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Modulation of pyramidal cell output in the medial prefrontal cortex by mGluR5 interacting with CB1

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Abstract

The medial prefrontal cortex (mPFC) serves executive cognitive functions such as decision-making that are impaired in neuropsychiatric disorders and pain. We showed previously that amygdala-driven abnormal inhibition and decreased output of mPFC pyramidal cells contribute to pain-related impaired decision-making (Ji et al., 2010). Therefore, modulating pyramidal output is desirable therapeutic goal. Targeting metabotropic glutamate receptor subtype mGluR5 has emerged as a cognitive-enhancing strategy in neuropsychiatric disorders, but synaptic and cellular actions of mGluR5 in the mPFC remain to be determined. The present study determined synaptic and cellular actions of mGluR5 to test the hypothesis that increasing mGluR5 function can enhance pyramidal cell output.

Whole-cell voltage- and current-clamp recordings were made from visually identified pyramidal neurons in layer V of the mPFC in rat brain slices. Both the prototypical mGluR5 agonist CHPG and a positive allosteric modulator (PAM) for mGluR5 (VU0360172) increased synaptically evoked spiking (E–S coupling) in mPFC pyramidal cells. The facilitatory effects of CHPG and VU0360172 were inhibited by an mGluR5 antagonist (MTEP). CHPG, but not VU0360172, increased neuronal excitability (frequency–current [$F-I$] function). VU0360172, but not CHPG, increased evoked excitatory synaptic currents (EPSCs) and amplitude, but not frequency, of miniature EPSCs, indicating a postsynaptic action. VU0360172, but not CHPG, decreased evoked inhibitory synaptic currents (IPSCs) through an action that involved cannabinoid receptor CB1, because a CB1 receptor antagonist (AM281) blocked the inhibitory effect of VU0360172 on synaptic inhibition. VU0360172 also increased and prolonged CB1-mediated depolarization-induced suppression of synaptic inhibition (DSI). Activation of CB1 with ACEA decreased inhibitory transmission through a presynaptic mechanism.

The results show that increasing mGluR5 function enhances mPFC output. This effect can be accomplished by increasing excitability with an orthosteric agonist (CHPG) or by increasing excitatory synaptic drive and CB1-mediated presynaptic suppression of synaptic inhibition (“dis-inhibition”) with a PAM (VU0360172). Therefore, mGluR5 may be a useful target in conditions of impaired mPFC output.

Keywords

mGluR5; Positive allosteric modulator; PAM; CB1; Prefrontal cortex; Synaptic transmission

1. Introduction

The medial prefrontal cortex (mPFC) serves executive functions such as decision-making, which are essential for selecting appropriate and inhibiting inappropriate actions. Cognitive dysfunctions can result in behavioral disinhibition, inflexibility and perseverance, which are key characteristics of neuropsychiatric disorders associated with emotional dysregulation such as addiction and anxiety disorders (Quirk and Beer, 2006; Hartley and Phelps, 2010; Dalley et al., 2011; Chudasama, 2011). Top-down cognitive control of emotional behavior has been studied extensively in experimental models of “extinction” of aversive behaviors such as conditioned fear and taste aversion (Berman and Dudai, 2001; Myers and Davis, 2007; Quirk et al., 2010; Hartley and Phelps, 2010; Herry et al., 2010). A new theory also views persistent pain as a state in which aversive emotional associations are continuously formed rather than extinguished (Apkarian et al., 2009).

Current concepts of cognitive control of emotions agree on the critical role of intact interactions between medial prefrontal cortex (mPFC) and amygdala (Ochsner and Gross, 2005; Dalley et al., 2011). The infralimbic region of the mPFC inhibits amygdala output to “extinguish” aversive behavior (Maren and Quirk, 2004; Likhtik et al., 2005; Akirav and Maroun, 2007; Sah and Westbrook, 2008; Pape and Pare, 2010; Sotres-Bayon and Quirk, 2010; Kim and Richardson, 2010; Herry et al., 2010). Increased mPFC activity correlates with successful control (extinction) of negative emotions (Mickley et al., 2005; Knapska and Maren, 2009; Kim et al., 2010; Chang et al., 2010) and decreased activity with cognitive control deficits in models of extinction (Mickley et al., 2007; Hefner et al., 2008; Kim et al., 2010; Chang and Maren, 2010; Sierra-Mercado et al., 2011) and behavioral disinhibition (Dalley et al., 2011).

Our previous study showed that amygdala-driven abnormal inhibition and decreased output of mPFC pyramidal cells contribute to pain-related impaired decision-making (Ji et al., 2010). The underlying mechanism is feedforward inhibition of mPFC pyramidal cells, resulting in cortical deactivation (Neugebauer et al., 2009; Ji et al., 2010; Ji and Neugebauer, 2011). Feedforward inhibition of the mPFC critically involves activation of metabotropic glutamate receptor subtype mGluR1, but not mGluR5, presynaptic to GABAergic interneurons that inhibit mPFC pyramidal cells (Sun and Neugebauer, 2011; Ji and Neugebauer, 2011).

The present study focused on the role of metabotropic glutamate receptor mGluR5 to increase mPFC output, because activation of mGluR5 has cognitive-enhancing effects in neuropsychiatric disorders (Homayoun and Moghaddam, 2010; Niswender and Conn, 2010; Vinson and Conn, 2011; Nicoletti et al., 2011). mGluR5 is expressed at particularly high levels in the mPFC mostly on postsynaptic elements (Romano et al., 1995; Muly et al., 2003). Activation of mGluR5 with orthosteric agonists increased spike firing (Fontanez-Nuin et al., 2011) and induced spontaneous, but not miniature, excitatory synaptic currents in layer V pyramidal cells in the prefrontal cortex (Marek and Zhang, 2008). Modulation of evoked synaptic transmission in mPFC cells by mGluR5 was not observed (Fontanez-Nuin et al., 2011). Pharmacological blockade of mGluR5 in the mPFC (Fontanez-Nuin et al., 2011) or knockout of mGluR5 (Xu et al., 2009) impaired cognitive control of negative emotions (fear extinction). The synaptic and cellular actions of mGluR5 on mPFC pyramidal cells remain to be determined.

We used a novel and highly selective mGluR5 positive allosteric modulator (PAM; VU0360172) (Rodriguez et al., 2010). mGluR5 PAMs are developed for clinical trials to treat cognitive deficits in neuropsychiatric disorders (Niswender and Conn, 2010; Hammond et al., 2010; Mueller et al., 2010). Unlike orthosteric agonists, allosteric modulators are devoid of activity in the absence of endogenous ligands; they exhibit less desensitization and fewer side effects from off target interactions (Gregory et al., 2011), thus allowing the activity- and target-dependent fine-tuning of cortical output.

Importantly, mGluR5 interacts with presynaptic cannabinoid receptor CB1 to depress inhibitory transmission (Lovinger, 2008; Kano et al., 2009). CB1 plays an important role in the cognitive control of emotions such as in the extinction of aversive associations (Lutz, 2009). Activation of CB1 in the infralimbic mPFC facilitated fear extinction whereas a CB1 antagonist or knockout of CB1 impaired this control process (Marsicano et al., 2002; Lin et al., 2009). In the rodent mPFC, CB1 is exclusively expressed in GABAergic interneurons (Marsicano and Lutz, 1999; Wedzony and Chocyk, 2009), and CB1 on axon terminals faces postsynaptic mGluR5 on pyramidal cells (Lafourcade et al., 2007). Thus, we tested the novel hypothesis that mGluR5 in concert with CB1 can increase mPFC pyramidal output.

2. Methods

2.1. Animals

Male Sprague Dawley rats (120–200 g) were housed in a temperature controlled room and maintained on a 12 h day/night cycle with unrestricted access to food and water and food. On the day of the experiment, rats were transferred from the animal facility and allowed to acclimate to the laboratory for at least 1 h. All experimental procedures conform to the guidelines of the International Association for the Study of Pain (IASP) and of the National Institutes of Health (NIH) and were approved by the Institutional Animal Care and Use Committee (IACUC).

2.2. Electrophysiology in brain slices

2.2.1. Slice preparation—Brain slices containing the medial prefrontal cortex (mPFC) were obtained from normal naïve rats as previously described (Ji et al., 2010; Sun and Neugebauer, 2011). Coronal brain slices (300–500 μ m) were cut at 3.0–3.2 rostral to bregma. At this level, slices contain both the prelimbic and infralimbic (recording site) regions of the mPFC. A single brain slice was transferred to the recording chamber and submerged in ACSF (31 ± 1 °C), which superfused the slice at ~ 2 ml/min. ACSF contained (in mM) NaCl 117, KCl 4.7, NaH_2PO_4 1.2, CaCl_2 2.5, MgCl_2 1.2, NaHCO_3 25, and glucose 11. The ACSF was oxygenated and equilibrated to pH 7.4 with a mixture of 95% O_2 /5% CO_2 . Only one or two brain slices per animal were used, one neuron was recorded in each slice, and a fresh slice was used for each new experimental protocol. Numbers in the text refer to the number of neurons tested for each parameter.

2.2.2. Patch-clamp recording—Whole-cell patch-clamp recordings were obtained from visually identified layer V pyramidal cells in the mPFC (~ 700 μ m lateral to the interhemispheric fissure) using infrared DIC-IR videomicroscopy as described previously (Ji et al., 2010; Sun and Neugebauer, 2011). Recording pipettes (3–5 M Ω tip resistance) made from borosilicate glass were filled with intracellular solution containing (in mM): 122 K-gluconate, 5 NaCl, 0.3 CaCl_2 , 2 MgCl_2 , 1 EGTA, 10 HEPES, 5 Na_2 -ATP, and 0.4 Na_3 -GTP. For recordings of IPSCs at -70 mV a high chloride internal solution (Pan et al., 1990) was used containing the following (in mM): 126 KCl, 10 NaCl, 1 MgCl_2 , 11 EGTA, 10 HEPES, 2 Mg -ATP, 0.25 Na_3 -GTP; pH was adjusted to 7.2–7.3 with KOH and osmolarity to 280 mOsm/kg with sucrose. Data acquisition and analysis of voltage and current signals was

done using a dual 4-pole Bessel filter (Warner Instr.), low-noise Digidata 1322 interface (Axon Instr.), Axoclamp-2B amplifier (Axon Instr.), Pentium PC, and pClamp9 software (Axon Instr.). Headstage voltage was monitored continuously on an oscilloscope to ensure precise performance of the amplifier. Neurons were voltage-clamped at -70 mV or 0 mV for the study of excitatory and inhibitory transmission, respectively. The calculated equilibrium potential for chloride in this system was -68.99 mV (Nernst equation, pClamp9 software). High ($G\Omega$) seal and low series (<20 M Ω) resistances were checked throughout the experiment (using pClamp9 membrane test function) to ensure high-quality recordings.

2.2.3. Synaptic transmission—Using concentric bipolar stimulating electrodes (Kopf Instr.), excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs), excitatory postsynaptic potentials (EPSPs), and action potentials (E–S coupling) were evoked in pyramidal cells by focal stimulation (150 μ s square-wave pulses; using an S88 stimulator; Grass Instruments) of presumed basolateral amygdala (BLA) afferents in the infralimbic cortex as described previously (Ji et al., 2010; Sun and Neugebauer, 2011). The stimulation electrode was placed in layer IV (500 μ m from the medial surface of the slice) of the infralimbic cortex where BLA afferents were identified by the fluorescent signal originating from anterogradely labeled fibers following stereotaxic injections of a fluorescent tracer (DiI, 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate, Molecular Probes Inc.; dissolved in *N,N*-dimethylformamide dimethyl acetal, Sigma–Aldrich Inc.; 2 μ l at 40 nl/min) into the BLA (3.3 caudal to bregma, 5.0 lateral to midline, depth 9.0) 10 – 12 days before brain tissue was taken. The distance between stimulation and recording sites was 600 – 800 μ m.

Input–output relationships of evoked EPSCs and IPSCs were obtained by increasing the stimulus intensity in 100 μ A steps. For evaluation of a drug effect on synaptically evoked responses, the stimulus intensity was adjusted to 50% of the intensity required for maximum responses. Peak amplitudes were measured and averaged across the sample of neurons.

Synaptically evoked spiking (E–S coupling) was measured in current-clamp mode. Action potentials were evoked by synaptic stimulation at near spike-threshold intensity, i.e., the stimulus intensity that evoked 2 – 3 spikes (action potentials) in a series of 10 trials. E–S coupling was measured as the number of spikes evoked by ten subsequent synaptic stimulations.

Miniature (in TTX 1 μ M) EPSCs and IPSCs (mEPSCs and mIPSCs) were recorded at -70 mV and 0 mV, respectively, as described previously (Ji et al., 2010; Sun and Neugebauer, 2011). A fixed length of traces (5 min) was analyzed for frequency and amplitude distributions using MiniAnalysis program 5.3 (Synaptosoft, Decatur, GA). The root mean square (RMS) of the background noise was computed for each set of data. Detection threshold for an event was set to 3 – 4 times the RMS value. Peaks were detected automatically, but each detected event was then visually inspected to prevent the inclusion of false data.

2.3. Drugs

The following drugs were used: mGluR5 positive allosteric modulator N-cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide hydrochloride ([VU0360172](#), [VU'172](#)) was synthesized as described previously (Rodriguez et al., 2010). mGluR5 agonist (R, S)-2-chloro-5-hydroxyphenylglycine ([CHPG](#); Doherty et al., 1997), mGluR5 antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine ([MTEP](#); Lea and Faden, 2006), CB1 agonist arachidonyl-2-chloroethylamide ([ACEA](#), Hillard et al., 1999), CB1 antagonist *N*-(morpholin-4-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide ([AM281](#), Lan et al., 1999), NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid ([AP5](#)); non-

NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium salt (NBQX); and GABA_A receptor antagonist [*R*-(*R**, *S**)]-6-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)furo[3,4-*e*]-1,3-benzodioxol-8(6H)-one (bicuculline) were purchased from Tocris Cookson, Ellisville, MO. All drugs were dissolved in ACSF to their final concentration on the day of the experiment. Selectivity and target concentrations have been established in the literature (for CHPG, Schoepp et al., 1999; CB1, Hillard et al., 1999; Lan et al., 1999; VU'172 Rodriguez et al., 2010; Pertwee, 2010) and our own studies on mGluR5 (Ren and Neugebauer, 2010; Li et al., 2011; Sun and Neugebauer, 2011).

Drugs were applied to the brain slice by gravity-driven superfusion in the ACSF (~2 ml/min). Solution flow into the recording chamber (1 ml volume) was controlled with a three-way stopcock. Drugs were applied for at least 15 min to establish equilibrium in the tissue. ACSF served as vehicle control in all experiments.

2.4. Statistical analysis

All averaged values are given as the mean $\pm$ SE. Statistical significance was accepted at the level $P < 0.05$. GraphPad Prism 3.0 software (GraphPad Software, San Diego, CA) was used for all statistical analysis. Student's *t*-test was used to compare two sets of data that have Gaussian distribution and similar variances. For multiple comparisons, one-way ANOVA or two-way ANOVA was used with appropriate posttests as indicated in the text and figure legends (Bonferroni posttests to compare selected pairs of data; Dunnett's posttests to compare all sets of data to a control value).

3. Results

Whole-cell patch-clamp recordings were made of visually identified layer V pyramidal cells in the infralimbic region of the mPFC. Both the prototypical mGluR5 agonist CHPG (500 μ M, $n = 5$ neurons; Fig. 1A) and a positive allosteric modulator for mGluR5 (VU0360172, VU'172; EC₅₀ = 90.6 nM, $n = 13$ neurons; Fig. 1B) increased synaptically evoked spiking (E-S coupling) in infralimbic mPFC pyramidal cells significantly (CHPG, $P < 0.001$; VU'172, $P < 0.05$ – 0.001 , Bonferroni posttests). An mGluR5 antagonist (MTEP; 10 μ M) inhibited the effects of CHPG ($n = 4$; $P < 0.01$, Bonferroni posttest) and VU'172 ($n = 4$; $P < 0.05$, Bonferroni posttest). Excitatory postsynaptic potentials (EPSPs) and action potentials (spikes) were evoked with near threshold stimulus intensity for spiking from a holding potential of -60 mV.

CHPG (500 μ M, $n = 5$ neurons; Fig. 2A), but not VU'172 (1 μ M, $n = 5$ neurons; Fig. 2B), increased frequency–current (F – I) function of mPFC pyramidal cells. Depolarization-induced spiking was measured in current-clamp. The effect of CHPG was statistically significant ($P < 0.0001$, $F_{1,64} = 17.83$, main effect of drug, two-way ANOVA with Bonferroni posttests).

VU'172, but not CHPG, increased excitatory synaptic transmission (EPSCs, Fig. 3) and decreased inhibitory synaptic transmission (IPSCs, Fig. 4) onto mPFC pyramidal cells. EPSCs were monosynaptic and depended on non-NMDA receptor activation because they were completely blocked by NBQX (10 μ M). IPSCs were blocked by bicuculline (10 μ M) and also inhibited by NBQX, indicating that they were glutamate-driven polysynaptic as shown in detail in our previous studies (Ji et al., 2010; Sun and Neugebauer, 2011). VU'172 (1 μ M, $n = 5$ neurons; Fig. 3B), increased input–output (I/O) relationships of evoked EPSCs significantly ($P < 0.0001$, $F_{1,88} = 102.28$, main effect of drug; two-way ANOVA). The action of VU'172 was postsynaptic because VU'172 (1 μ M, $n = 4$ neurons; Fig. 3C) increased amplitude, but not frequency, of miniature EPSCs (mEPSCs; in TTX, 1 μ M)

significantly ($P < 0.05$, paired t -test). CHPG (500 μM , $n = 6$ neurons; Fig. 3A) had no effect on excitatory transmission ($P > 0.05$, $F_{1,110} = 3.75$, main effect of drug; two-way ANOVA).

VU'172 (1 μM , $n = 6$ neurons; Fig. 4BS) decreased I/O relationships of evoked IPSCs significantly ($P < 0.001$, $F_{1,110} = 14.34$, main effect of drug; two-way ANOVA). The inhibitory effect on synaptic inhibition involved activation of endocannabinoid CB1 receptors because a selective CB1 antagonist (AMN281, 1 μM ; $n = 4$ neurons) reversed the effect of VU'172 significantly ($P < 0.0001$, $F_{1,88} = 36.98$, main effect of drug; two-way ANOVA) but had no effect by itself ($n = 5$ neurons; $P > 0.05$, paired t -test). CHPG (500 μM , $n = 6$ neurons; Fig. 4A) had no effect on inhibitory transmission ($P > 0.05$, $F_{1,110} = 1.13$, main effect of drug; two-way ANOVA).

Next we sought to demonstrate the interaction between mGluR5 PAM and CB1 to modulate synaptic inhibition of mPFC pyramidal cells. Presynaptic depression is a key function of the CB1 receptor that involves a retrograde signaling process in which endocannabinoids are released from the postsynaptic site to activate CB1 on the presynaptic terminal and inhibit transmitter release (Lovinger, 2008; Lutz, 2009; Kano et al., 2009; Di Marzo, 2011). One mechanism to activate CB1-mediated inhibition of GABAergic transmission is postsynaptic calcium influx following depolarization, resulting in endocannabinoid synthesis and release ("depolarization-induced suppression of inhibition", DSI) (Kano et al., 2009).

To do so, we recorded IPSCs with a high chloride internal solution at -70 mV and induced DSI by a brief 1 s depolarization (Lovinger, 2008; Kano et al., 2009). DSI was blocked by a selective CB1 receptor antagonist (AM281, 1 μM ; $n = 5$ neurons; $P < 0.0001$, $F_{1,224} = 171.40$, main effect of drug; two-way ANOVA; Fig. 5A). VU'172 (1 μM , $n = 8$ neurons) enhanced CB1-mediated DSI significantly ($P < 0.0001$, $F_{1,392} = 164.14$, main effect of drug; two-way ANOVA), increasing its magnitude and duration. Next we sought to determine the site of action of CB1 on inhibitory transmission. A selective CB1 agonist (ACEA, 10 nM) decreased frequency, but not amplitude, of mIPSCs ($n = 5$ neurons, $P < 0.05$, paired t -test; Fig. 6A–C). The results show that CB1 act presynaptically to control inhibitory transmission onto mPFC pyramidal cells.

Next we tested the consequence of CB1-mediated presynaptic inhibition of inhibitory transmission on the output function of pyramidal cells (Fig. 6D, E). ACEA increased synaptically evoked spiking (E–S coupling) significantly ($n = 3$ neurons; $P < 0.05$, paired t -test; Fig. 6D, individual example; Fig. 6E, summary). The data show that disinhibition increases pyramidal output. The differential inhibitory and facilitatory effects of ACEA on inhibitory and excitatory inputs, respectively, argue against non-specific effects such as rundown.

4. Discussion

This study is the first to analyze synaptic and cellular effects of mGluR5 on pyramidal cells in the medial prefrontal cortex (mPFC) using a positive allosteric modulator (PAM). The key novel findings are as follows: activation of mGluR5 with a presumed orthosteric agonist (CHPG) or PAM (VU0360172, VU'172) increased pyramidal cell output (E–S coupling). CHPG did so by increasing neuronal excitability (F – I function) whereas VU'172 enhanced excitatory transmission while decreasing inhibitory transmission. The inhibitory effect of VU'172 on synaptic inhibition involved activation of presynaptic CB1 receptors. The significance of these novel results is that they identify mGluR5 as a useful target to increase mPFC output; and they show the underlying mechanism(s) of action.

Group I mGluRs can modulate excitatory and inhibitory transmission in the cortex and have emerged as important targets for the treatment of neuropsychiatric disorders associated with

cognitive deficits (Homayoun and Moghaddam, 2010; Niswender and Conn, 2010; Lesage and Steckler, 2010; Vinson and Conn, 2011; Nicoletti et al., 2011). Dysfunction of the medial prefrontal cortex (mPFC) has been identified as a key neurobiological correlate of cognitive inflexibility that is associated with neuropsychiatric disorders such as drug addiction, obsessive-compulsive disorder, and schizophrenia (Clarke et al., 2004; Bowie and Harvey, 2006; Gu et al., 2008; Stalnaker et al., 2009; Goto et al., 2010). Perseveration of high-risk decision-making in a gambling task was also shown in pain patients (Apkarian et al., 2004) and in animal pain models (Galhardo and Pais-Vieira, 2005; Ji et al., 2010). Pain-related functional and structural abnormalities were detected in the mPFC (Metz et al., 2009; Apkarian et al., 2009; Ji et al., 2010). Failure to activate the mPFC is associated with visceral hypersensitivity in patients (Mayer et al., 2005). For those disorders, modulation of mPFC function may be a useful therapeutic strategy, but better knowledge of suitable targets is required.

Positive allosteric modulators (PAMs) for mGluR5 hold promise for the treatment of cognitive deficits in schizophrenia (Vinson and Conn, 2011) and have been shown to enhance certain forms of synaptic plasticity and learning and memory, including hippocampal LTP and/or LTD (Ayala et al., 2009; for VU172 see Noetzel et al., 2011) and performance in the Morris water maze, in a spatial alternation task, novel object recognition task and the five-choice serial reaction time test (Cleva and Olive, 2011). Unlike orthosteric agonists, allosteric modulators are devoid of activity in the absence of endogenous ligands; they do not exhibit desensitization and are believed to have fewer side effects from off target interactions (Mueller et al., 2010). Their synaptic and cellular actions in the mPFC, however, remain to be determined and were addressed in this study.

Both mGluR1 and mGluR5 subtypes are expressed in the PFC at pre- and postsynaptic sites (Cauli et al., 2000; Muly et al., 2003). Older studies using non-subtype-selective mGluR agonists reported mixed effects on excitatory and inhibitory transmission in frontal cortical areas. 1S,3R-ACPD increased network activity-dependent spontaneous inhibitory transmission onto presumed GABAergic interneurons (Zhou and Hablitz, 1997) and onto pyramidal cells (Chu and Hablitz, 1998) in unspecified neocortical or frontal cortical areas and increased GABA release in the PFC (Segovia and Mora, 2005). On the other hand, 1S,3R-ACPD inhibited evoked excitatory and inhibitory synaptic potentials in layer 2/3 frontal cortical neurons (Burke and Hablitz, 1994) and increased excitability of these neurons (Burke and Hablitz, 1996).

Modulation of evoked excitatory and/or inhibitory synaptic transmission in mPFC neurons by mGluR5 has not been reported before. An mGluR1/5 agonist (DHPG) increased network activity-dependent spontaneous excitatory transmission in layer V mPFC pyramidal cells (Marek and Zhang, 2008). The lack of direct effects on excitatory synaptic transmission in the presence of TTX is not inconsistent with our finding that CHPG did not modulate monosynaptic excitatory synaptic transmission. The indirect facilitatory synaptic effect of DHPG was blocked by an mGluR5 antagonist (MPEP) (Marek and Zhang, 2008), whereas DHPG-induced glutamate release in the mPFC was blocked by an mGluR1 antagonist (LY367385) (Melendez et al., 2005). The orthosteric mGluR5 agonist CHPG increased intrinsic bursting of layer 5 pyramidal cells in the infralimbic region of the mPFC (Fontanez-Nuin et al., 2011). A systemically administered PAM for mGluR5 (CDPPB) increased spontaneous action potential firing of putative pyramidal cells in the mPFC of awake animals but blocked the excitatory effect of an NMDA antagonist (MK801) in these cells (Lecourtier et al., 2007). Conversely, systemic administration of an mGluR5 antagonist (MPEP) decreased the spontaneous firing rate of putative mPFC pyramidal cells in awake animals and potentiated the effects of an NMDA receptor antagonist (Homayoun and Moghaddam, 2006). These studies did not determine the site of drug action and also

reported opposite effects of these compounds in some neurons. The results were interpreted to suggest that mGluR5 exerts state-dependent modulation of mPFC activity that is controlled by NMDA receptor-driven GABAergic inhibition (Homayoun and Moghaddam, 2010).

The present study found that CHPG increased excitability and output of mPFC pyramidal cells, which is generally consistent with previous reports on mGluR5 effects in the mPFC (Lecourtier et al., 2007; Marek and Zhang, 2008; Fontanez-Nuin et al., 2011) as described in the previous paragraph. CHPG has low affinity and requires high micromolar concentrations (Nicoletti et al., 2011) and the increase of neuronal excitability independent of afferent modulation may indicate a broad non-differential effect on mPFC function. In support of this notion, facilitatory mGluR5-mediated effects of DHPG on spontaneous excitatory transmission were indirect and network activity-dependent (Marek and Zhang, 2008). Further, DHPG increased synaptic inhibition through an indirect glutamate-driven interneuron-mediated action involving mGluR1 but not mGluR5 (Sun and Neugebauer, 2011). These observations may suggest that the broad and complex effects of orthosteric agonists negatively impact their usefulness as tools to tweak output function of mPFC cells.

Therefore, we used a novel orally active PAM for mGluR5 (VU0360172) (VU'172; Rodriguez et al., 2010) to address the hypothesis that mGluR5 can enhance mPFC cell output by increasing the excitatory drive onto pyramidal cells. The key novel finding of the present study is that VU'172 not only increased excitatory transmission through a postsynaptic mechanism but also inhibited inhibitory transmission through an indirect action that depends on presynaptic CB1. This effect is different than that of CHPG which did not affect synaptic transmission. The reason for the observed differences is not clear. CHPG competes with the endogenous ligand whereas PAMs enhance the effect of glutamate. The allosteric modulator induces a conformational change that creates essentially a "new" receptor type with different affinity, efficacy or coupling to downstream signaling cascades. Homo- and/or hetero-dimerization have also been suggested to affect allosteric modulation (for Discussion see Melancon et al., 2012).

CHPG is a weak partial agonist of mGluR5 that induces a maximal response of approximately 60% of the response to glutamate with an EC₅₀ value of 750 μ M (Doherty et al., 1997; Schoepp et al., 1999). We have confirmed this weak efficacy and low potency of CHPG relative to DHPG in multiple cell lines expressing mGluR5 and in other preparations (Conn et al., unpublished findings). Because of this low potency and efficacy we anticipate that the concentrations of CHPG used in the current studies (500 μ M) are likely to induce a very weak activation of mGluR5 that falls well below 50% of the maximal response to DHPG. Unfortunately, the solubility limits of CHPG make it difficult to achieve higher concentrations in the slice preparation used. Thus, depending on the baseline concentrations of extracellular glutamate in the slice preparation, it is conceivable that VU'172 could potentiate responses to glutamate-induced activation of mGluR5 to a level that is higher than those achieved with 500 μ M CHPG alone. However, the present data do not provide clear mechanistic insight into the observed actions of CHPG.

It is also possible that the lack of effect of CHPG on synaptic transmission could involve desensitization. mGluR5 undergoes rapid agonist (CHPG) induced desensitization that is mediated by protein kinase C activation (Gereau and Heinemann, 1998; Alagarsamy et al., 1999), whereas PAMs are believed to lack this effect (Melancon et al., 2012). We were unable to detect desensitization because CHPG had no effect on synaptic transmission at any time point with measurements starting 3 min after the onset of drug application. However, we cannot rule out the possibility that desensitization occurred extremely rapidly and escaped detection in our rather slow perfusion system.

In our previous study we found that mGluR1, but not mGluR5, is involved in feedforward synaptic inhibition of mPFC pyramidal cells through action on interneurons and in pain-related abnormal inhibition of the mPFC (Sun and Neugebauer, 2011; Ji and Neugebauer, 2011). In the rodent brain, mGluR1 is expressed at low to moderate levels in the frontal cortex and is associated almost exclusively with interneurons and their presence in axons and glia has also been reported, whereas mGluR5 is expressed at particularly high levels in the mPFC and mostly on postsynaptic elements of pyramidal cells and interneurons (Muly et al., 2003). Importantly, mGluR5 can interact with presynaptic CB1 to depress inhibitory transmission (Lovinger, 2008; Kano et al., 2009) and produce hippocampal LTD (see Izumi and Zorumski, 2011). In the rodent mPFC, CB1 is exclusively expressed in GABAergic interneurons (Marsicano and Lutz, 1999; Wedzony and Chocyk, 2009), and presynaptic CB1 receptors on axon terminals face postsynaptic mGluR5 on pyramidal cells (Lafourcade et al., 2007). Presynaptic depression is a key function of the CB1 receptor that involves a retrograde signaling process in which endocannabinoids are released from the postsynaptic site to activate CB1 on the presynaptic terminal and inhibit transmitter release. This is consistent with our finding that activation of CB1 with ACEA decreased inhibitory transmission through a presynaptic mechanism. CB1-mediated inhibition of GABAergic transmission can be activated by postsynaptic calcium influx following depolarization, resulting in endocannabinoid synthesis and release ("depolarization-induced suppression of inhibition", DSI) (Kano et al., 2009). Under certain conditions CB1-mediated DSI requires mGluR5 activation (Sheinin et al., 2008). Our data show that mGluR5 enhances CB1-mediated DSI. Details of this interaction remain to be determined but may involve endocannabinoid biosynthesis through the phospholipase C – diacylglycerol pathway, which has been shown to be one mechanism through which group I mGluRs activate CB1-mediated inhibition of neurotransmitter release (Lovinger, 2008; Kano et al., 2009). Interestingly, a previous study speculated that some actions of DHPG on mPFC cells involved GABAergic mechanisms (Marek and Zhang, 2008).

It should be noted that the results of the present study were obtained with selective compounds. CHPG is still the only selective orthosteric agonist for mGluR5 but displays low affinity and requires high concentrations (Schoepp et al., 1999; Nicoletti et al., 2011). We have used CHPG successfully in previous studies to distinguish mGluR5 and mGluR1 functions (Neugebauer et al., 2003; Li and Neugebauer, 2004; Li et al., 2011). VU0360172 is a chemically optimized orally active analog of VU0092273, one of the most potent and selective mGluR5 PAMs that bears structural resemblance to the prototypical negative allosteric modulator MPEP and binds to the MPEP site (Rodriguez et al., 2010). MTEP inhibited the effects of CHPG and VU0092273 on synaptically evoked spiking to a similar extent, confirming the involvement of mGluR5. AM281 is a potent and highly selective competitive CB1 antagonist that is able to penetrate the blood brain barrier (Lan et al., 1999). A synthetic analog of *N*-arachidonylethanolamine (AEA or anandamide), ACEA is a highly potent and one of the most selective agonists for CB1 (Hillard et al., 1999). It should be noted, however, anandamides can activate CB1 and TRPV1 (Di Marzo, 2010) and ACEA has been shown to activate TRPV1 channels in peripheral tissues (Baker and McDougall, 2004; Ruparel et al., 2011) and in the brain (Casarotto et al., 2012). Still, it is unlikely that actions of ACEA on TRPV1 can explain the results of the present study for the following reasons. TRPV1 activation counteracts endocannabinoid-mediated retrograde inhibition of GABAergic transmission in the striatum (Di Marzo, 2011). CB1 and TRPV1 receptors have opposing effects in the prefrontal cortex (Rubino et al., 2008; Giordano et al., 2011). ACEA produces dose-dependent opposite effects through actions at CB1 and TRPV1 in the PAG (Casarotto et al., 2012).

Finally, we would like to add some notes of caution. While the stimulation electrode was positioned on labeled fibers from the BLA we cannot exclude the possibility that

glutamatergic afferents from other areas were stimulated, which is a limitation of this approach. Rundown is another issue that needs to be considered in whole-cell patch-clamp studies. For the following reasons we do not believe rundown accounted for the observed drug effects: Rundown is primarily a concern with depressing drug effects, which were not observed with CHPG. Both VU0360172 and ACEA produced suppression of inhibitory transmission but also had excitatory effects on glutamatergic transmission and E–S coupling. The differential effects strongly argue against a non-specific rundown effect. Furthermore, the effects of VU0360172 were blocked with antagonists (MTEP and AM281).

In conclusion, the novelty and significance of this study is that mGluR5 was identified as a useful target to fine-tune mPFC output. The synaptic mechanisms of mGluR5 PAM effects involve increase of excitatory drive onto pyramidal cells through a postsynaptic action and concomitant decrease of synaptic inhibition through a CB1-dependent presynaptic mechanism. The results emphasize the potential value of mGluR5 modulators to restore “normal” levels of mPFC output that is impaired in clinically relevant conditions.

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Fig. 1.

Synaptically evoked spiking. CHPG (A) and VU0360172 (VU'172; (B) increased synaptically evoked spiking (E-S coupling) in mPFC pyramidal cells. An mGluR5 antagonist (MTEP) inhibited the effects of CHPG and VU'172. Individual traces (10 each) show current-clamp recordings of excitatory postsynaptic potentials (EPSPs) and action potentials (spikes) evoked with near threshold stimulus intensity for spiking from a holding potential of -60 mV before (predrug) and during drug application. (A) CHPG (500 μ M) alone ($n = 8$ neurons) and coapplied with MTEP (10 μ M; $n = 4$ neurons). Bar histograms (mean $\pm$ SE) show probability of synaptically evoked spikes calculated as follows: (number of trials with evoked spikes)/(number of trials). **, *** $P < 0.01$, 0.001, Bonferroni posttests. (B) VU'172 alone ($EC_{50} = 90.6$ nM, $n = 13$ neurons, 4–5 data points per concentration). Concentration–response curves for VU'172 were obtained by nonlinear regression analysis using the formula $y = A + (B - A) / [1 + (10^{C/10^x})^D]$, where A is the bottom plateau, B top plateau, $C = \log(EC_{50})$, and D is the slope coefficient (GraphPad Prism software). VU'172 (1 μ M) coapplied with MTEP (10 μ M; $n = 4$ neurons). *, *** $P < 0.05$, 0.001, compared to predrug; # $P < 0.05$, compared to VU'172 alone, Bonferroni posttests.

Fig. 2. Neuronal excitability. CHPG (A), but not VU0360172 (B), increased frequency–current (F–I) function of mPFC pyramidal cells. Individual traces show current-clamp recordings of action potentials generated by direct intracellular current injections (500 ms) of 0 pA and 300 pA from a holding potential of -60 mV before (predrug) and during drug application. Scale bars, 50 mV, 100 ms. Graphs show input–output functions (F–I relationships) averaged for the sample of neurons. (A) CHPG (500 μ M, $n = 5$ neurons). $P < 0.0001$, $F_{1,64} = 17.83$, main effect of drug (two-way ANOVA with Bonferroni posttests). (B) VU'172 (1 μ M, $n = 5$ neurons). $P > 0.05$, $F_{1,64} = 0.35$, main effect of drug (two-way ANOVA).

Fig. 3.

Excitatory synaptic transmission. VU'172 (A), but not CHPG (B), increased evoked excitatory synaptic currents (EPSCs) in mPFC pyramidal cells. Individual traces recorded in voltage-clamp show EPSCs (average of 8–10) evoked with a stimulation intensity of 0.9 mA. Scale bars, 100 pA, 30 ms. Graphs show input–output (*I/O*) relationships obtained by measuring peak amplitudes of EPSCs as a function of afferent fiber stimulus intensity. (A) CHPG (500 μM, $n = 6$ neurons) had no effect on evoked EPSCs. $P > 0.05$, $F_{1,110} = 3.75$, main effect of drug (two-way ANOVA). (B) VU'172 (1 μM, $n = 5$ neurons) increased EPSCs significantly. $P < 0.0001$, $F_{1,88} = 102.28$, main effect of drug (two-way ANOVA). *** $P < 0.001$ (Bonferroni posttests). (C) VU'172 also increased amplitude, but not frequency, of miniature EPSCs (mEPSCs; in TTX, 1 μM). Current traces show mEPSCs before and during application of VU'172. Scale bars, 10 pA, 2.5 s. Bar histograms show mean amplitude and frequency ($\pm$ SE) averaged for the sample of neurons ($n = 4$). * $P < 0.05$ (paired *t*-test).

Fig. 4.

Inhibitory synaptic transmission. VU'172 (A), but not CHPG (B), decreased evoked inhibitory synaptic currents (IPSCs) in mPFC pyramidal cells through a CB1-dependent action. Individual traces recorded in voltage-clamp show IPSCs (average of 8–10) evoked with a stimulation intensity of 0.9 mA. Graphs show input–output (*I/O*) relationships obtained by measuring peak amplitudes of IPSCs as a function of afferent fiber stimulus intensity. (A) CHPG (500 μ M, $n = 6$ neurons) had no effect on IPSC input–output function. $P > 0.05$, $F_{1,110} = 1.13$ (two-way ANOVA). B, VU'172 (1 μ M, $n = 6$ neurons) decreased IPSCs significantly. $P < 0.001$, $F_{1,110} = 14.34$ (two-way ANOVA). Coapplication of a CB1 receptor antagonist (AM281, 1 μ M; $n = 4$ neurons) reversed the effect of VU'172 ($P < 0.0001$, $F_{1,88} = 36.98$, two-way ANOVA). ** $P < 0.01$ (Bonferroni posttests).

Fig. 5.

Depolarization-induced suppression of synaptic inhibition (DSI). VU0360172 enhanced CB1-mediated DSI. Brief (1 s) Depolarization of mPFC pyramidal cells decreased IPSCs (DSI) recorded in voltage-clamp at -70 mV with a high chloride internal solution (see Methods). Individual traces show IPSCs (average of 8–10) evoked with a stimulation intensity of 0.9 mA. Graphs show the time course of evoked IPSCs before and after brief depolarization in presence of drugs or vehicle (ACSF, “DSI vehicle”). IPSC amplitudes are normalized to pre-DSI control values averaged for the sample of neurons (mean $\pm$ SE). (A) DSI was blocked by a CB1 receptor antagonist (AM281, 1 μ M; $n = 5$ neurons). $P < 0.0001$, $F_{1,224} = 171.40$ (two-way ANOVA). (B) VU-172 (1 μ M, $n = 8$ neurons) increased DSI significantly. $P < 0.0001$, $F_{1,392} = 164.14$ (two-way ANOVA). Current traces show IPSCs before and 3 s and 48 s after depolarization.

Fig. 6.

Presynaptic CB1 increase synaptically evoked spiking. (A–C) A selective CB1 agonist (ACEA, 10 nM) decreased frequency, but not amplitude, of miniature IPSCs (mIPSCs, in TTX, 1 μ M; $n = 5$ neurons). (A) current traces show mIPSCs before and during application of ACEA. Scale bars, 10 pA, 2.5 s. Bar histograms show mean amplitude (B) and frequency (C) averaged for the sample of neurons (mean $\pm$ SE). * $P < 0.05$ (paired t -test). (D, E) ACEA increased synaptically evoked spiking (E–S coupling). (D) individual traces (10 each) show current-clamp recordings of EPSPs and action potentials (spikes) evoked with near threshold stimulus intensity for spiking from a holding potential of -60 mV before (predrug) and during drug application. (E) bar histograms (mean $\pm$ SE) show probability of synaptically evoked spikes before and during ACEA ($n = 3$ neurons) calculated as follows: (number of trials with evoked spikes)/(number of trials). * $P < 0.05$, paired t -test.