrenia (Marek and Aghajanian, 1998b).

One possible approach to suppressing glutamate release is through metabotropic glutamate (mGlu) receptors because many subtypes function as presynaptic autoreceptors on glutamatergic terminals (Conn and Pin, 1997). The mGlu receptors are a novel family of glutamate G-protein coupled receptors that are commonly separated into three classes based on both pharmacology and signal transduction pathways (Conn and Pin, 1997; Schoepf et al., 1999). Group I mGlu receptors (e.g., mGlu1 and mGlu5) are coupled to phospholipase C and phosphoinositide hydrolysis. In contrast, both group II (e.g., mGlu2 and mGlu3) and group III (e.g., mGlu4, mGlu6, mGlu7, and mGlu8) are negatively coupled to cAMP formation and are thought to function as inhibitory presynaptic autoreceptors that may play a role in synaptic plasticity (Conn and Pin, 1997; Li et al., 1998). This group II and group III mGlu receptors have overlapping, but distinct, patterns of mRNA expression in the rat central nervous system (Ohishi et al., 1993a,b, 1995; Saugstad et al., 1997).

Previously, a relatively nonspecific group II/III mGlu agonist, (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; 200 μM), was found to suppress 5-HT<sub>1A</sub>-induced EPSCs (Aghajanian and Marek, 1997). Recently, novel conformationally constrained analogs of glutamate that are selective for the group II mGlu receptors at low concentrations, including two agonists, (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740) and (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268), and an antagonist, (2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-xanthyl-9-yl)propanoic acid (LY341495), have been developed (Monn et al., 1997, 1999; Schoepf et al., 1997; Fitzjohn et al., 1998; Kingston et al., 1998). We now provide evidence that physiological as well as pharmacological activation of mGlu2/3 receptors suppresses glutamate release induced by 5-HT<sub>2A</sub> receptor activation in the medial prefrontal cortex (transitional neocortex including both the prelimbic area of the medial prefrontal cortex and the anterior cingulate). Furthermore, a striking laminar overlap was found in the medial prefrontal cortex compared with other cortical regions for 5-HT<sub>2A</sub> and mGlu2/3 receptor binding that may be relevant to targeting drugs for neuropsychiatric syndromes involving the prefrontal cortex.

**Materials and Methods**

**Autoradiography.** Whole brains were obtained from adult male Sprague-Dawley rats (120–200 g), rapidly frozen in powdered dry ice, and mounted on cryostat chucks. Coronal sections (20 μm) were cut from the prefrontal cortex and thaw-mounted on gelatin-coated slides. Sections were stored at −20°C before use. For [H]LY354740 binding, tissue sections were preincubated in ice-cold 10 mM potassium phosphate buffer with 100 mM potassium bromide (phosphate/bromide buffer), pH 7.6, for 30 min to remove endogenous receptor ligands, then rapidly dried under a stream of cool air. Then the sections were incubated for 60 min at room temperature in phosphate/bromide buffer with 50 nM [H]LY354740 (custom prepared by Amershaw, Buckinghamshire, England). Nonspecific binding was determined using adjacent sections with 1 mM 1-glutamate in the buffer solution. After incubation, each slide was rinsed rapidly three times with 2 ml of ice-cold phosphate/bromide buffer followed by a final rinse with 2 ml of ice-cold glutaraldehyde (49%)/acetone mixture (1:19, v/v), then quickly dried under a stream of cool air. Rinse time was <10 s per slide. Sections were opposed, with tritium microscapes, to tritium-sensitive film (Hyperfilm-<sup>3</sup>H, Amersham, Piscataway, NJ) for 14 days.

For measurement of 5HT<sub>2A</sub> binding sites, tissue sections from the same rats were incubated for 90 min at room temperature in a pH 7.4 buffer (50 mM Tris/HCl, 4 mM CaCl<sub>2</sub>, 0.1% ascorbate, and 0.1% bovine serum albumin) with 0.5 nM [H]DOI (NEN Life Science Products, Inc., Boston, MA). Nonspecific binding was defined by incubation of adjacent sections in buffer and radioligand with 1 μM M100,907 (formerly known as MDL100,907) added. After incubation, the slides were rinsed twice for 10 min in ice-cold assay buffer followed by a brief immersion in ice-cold purified water (Milli-Q Millipore, Bedford, MA), then dried under a stream of warm air. Tissue sections were opposed, with [H]Microscapes, to [H]-sensitive film (Amersham, Piscataway, NJ) for 30 h.

Quantitative autoradiogram analysis was performed with a computer-assisted image analyzer (MCID; Imaging Research Inc., St. Catherine, Ontario). Optical density values were converted to femtomole per milligram of protein by using a computer-generated regression analysis. Values displayed in Fig. 4 represent data averaged from four rats with six sections per rat. This data was analyzed with a one-way ANOVA and testing of layers significantly different from layer Va with the Neumann-Keuls multiple comparison test (GraphPad Prism; GraphPad Software, Inc., San Diego, CA).

**Membrane Binding Methods.** Well washed crude membranes of the rat forebrain were prepared as described previously (Wright et al., 1994). Cell membranes from human mGlu2- and human mGlu3-expressing RGT cells (cells transfected with a rat glutamate transporter) were obtained as described previously (Johnson et al., 1999). Frozen tissue pellets were thawed on the day of assay and washed three times with ice-cold assay buffer (phosphate/bromide buffer). To start the reaction, rat tissue (0.15–0.20 mg of protein) or mGlu2 or mGlu3 tissue (0.04–0.06 mg of protein) was added to deep-well polystyrene microtiter plates or 5-ml scintillation vials (for rat brain tissue) that contained [H]LY354740 (10 nM) and appropriate concentrations of test compounds in assay buffer. Final assay volume
was 0.5 ml. Nonspecific binding was defined with 1 mM l-glutamate. Samples were incubated on ice for 60 min and bound radioligand was separated from free radioligand by rapid filtration with washes with 1 ml of ice-cold assay buffer using a Whatman GF-B filter for mGlu2 or mGlu3 binding. For rat brain tissue, bound [3H]LY354740 was separated from free [3H]LY354740 by centrifugation as described previously (Wright et al., 1994). Protein concentration was determined by using the Pierce Coomassie micro assay (Rockford, IL). Kᵢ values were determined by using nonlinear regression in the GraphPad Prism program (GraphPad Software, Inc.).

**Electrophysiology.** Brain slices were prepared from male Sprague-Dawley rats (120–200 g) as described previously (Aghajanian and Rasmussen, 1989). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg; i.p.) and decapitated. Coronal slices (500 µm) were cut with an oscillating-blade tissue slicer at a level corresponding to approximately 2.5 mm anterior to bregma ( Paxinos and Watson, 1986). A slice containing the medial prefrontal cortex 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 126 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 2 mM MgSO₄, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, and 10 mM D-glucose.

Intracellular recording and single-electrode voltage clamping were conducted in layer V pyramidal cells by using an Axoclamp-2A (Axon Instruments, Inc., Foster City, CA) as previously described (Aghajanian and Marek, 1997). Stubby electrodes (~8 mm, shank to tip) with relatively low capacitance and resistance (30–60 MΩ) were filled with 1 M potassium acetate. The cells were voltage clamped at −70 mV. The EPSCs recorded under these conditions do not appear to be contaminated by reversed inhibitory postsynaptic currents for the following reasons. Only 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 inhibitory postsynaptic currents. In contrast, the 5-HT-induced EPSCs recorded with nonchloride-containing electrodes (i.e., potassium acetate or gluconate) at holding potentials near Ec₅₀ are completely blocked by the AMPA/kainate antagonist LY293558 (Aghajanian and Marek, 1997). The voltage-clamp signals were low-pass filtered (1000 Hz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments, Inc.). EPSC frequencies were obtained from 10 successive episodes (1-s duration) during the baseline and drug treatment periods. Evoked potentials were obtained while holding cells at −80 mV and stimulating the forceps constant depolarizing pulse, had the previously reported characteristics (McCormick et al., 1985; Connors and Gutnick, 1990) of regularly spiking pyramidal cells.

Electrophysiological Characteristics of Cortical Layer V Pyramidal Cells. Layer V pyramidal cells were recorded in a zone ca. 1/2 to 2/3 the distance between the pial surface and the subcortical white matter. The pyramidal cells in the present study had the following characteristics: resting potential, −70.7 ± 0.8 mV; action potential amplitude, 81.0 ± 0.8 mV; action potential duration (at half-amplitude), 0.78 ± 0.02 ms; input resistance (−0.4 ± 0.02 ms); TTX-sensitive, 34.3 ± 2.3 MΩ (n = 71). All of the cells in the present series, except a single cell with an intrinsically bursting firing pattern to a constant depolarizing pulse, had the previously reported characteristics (McCormick et al., 1985; Connors and Gutnick, 1990) of regularly spiking pyramidal cells.

**Results**

**Binding Affinity of mGlu2/3 Agonists and Antagonist.** The mGlu2/3 agonist LY354740 potently bound to human mGlu2 and mGlu3 receptors with a slight selectivity for mGlu2 compared with mGlu3 (Table 1; Fig. 1), consistent with a previous report of a ~5-fold selectivity of LY354740 for mGlu2 versus mGlu3 in a functional assay (Schoepp et al., 1997). LY379268, another mGlu2/3 agonist, potently bound to human mGlu2 and mGlu3 receptors with a slight selectivity for mGlu2 versus mGlu3 (Table 1; Fig. 1), but selectivity for LY379268 at mGlu2 versus mGlu3 receptors has not been observed in a functional assay or in a binding assay (Monn et al., 1999) using the antagonist LY341495 as the radioligand (Johnson et al., 1999). LY341495 bound with a nanomolar potency to both mGlu2 and mGlu3, but with ~3-fold selectivity for mGlu2 versus mGlu2. All three mGlu2/3 ligands fully displaced [3H]LY354740 from rat forebrain binding sites with nanomolar potency (Fig. 1). Importantly, the rank order of potency for all three mGlu2/3 ligands at displacing the radioligand from the rat forebrain binding sites was similar to that found with human mGlu2/3 receptors transfected into the RGT cells.

**Electrophysiological Characteristics of Cortical Layer V Pyramidal Cells.** Layer V pyramidal cells were recorded in a zone ca. 1/2 to 2/3 the distance between the pial surface and the subcortical white matter. The pyramidal cells in the present study had the following characteristics: resting potential, −70.7 ± 0.8 mV; action potential amplitude, 81.0 ± 0.8 mV; action potential duration (at half-amplitude), 0.78 ± 0.02 ms; input resistance (−0.4 ± 0.02 ms); TTX-sensitive, 34.3 ± 2.3 MΩ (n = 71). All of the cells in the present series, except a single cell with an intrinsically bursting firing pattern to a constant depolarizing pulse, had the previously reported characteristics (McCormick et al., 1985; Connors and Gutnick, 1990) of regularly spiking pyramidal cells.

The mGlu2/3 Antagonist LY341495 Enhances the Frequency and Amplitude of 5-HT-Induced EPSCs. We had previously observed that the AMPA/kainate antagonist LY293558 and the nonselective mGlu agonist (1S,3S)-1-amino-cyclopropane-1,3-dicarboxylic acid (ACPD) completely blocked 5-HT-induced EPSCs in a fashion suggesting that

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kᵢ (nM)</th>
<th>95% CI</th>
<th>Kᵢ (nM)</th>
<th>95% CI</th>
<th>Kᵢ (nM)</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>LY354740</td>
<td>47.8 ± 15</td>
<td>24.7–64.4</td>
<td>65.8 ± 27</td>
<td>66.2–146</td>
<td>21.4 ± 2.0</td>
<td>17.2–27.5</td>
</tr>
<tr>
<td>LY379268</td>
<td>6.65 ± 1.3</td>
<td>4.03–9.08</td>
<td>12.8 ± 5.3</td>
<td>5.73–16.9</td>
<td>1.45 ± 0.18</td>
<td>0.85–2.08</td>
</tr>
<tr>
<td>LY341495</td>
<td>6.01 ± 1.3</td>
<td>3.40–9.96</td>
<td>2.52 ± 0.6</td>
<td>1.39–3.97</td>
<td>0.68 ± 0.1</td>
<td>0.31–1.08</td>
</tr>
</tbody>
</table>

Affinities for displacement by LY354740, LY379268 and LY341495 at cloned human mGlu receptors (mGlu2 and mGlu3) and rat brain homogenates of [3H]LY354740 binding. All values displayed are means ± S.E. (n = 3).
these drugs may be acting at a postsynaptic and a presynaptic site, respectively (Aghajanian and Marek, 1997). To test whether tonic release of endogenous glutamate might be acting on inhibitory autoreceptors to reduce the sustained increase in EPSCs induced by 5-HT, the effects of the highly selective mGlu2/3 antagonist LY341495 (Kingston et al., 1998) on 5-HT-induced EPSCs were investigated with intracellular recordings from layer V pyramidal cells of the medial prefrontal cortex. It should be noted that LY341495 (≤1 μM) does not block the suppressant action of the group III mGlu receptor agonist L-serine-O-phosphate (L-SOP) on 5-HT-induced EPSPs (unpublished observations, G.J.M.). The mGlu2/3 antagonist LY341495 (20 min) enhanced the frequency of 5-HT-induced EPSCs by 30 to 65% (0.1–30 μM; Fig. 2; Table 2); a significant increase in the frequency of EPSCs was observed first at 100 nM, although a trend was present for a significant increase at 30 nM (P = .09, n = 4). The classical interpretation of a change in the frequency of synaptic currents is via an effect on the presynaptic terminal (Van der Kloot, 1991). LY341495 also increased the amplitude of 5-HT-induced EPSCs by 12 to 21% (100 nM to 30 μM; Fig. 2; Table 1). In 16 of the 19 cells (4 cells were tested with two or more concentrations), LY341495 significantly increased the EPSC amplitude for the 5-HT-induced EPSCs (Kolmogorov-Smirnov test, P < .05, n = 4). A change in the amplitude of synaptic currents could be mediated by either a presynaptic or postsynaptic effect (Van der Kloot, 1991). LY341495 did not alter the amplitude of electrically evoked early EPSPs (data not shown, n = 2) or the amplitude of spontaneously occurring EPSCs (contrast Fig. 2, A1 and A3; Table 2) observed under basal conditions in 17 of the 19 cells tested (Kolmogorov-Smirnov test, P < .05). Together, these results suggest that mGlu2 and/or mGlu3 function as autoreceptors that are tonically activated by physiological concentrations of glutamate released via 5-HT2A receptor activation.

mGlu 2/3 Agonists Suppress 5-HT-Induced EPSCs and Electrically Evoked EPSPs. Intracellular recordings from layer V pyramidal cells of the medial prefrontal cortex were used to test mGlu2/3 agonists for both the suppression of 5-HT-induced EPSCs and the increase in electrically evoked late EPSPs after application of the 5-HT2A agonist DOI. LY354740 (Schoepp et al., 1997) was virtually equipotent at suppressing 5-HT-induced EPSCs (n = 5) and DOI-enhanced electrically evoked late EPSPs (n = 5) (EC50 values: 89.1 versus 85.3 nM). In contrast, LY354740 was 3- to 5-fold less potent at suppressing electrically evoked early EPSPs (EC50 values: 235 and 454 nM; Table 3; Fig. 3C; Fig. 4, bottom). LY366563 (Monn et al., 1997; Schoepp et al., 1998) (1 μM), the inactive enantiomer of LY354740, did not suppress 5-HT-induced EPSCs in any of the four cells from the medial prefrontal cortex tested (data not shown). The suppressant effect of LY354740 on 5-HT-induced EPSCs showed regional variability in that neurons from the medial prefrontal cortex were 4-fold more sensitive to this drug than were a random sample of cells (n = 3) from the Fr1, Fr3, and Par1 areas of the frontoparietal neocortex (Paxinos and Watson, 1986) (EC50 = 398 ± 142, n = 3). Another mGlu2/3 agonist, LY379268 (Table 3), was approximately equipotent in suppressing 5-HT-induced EPSCs and the electrically evoked early EPSPs and late EPSPs after DOI application (EC50 values: ~230–276 nM, n = 4).

A Selective mGlu2/3 Antagonist Blocks the Effects of LY354740. A concentration of mGlu2/3 antagonist LY341495 (1 μM, G.J.M., unpublished observations) was tested...
against the suppressant action of the mGlu2/3 agonist LY354740. LY341495 resulted in a robust rightward shift in the concentration-response curve for the suppression by LY354740 against the frequency of 5-HT-induced EPSCs, yielding a pA2 value in the nanomolar range (pA2 = 7.27, n = 5, Fig. 4). LY341495, which is ~3-fold more potent at mGlu3 than mGlu2 receptors, was ~4-fold more potent at blocking the suppressant effect of LY354740 on electrically evoked early EPSPs (pA2 = 7.9, n = 5, Fig. 4) than on 5-HT-induced EPSCs (Table 1). The antagonism by LY341495 against suppression by LY354740 for both the 5-HT-induced EPSCs and the electrically evoked early EPSPs was surmountable by higher concentrations of LY354740, which is consistent with competitive antagonism. The potency of LY341495 in blocking the suppressant action of LY354740 on electrically evoked late EPSPs after DOI application was not tested.

Presynaptic versus Postsynaptic Action of LY354740. The 5-HT-induced EPSCs are mediated via AMPA/kainate receptors on layer V pyramidal cells because they are completely blocked by the AMPA/kainate antagonist LY293558 (Aghajanian and Marek, 1997). To evaluate whether a postsynaptic action could be involved in the suppression by mGlu agonists of the 5-HT-induced EPSCs, the effect of LY354740 on inward currents induced by bath application of AMPA (5 µM) was examined in layer V pyramidal cells. After treatment of the slice with TTX (2 µM) to block impulse flow, LY354740 did not block AMPA-induced inward currents (AMPA, 243 ± 74 pA; LY354740 and AMPA, 233 ± 75 pA; n = 4; Fig. 5). LY354740 did induce small outward currents in two of the four cells after TTX treatment (40 and 60 pA). The effects of LY354740 and AMPA after TTX treatment were similar when the experiment was repeated by
applying the drugs in ACSF containing no Ca\(^{2+}\) \((n = 2\), data not shown).

A more direct test of whether the suppressant effect of LY354740 on 5-HT-induced EPSCs was mediated via a presynaptic site involved analyzing the cumulative probability distributions of the EPSC amplitudes. In four of five cells, a concentration of LY354740 at approximately the EC\(_{50}\) for reducing the frequency of 5-HT-induced EPSCs did not cause a significant change \((P > 0.05)\) in the EPSC amplitude as assessed with the Kolmogorov-Smirnov test (Fig. 5B). The selective blockade in the frequency rather than the amplitude of 5-HT-induced EPSCs also argues for a presynaptic locus of action for LY354740 (Van der Kloot, 1991).

\[125\text{I}]\text{DOI} \text{ and [3H]LY354740 Autoradiography.}\ To begin an examination of the neuroanatomical basis of the interaction between 5-HT\(_{2A}\) receptors and the group II mGlu receptors, we compared the autoradiographic pattern of binding for \([125\text{I}]\text{DOI}\) and \([3\text{H}]\text{LY354740}\) (Schoepp et al., 1997) in coronal brain sections including the medial prefrontal cortex by using concentrations of radiolabeled ligands to specifically label 5-HT\(_{2A}\) and mGlu2/3 receptors, respectively (Figs. 6 and 7). Both \([125\text{I}]\text{DOI}\) and \([3\text{H}]\text{LY354740}\) bound to the superficial and mid-layer of the medial prefrontal cortex. The laminar distribution of \([125\text{I}]\text{DOI}\) is consistent with previous observations for peaks in specific 5-HT\(_{2A}\) receptor binding in layers I and Va of the neocortex and transitional cortex (Blue et al., 1988). In all areas of the neocortex and transitional neocortex, the \([3\text{H}]\text{DOI}\) binding was highest in layer Va and significantly lower in the intermediate layers (layers II–IV). In contrast to the medial prefrontal cortex where \([3\text{H}]\text{LY354740}\) binding was significantly lower in layers II/III than in layer Va, in the frontoparietal region e.g., Par1), \([3\text{H}]\text{LY354740}\) binding was significantly higher in layers II/III/IV than in layer Va.

### Discussion

The most striking finding from the present studies was that, in the absence of an exogenous agonist, the potent group II mGlu antagonist LY341495 increased the frequency and amplitude of 5-HT-induced EPSCs. This increase occurred at concentrations of LY341495 \((\leq 1 \mu M)\) that do not block the suppressant action on 5-HT-induced EPSCs by the group III mGlu agonist L-SOP. The mGlu antagonist did not alter the amplitude of either the basal EPSCs or the electrically evoked early EPSPs, these being situations where no tonic activation of presynaptic autoreceptors by endogenous glutamate would be expected. However, when a vigorous and sustained increase in EPSCs occurred during the application of 5-HT, the frequency and amplitude of synaptic currents was almost always enhanced by the mGlu antagonist. These findings are consistent with the hypothesis that mGlu2/3 receptors function as inhibitory autoreceptors in cortical glutamatergic terminals whose transmitter release is positively regulated by 5-HT\(_{2A}\) receptor activation. These observations appear to be similar to the previous demonstration in the hippocampus of a use-dependent activation of presynaptic mGlu receptors that function to decrease excitatory amino acid release (Scanziani et al., 1997).

Conversely, mGlu2/3 agonists suppress glutamate release induced by 5-HT\(_{2A}\) receptor activation from nerve endings that terminate onto cortical layer V pyramidal cells. Two potent and selective mGlu2/3 agonists, LY354740 (Mann et al., 1997; Schoepp et al., 1997) and LY379268 (Mann et al., 1999), suppressed the increase in EPSCs induced by 5-HT and the enhancement of electrically evoked late EPSPs induced by the partial 5-HT\(_{2A}\) agonist DOI with a similar potency. This action was not shared by LY366553, the inactive (−)-isomer of LY354740. Furthermore, the selective mGlu2/3 antagonist LY341495 blocked the suppressant action of LY354740 on 5-HT-induced EPSCs or electrically evoked early EPSPs with potencies consistent with pharmacological antagonism of mGlu2/3 receptors (Kingston et al., 1998).

Although both mGlu2/3 agonists LY354740 and LY379268 suppressed 5-HT-induced EPSCs, the 3-fold greater potency of LY354740 over LY379268 is surprising in light of the greater affinity of LY379268 over LY354740 at mGlu2, mGlu3, and rat forebrain binding sites. In this context, it should be noted that LY354740 and LY379268 are conformationally constrained analogues of glutamic acid (Mann et al., 1997, 1999). Perhaps differences in the potency of these agonists in receptor binding assays compared with functional assays in tissue slices could reflect differences in the ability of these compounds to access the receptor due to differing affinities at other synaptic sites such as glutamate transporters.

The mGlu2/3 agonists appear to act on a presynaptic site to decrease glutamate release. In four of five cells, LY354740 did not alter the amplitude of 5-HT-induced spontaneous EPSCs at a concentration that decreased frequency by 50%.

### Table 2

<table>
<thead>
<tr>
<th>LY341495</th>
<th>n</th>
<th>Basal</th>
<th>5-HT</th>
<th>LY341495</th>
<th>LY341495/5-HT</th>
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<td>EPSC frequency</td>
<td></td>
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<tr>
<td>30 nM ((n = 4))</td>
<td>4</td>
<td>5.1 ± 0.9</td>
<td>34.7 ± 13.7(^a)</td>
<td>5.3 ± 1.2</td>
<td>42.8 ± 16.8</td>
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<tr>
<td>100 nM ((n = 6))</td>
<td>5</td>
<td>8.4 ± 1.3</td>
<td>18.5 ± 6.2(^a)</td>
<td>5.0 ± 1.2</td>
<td>24.2 ± 7.1(^b)</td>
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<tr>
<td>1 \mu M ((n = 6))</td>
<td>6</td>
<td>6.3 ± 1.3</td>
<td>31.6 ± 9.5(^a)</td>
<td>4.5 ± 1.4</td>
<td>45.3 ± 10.4(^b)</td>
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<tr>
<td>10 \mu M ((n = 5))</td>
<td>5</td>
<td>5.4 ± 1.4</td>
<td>18.8 ± 5.1(^a)</td>
<td>7.6 ± 3.6</td>
<td>31.3 ± 9.3(^b)</td>
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<tr>
<td>30 \mu M ((n = 4))</td>
<td>4</td>
<td>4.9 ± 1.3</td>
<td>21.1 ± 7.8(^a)</td>
<td>4.4 ± 1.9</td>
<td>34.6 ± 10.0(^b)</td>
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<tr>
<td>EPSC amplitude</td>
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<tr>
<td>30 nM</td>
<td>4</td>
<td>67.4 ± 5.0</td>
<td>83.1 ± 7.8</td>
<td>63.4 ± 6.5</td>
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<td>1 \mu M</td>
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<td>57.8 ± 3.9</td>
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<tr>
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<td>81.4 ± 5.6</td>
<td>77.1 ± 6.0</td>
<td>98.3 ± 5.4(^b)</td>
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<tr>
<td>30 \mu M</td>
<td>4</td>
<td>68.2 ± 3.5</td>
<td>80.0 ± 4.9(^a)</td>
<td>68.1 ± 8.6</td>
<td>92.1 ± 6.9(^b)</td>
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\(^a\) \(P < 0.05\) compared to basal value.

\(^b\) \(P < 0.05\) compared to 5-HT value. Note that there were no significant differences between the basal value compared to the respective LY341495 value.
Changes in the frequency of synaptic currents is generally attributed to a presynaptic locus, whereas changes in the amplitude of synaptic currents can be attributed to either a presynaptic or postsynaptic locus (Van der Kloot, 1991). Furthermore, LY354740 did not suppress the inward current induced by bath application of AMPA following blockade of synaptic transmission by a combination of TTX and Ca²⁺-free ACSF. Effects of the mGlu2/3 agonists at presynaptic sites would be consistent with evidence suggesting that activation of either mGlu2 or mGlu3 receptors suppresses glutamate release at corticostriatal synapses (Lovinger and McCool, 1995).

At this time, however, we cannot rule out the possibility that the mGlu agonists and antagonists might have postsynaptic effects to account for the alteration in 5-HT-induced EPSC frequency and amplitude. The apical dendrites contain Na⁺ and Ca²⁺ conductances that are thought to play a dramatic role in the amplification of distal synaptic signals

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**Fig. 3.** LY354740: suppression of synaptic currents/potentials. A, the effects of 5-HT; then the effects of the mGlu2/3 agonist LY354740 on 5-HT-induced EPSCs in the same cell. The command potential in this cell was set at −70 mV. B, the effect of LY354740 on electrically evoked EPSPs after DOI treatment (3 μM). This cell was held at −80 mV during the entire experiment. C, summary for LY354740 on the normalized frequency of 5-HT-induced spontaneous EPSCs, the amplitude of electrically evoked early EPSPs, and the amplitude of the electrically evoked late EPSPs after DOI treatment.
ly27 (top). The electrical stimulus was adjusted in these experiments to evoke an early EPSP at ~70% of the amplitude necessary to elicit a spike (e.g., 13.2 ± 2.7 and 13.8 ± 1.6 mV, respectively). The EC_{50} for LY354740 was 454 nM (n = 4); in a separate group of cells in the presence of LY341495, the EC_{50} for LY354740 was 36,684 nM (n = 4) to yield a pA_{2} value of 7.9. LY354740 was tested in separate groups of cells in the absence and presence of LY341495 because the slow washout of 5-HT-containing axons and 5-HT_{2A} receptors (Blue et al., 1988; Aghajanian and Marek, 1997). By inference, these considerations suggest that mGlu2/3 receptors play a particularly important role in the integration of synaptic activity by apical dendrites of the layer V pyramidal cells.

As an initial step to determine the underlying neuroanatomical basis for the interactions between mGlu2/3 and 5-HT_{2A} receptors, we used autoradiography of coronal sections including the medial prefrontal cortex with [3H]LY354740 and [125I]DOI as ligands for these receptors, respectively. We observed a striking similarity in the laminar distribution of mGlu2/3 binding and 5-HT_{2A} binding in the medial prefrontal cortex. Previously, the highest density of 5-HT_{2A} receptors in the neocortex and transitional cortex has been reported to be in layers I and V (Blue et al., 1988). Similarly, the highest density of mGlu2/3 receptor binding in the medial prefrontal cortex also was in layers 1 and Va. In contrast, the peak mGlu2/3 binding in the Fr1 and Par1 regions of the frontoparietal cortex was present in layers II–IV, superficial to the highest density of DOI binding in layer Va. In this context, the lower potency of LY354740 in suppressing 5-HT-induced EPSCs in the frontoparietal region compared with the medial prefrontal cortex might be attributed to a lower density of mGlu2/3 receptors on the nerve endings innervating the apical dendrites of layer V pyramidal cells in the former region. Additional work will be necessary to determine whether mGlu2/3 and 5-HT_{2A} receptors are localized in the same nerve terminals in the medial prefrontal cortex and other cortical regions.

The pattern of [3H]LY354740 binding in the medial prefrontal cortex appears similar to immunostaining for mGlu2 receptors from coronal sections including the medial prefrontal cortex (Ohishi et al., 1997). This may provide an initial suggestion as to the role of mGlu2 versus mGlu3 receptors on the nerve endings innervating the apical dendrites of layer V pyramidal cells in the former region. Activation of group II mGlu receptors in the apical dendritic compartment of pyramidal cells, despite the small and inconsistent outward currents observed in somatic recordings, might suppress these Na^+ and Ca^{2+} conductances either directly via effects on Ca^{2+} conductances or indirectly via effects on K^+ conductances. Thus, the effect of mGlu agonists could be on the apical dendrite of pyramidal cells that were being recorded. Alternatively, the effect of mGlu agonist could be on the apical dendrite of neighboring pyramidal cells of the neuron from which the recording was taken. However, nei-
1999). In contrast to mGlu2, the presence of mGlu3 receptor mRNA in the thalamus appears largely restricted to GABAergic cells in the reticular nucleus of the thalamus (Ohishi et al., 1993b; Petralia et al., 1996) that do not project to the neocortex. This differential localization suggests that the suppression of 5-HT-induced spontaneous EPSCs by the group II mGlu agonists may be mediated mainly via activation of mGlu2. On the other hand, mRNA for mGlu3 appears to be present in almost all pyramidal cells of the neocortex. Thus, activation of mGlu3 by the group II mGlu receptor agonists could mediate a significant component of the suppression of early evoked EPSPs (in layer V pyramidal cells) to electrical stimulation of the forceps minor that would excite primarily corticofugal fibers arising from both layer V and VI.

At this time, the factor that accounts for the 3- to 5-fold selectivity of LY534740 for suppression of 5-HT-induced EPSCs versus electrically evoked early EPSPs (Figs. 3 and 4; Table 3) remains to be explained. Several recent studies have found a 4- to 5-fold selectivity for LY534740 in suppressing the inhibition of adenylyl cyclase in cell lines expressing mGlu2 versus mGlu3 receptors (Schoepp et al., 1997; Monn et al., 1999). In contrast, the mGlu2/3 antagonist LY341495, which is 3-fold less potent at mGlu2 versus mGlu3 receptors, was 4-fold less potent at suppressing 5-HT-induced EPSCs than electrically evoked early EPSPs. These considerations suggest that group II mGlu agonists might suppress 5-HT-induced EPSCs and electrically evoked early EPSPs via activation of mGlu2 and mGluR3, respectively. However, LY379268 lacked selectivity in suppressing electrically evoked early EPSPs versus 5-HT-induced EPSPs. A recent report found that this mGlu agonist was slightly less than 2-fold selective in suppressing adenylyl cyclase activity in cell lines expressing mGlu2 versus mGlu3 receptors (Monn et al., 1999). The relationship between the functional activity of these mGlu receptors in cell lines versus in native tissue remains to be determined. It is clear that definitive identification of the receptors involved in these responses requires agonists/antagonists with greater selectivity or the use of transgenic mice with disruption of mGlu2 or mGlu3 receptors.

Clinical applications for the mGlu2/3 agonist LY534740 have previously been raised for the treatment of anxiety disorders, nicotine withdrawal, and schizophrenia (Helton et al., 1997, 1998; Moghaddam and Adams, 1998). Additional clinical implications of a physiological interaction between 5-HT2A and mGlu receptors in the medial prefrontal cortex is intriguing because the medial prefrontal cor-

### Table 3

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<tr>
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<th>LY354740</th>
<th>LY379268</th>
<th>LY341495</th>
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<tr>
<td>Inhibition of forskolin-activated adenylyl cyclase</td>
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<tr>
<td>Human mGlu2 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>5.1 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human mGlu3 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>24.3 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5-HT-induced EPSCs and evoked EPSPs</td>
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<tr>
<td>5-HT-induced EPSCs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89 ± 30 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>231 ± 54 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>54 ± 7.0 (K&lt;sub&gt;B&lt;/sub&gt;)</td>
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<tr>
<td>Evoked late EPSPs after DOI&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85 ± 22 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>260 ± 41 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Evoked early EPSPs&lt;sup&gt;d&lt;/sup&gt;</td>
<td>235 ± 108 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>276 ± 84 (EC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>13 ± 6.7 (K&lt;sub&gt;B&lt;/sub&gt;)</td>
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</tbody>
</table>

N.D., not determined.

<sup>a</sup> Potency (EC<sub>50</sub>, nM) for suppression of forskolin-activated adenylyl cyclase for LY354740 (Schoepp et al., 1997) and LY379268 (Monn et al., 1999).

<sup>b,c,d</sup> EC<sub>50</sub> values (nM) for agonists at suppressing 5-HT-induced EPSCs, evoked late EPSCs after DOI, and evoked early EPSPs. “Apparent” K<sub>B</sub> value of antagonist for blocking suppressant action of LY354740 (nM) as determined from pA<sub>2</sub> value.

**Fig. 5.** A, LY354740 does not alter the steady state inward current induced by AMPA following block of synaptic transmission. An example of the relative lack of suppression by LY354740 (1 μM, 5 min pretreatment) of the steady-state inward current induced by AMPA (5 μM, 45 s) in a layer V medial prefrontal pyramidal cell following bath perfusion of TTX (2 μM) to block impulse flow. The command potential was set at −70 mV in this cell. B, an EPSC amplitude cumulative frequency plot in a single cell for the effect of 5-HT versus a combination of 5-HT and a concentration of LY354740 that decreased the EPSC frequency by 50%.
tex is believed to be involved in the pathophysiology of mood disorders and schizophrenia (Kroeze and Roth, 1998; Marek and Aghajanian, 1998b). Indeed, most of the “atypical antidepressants” (e.g., mirtazepine, mianserin, nefazodone, trazodone, and iprindole) share 5-HT$_{2A}$ antagonism as their most potent common pharmacological action (Richelson and Nelson, 1984; Wander et al., 1986; Eison et al., 1990; Marek et al., 1992; de Boer, 1996). Clozapine and the newest generation of “atypical antipsychotics” (e.g., olanzepine, risperidone, and the putative antipsychotic

Fig. 6. Autoradiography of $[^3H]$LY354740 binding (top) and $[^{125}I]$DOI binding (bottom) after subtraction of background binding in a coronal slice through medial prefrontal cortex (transitional cortex) and frontoparietal neocortex at a level corresponding to the physiological studies. Note the superficial and midcortical increase in $[^{125}I]$DOI binding, which is known to be present in layers I and Va of the neocortex and transitional cortex. The laminar distribution of $[^3H]$LY354740 binding and $[^{125}I]$DOI binding was similar in the medial prefrontal cortex, but not in the frontoparietal cortex.
and Va of the anterior cingulate appears to be contiguous with the density of layer Va binding of the respective area, P sections were obtained from each of four rats to calculate the averaged are presented in fmol of ligand bound/mg protein (mean ± S.E.). Six sections were obtained from each of four rats to calculate the averaged data. The increased density of 5-HT_{TA} receptor binding that was found in layers I and Va of the neocortex and anterior cingulate (Blue et al., 1988) was used as a template to identify the different cortical layers from the cortical regions of interest (Paxinos and Watson, 1986). Note that the increased density of 5-HT_{TA} receptor binding that was found in layers I and Va of the anterior cingulate appears to be contiguous with the prelimbic and infralimbic cortical areas, * significantly different from density of layer Va binding of the respective area, P < 0.05, ** significantly different from density of layer Va binding of the respective area, P < 0.01.

mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: An in situ hybridization study. *J Comp Neurol* 335:252–266.


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**mGluR2/3 Agonists Suppress 5-HT-Induced EPSCs**

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