Published in final edited form as:

Neuropharmacology. 2015 August ; 95: 388–394. doi:10.1016/j.neuropharm.2015.04.003.

Group II mGluRs modulate baseline and arthritis pain-related synaptic transmission in the rat medial prefrontal cortex

Takaki Kiritoshi¹ and Volker Neugebauer^{1,2}

¹Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center (TTUHSC), School of Medicine, 3601 4th Street, Mail Stop 6592, Lubbock, Texas 79430-6592

²Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center (TTUHSC), School of Medicine, 3601 4th Street, Mail Stop 6592, Lubbock, Texas 79430-6592

Abstract

The medial prefrontal cortex (mPFC) serves executive functions that are impaired in neuropsychiatric disorders and pain where abnormal synaptic inhibition of mPFC pyramidal cells has been linked to cognitive deficits. Therefore, restoring normal transmission is a desirable goal. Group II metabotropic glutamate receptors mGluR 2 and 3 are highly expressed in the mPFC, modulate excitatory and inhibitory transmission, and have been targeted for the treatment of neuropsychiatric disorders, but their pain-related modulatory effects in the mPFC remain to be determined. Here we evaluated the ability of an mGluR2/3 agonist and antagonist to restore pyramidal output in an arthritis pain model.

Whole-cell patch-clamp recordings were made from visually identified layer V mPFC pyramidal cells in rat brain slices. A selective group II mGluR agonist (LY379268) decreased synaptically evoked spiking in slices from normal and arthritic rats (5–6 h postinjection of kaolin/carrageenan into one knee). Effects were concentration-dependent and reversed by a selective antagonist (LY341495). LY379268 decreased monosynaptic excitatory postsynaptic currents (EPSCs) and glutamate-driven inhibitory postsynaptic currents (IPSCs) in the arthritis model. Effects on EPSCs preceded those on IPSCs and could explain the overall inhibitory effect on pyramidal output. LY379268 decreased frequency, but not amplitude, of miniature EPSCs without affecting miniature IPSCs. LY341495 by itself increased synaptically evoked spiking under normal conditions and in the pain model.

We conclude that group II mGluRs act on glutamatergic synapses to inhibit direct excitatory transmission and feedforward inhibition onto pyramidal cells. Their net effect is decreased pyramidal cell output. The facilitatory effect of a group II antagonist suggests that the system may

© 2015 Published by Elsevier Ltd.

Corresponding author: Volker Neugebauer, M.D., Ph.D., Professor and Chair, Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center | School of Medicine, 3601 4th Street | Mail Stop 6592, Lubbock, Texas 79430-6592, Phone: (806) 743-3880, Fax: (806) 743-2744, volker.neugebauer@ttuhsc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

be tonically active to control pyramidal output and does not change in the pain model. Failure to release the inhibitory tone in conditions that require enhanced cortical function and output, such as pain control, could be a mechanism for the development or persistence of a disease state.

Keywords

Medial prefrontal cortex; pain; synaptic transmission; mGluR; patch-clamp

1. Introduction

The medial prefrontal cortex (mPFC) serves executive control functions such as decision-making and behavioral control, which are impaired in pain patients [4;30] and in animal models of pain [21;41]. Work from our group [18;20;21] and others [29] showed dysfunction of the medial prefrontal cortex in models of inflammatory and neuropathic pain. Abnormal synaptic inhibition from the amygdala was identified as a cause of decreased pyramidal cell output in a rodent model of arthritis pain [21]. Conversely, increasing pyramidal cell activity inhibited amygdala activity and pain behaviors [20]. To achieve this, a complex strategy was used to increase excitatory drive by metabotropic glutamate receptor mGluR5 activation and inhibition of synaptic inhibition through cannabinoid CB1 receptor activation [20;24].

Here we tested the usefulness of targeting another mGluR subtype to normalize synaptic transmission and increase pyramidal cell output in a pain model. Group II mGluRs, consisting of mGluR2 and mGluR3 subtypes, couple negatively to adenylyl cyclase, cAMP and PKA activation via G_i proteins and serve as presynaptic auto- or hetero-receptors [36;38]. Group II mGluRs are highly expressed in the prefrontal cortex with mGluR2 showing a bilaminar distribution (layer I and V), particularly in the mPFC, and mGluR3 being present on GABAergic axons and in pyramidal cells [27;44]. Activation of group II mGluRs produces well-established antinociceptive, anxiolytic and antidepressant effects [9;31;36]. Preclinical studies also suggested beneficial effects of group II mGluR agonists on positive and negative symptoms of schizophrenia but clinical trials failed [37]. Interestingly, group II mGluR antagonists and negative allosteric modulators also showed antidepressant effects and in particular on outcome measures of cognitive dysfunction [2;6;12;37], possibly involving the prefrontal cortex [2]. Group II mGluR antagonists had beneficial effects in an Alzheimer's Disease mouse model, improving cognitive functions and anxiety [22]. However, mGluR3 negative allosteric modulators impaired extinction of conditioned fear [43]. These findings suggest that group II mGluRs play complex roles in cognitive-affective functions and disorders and could also be useful targets to normalize deficits associated with pain such as medial prefrontal cortical dysfunction.

However, the role of group II mGluRs in pain-related synaptic transmission in the mPFC remains to be determined. Synaptic effects of group II mGluRs in the prefrontal cortex include a decrease of serotonin-induced or electrically-evoked excitatory synaptic responses of layer V pyramidal cells by group II agonists and enhancement by group II antagonists [27]. A recent study found that presynaptic mGluR2 mediates acute synaptic inhibition of excitatory transmission in the mPFC whereas long long-term depression depends on

postsynaptic mGluR3 [43]. In this study, we examined the effects of a selective group II mGluR agonist (LY379268) and antagonist (LY341495) on excitatory and inhibitory synaptic transmission and output of infralimbic mPFC pyramidal cells in brain slices from normal rats and from rats with arthritis. The goal was to determine whether activation or inhibition of group II mGluR function would be able to restore pyramidal cell output in the pain model.

2. Materials and Methods

2.1 Animals

Male Sprague Dawley rats (120–200 g) were housed in standard plastic cages in a temperature controlled room and maintained on a 12 h day/night cycle. Water and food were available without restriction. On the day of the experiment, rats were transferred from the animal facility and allowed to acclimate to the laboratory for at least 1 hr. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at TTUHSC and conform to the guidelines of the International Association for the Study of Pain (IASP) and of the National Institutes of Health (NIH)

2.2 Arthritis pain model

Arthritis was induced as described in detail previously [35]. A kaolin suspension (4%, 100 μ l) was injected slowly into the joint cavity through the patellar ligament with the use of a syringe and needle (1 ml, 25 gauge, 5/8 in). After repetitive flexions and extensions of the knee for 15 min, a carrageenan solution (2%, 100 μ l) was injected into the knee joint cavity, and the leg was flexed and extended for another 5 min. This treatment paradigm reliably leads to a localized inflammation confined to one knee joint within 1–3 h, persists for weeks, and is significantly associated with pain behaviors [35]. Brain slices were obtained from normal naïve rats and from rats with arthritis 5–6 h postinduction.

2.3 Electrophysiology in brain slices

2.3.1 Slice preparation—Brain slices containing the medial prefrontal cortex (mPFC) were obtained from normal naïve rats as previously described [21;24;42]. 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 regions of the mPFC. A single brain slice was transferred to the recording chamber and submerged in ACSF (31 $\pm$ 1°C) superfusing the slice at $\sim$ 2 ml/min. ACSF contained (in mM) NaCl 117, KCl 4.7, NaH₂PO₄ 1.2, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, and glucose 11. The ACSF was oxygenated and equilibrated to pH 7.4 with a mixture of 95% O₂/5% CO₂. Only one or two brain slices per animal were used. Only 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.

2.3.2 Patch-clamp recording—Whole-cell patch-clamp recordings were obtained from visually identified layer V pyramidal cells in the infralimbic mPFC ($\sim$ 700 μ m lateral to the interhemispheric fissure) using infrared DIC-IR videomicroscopy as described previously [21;24;42]. Recording electrodes (3–5 M Ω tip resistance) were made from borosilicate glass and filled with intracellular solution containing (in mM): 122 K-gluconate, 5 NaCl, 0.3

CaCl₂, 2 MgCl₂, 1 EGTA, 10 HEPES, 5 Na₂-ATP, and 0.4 Na₃-GTP; pH was adjusted to 7.2-7.3 with KOH and osmolarity to 280 mOsm/kg with sucrose. For the recording of inhibitory synaptic currents (IPSCs) QX-314 (5 mM) was included in the internal solution. Data acquisition and analysis 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. If series resistance (monitored with pClamp9 software) changed more than 10%, the neuron was discarded.

2.3.3 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 afferent fibers in layer IV (500 μ m from the medial surface of the slice) of the infralimbic cortex as described previously [21;24;42]. Our previous studies identified anterogradely labeled afferents from the basolateral amygdala (BLA) to the infra- and pre-limbic regions of the mPFC following stereotaxic injections of a fluorescent tracer (DiI) into the BLA [21;42]. That information guided the positioning of the stimulation electrode in the present study where no labeling was done. Neurons were voltage-clamped at -70 mV or 0 mV for the study of EPSCs and IPSCs, respectively. The calculated equilibrium potential for chloride in this system was -68.99 mV (Nernst equation, pClamp9 software).

Input-output relationships of evoked EPSCs and IPSCs were obtained by increasing the stimulus intensity in 100 μ A steps. Evoked IPSCs and EPSCs were measured in different cells and isolated by holding potentials. Glutamatergic and/or GABAergic nature of the PSCs was verified pharmacologically at the end of the experiment. 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 about 5 ± 1 spikes (action potentials) in a series of 10 subsequent synaptic stimulations ($\sim 50\%$). E-S coupling was measured as the number of spikes evoked in 10 trials.

Miniature (in TTX 1 μ M) EPSCs and IPSCs (mEPSCs and mIPSCs) were measured in different cells and isolated by holding potentials of -70 mV and 0 mV, respectively, as described previously [21;24;42]. 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 also visually inspected. Miniature EPSCs and IPSCs were measured 5-10 min before and 10-15 min during drug application.

2.4 Drugs

The following drugs were used: Group II mGluR agonist, LY379268, (1*R*,4*R*,5*S*,6*R*)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid; group II mGluR antagonist, LY341495 disodium salt, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propanoic acid disodium salt; NMDA receptor antagonist, AP5, DL-2-amino-5-phosphonopentanoic acid; non-NMDA receptor antagonists, CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione disodium, NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide disodium salt; and GABA_A receptor antagonist, bicuculline, [*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(6*H*)-one. They were purchased from Tocris Bioscience (R&D Systems, Minneapolis, MN). 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 LY379268 [5;8] and LY341495 [11;23].

2.5 Drug application

Drugs were applied by gravity-driven superfusion of the brain slice in 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.

2.6 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 (Graph-Pad, San Diego, CA) was used for all statistical analyses except for cumulative distribution analysis of miniature synaptic events in the patch-clamp studies where the Kolmogorov-Smirnov test was used (MiniAnalysis program 5.3, Synaptosoft Inc.). Statistical analysis was performed on the raw data. Student's *t*-test was used to compare two sets of data that had Gaussian distribution and similar variances. For multiple comparisons, ANOVA (repeated measures where appropriate) was used with Bonferroni posttests as indicated in the text and figure legends.

3. Results

3.1 Group II mGluR agonist effects on synaptically evoked spiking

Synaptically evoked spiking (E-S coupling) of layer V pyramidal cells in the infralimbic mPFC was recorded in current clamp in brain slices from normal rats and from rats with arthritis pain (5-6 h postinduction). Excitatory postsynaptic potentials (EPSPs) and action potentials (spikes) were evoked with near threshold stimulus intensity for spiking (i.e., ~50% success rate) from a holding potential of -60 mV (see Methods 2.3.3). A selective group II mGluR agonist (LY379268, 100 nM; [5;8]) decreased synaptically evoked spiking in brain slices from normal rats ($n = 5$ neurons) and in brain slices from rats with arthritis (5-6 h postinduction; $n = 6$ neurons). The inhibitory effects occurred within a few minutes and were reversed by a selective group II mGluR antagonist (LY341495, 1 μ M; [11;23]). Figure 1 shows individual examples (Fig. 1A, B) and the time course of agonist and antagonist effects averaged for the sample of neurons in brain slices from normal rats ($n = 5$) and from arthritic rats ($n = 5$; Fig. 1C). The inhibitory effects of LY379268 and antagonism

by LY341495 were significant ($P < 0.05$ - 0.001 , $F_{1,40} = 14.73$, normal; $F_{1,40} = 8.99$, arthritis; repeated measures ANOVA with Bonferroni posttests, compared to predrug values at 0 min). Another set of experiments ($n = 5$ neurons recorded in brain slices from arthritis rats) showed that the inhibitory effects of LY379268 persisted during prolonged application of the agonist without the antagonist (Fig. 1C; gray curve, “arthritis - no antag.”; $P < 0.05$ - 0.001 , $F_{1,40} = 10.71$, repeated measures ANOVA with Bonferroni posttests, compared to predrug values at 0 min). The inhibitory effect of LY379268 was concentration-dependent (Fig. 1D) with apparent EC_{50} values of 2.54 nM (normal, $n = 3$ -6 neurons per concentration) and 3.0 nM (pain model, $n = 3$ -6 neurons per concentration). Each concentration was tested only once except when no effect was detected. In that case the perfusion was switched to the next higher concentration.

3.2 Group II mGluR antagonist effects on synaptically evoked spiking

A group II mGluR antagonist (LY341495, 1 μ M) by itself increased synaptically evoked spiking under normal conditions ($n = 5$ neurons) and in the arthritis pain model (5-6 h postinduction; $n = 5$ neurons). Layer V mPFC pyramidal cells were recorded in current clamp and excitatory postsynaptic potentials (EPSPs) and action potentials (spikes) were evoked with near threshold stimulus intensity for spiking (i.e., ~50% success rate) from a holding potential of -60 mV. Individual examples of a pyramidal cell recorded in a brain slice from a normal rat (Fig. 2A) and another cell in a brain slice from an arthritic rat (Fig. 2B) show that LY341495 (1 μ M) increased E-S coupling. Summary of LY341495 effects in the sample of neurons in brain slices from normal ($n = 5$ neurons, Fig. 2C) and arthritic rats ($n = 5$ neurons, Fig. 2D) show that LY341495 (1 μ M) increased E-S coupling significantly ($P < 0.05$, paired t-test).

3.3 Group II mGluR agonist effects on excitatory and inhibitory synaptic transmission

Monosynaptic EPSCs and glutamate-driven IPSCs were evoked by stimulating fibers in layer IV that we identified in our previous studies as containing afferents from the basolateral amygdala (see Methods 2.3.3; [21;42]). Figure 3 shows that EPSCs were blocked completely by antagonists for NMDA and non-NMDA receptors (AP5, 50 μ M, and CNQX, 20 μ M; Fig. 3A). IPSCs were glutamate-driven, which is consistent with feedforward inhibition as shown in detail in our previous studies [21;42], because they were blocked by a non-NMDA receptor antagonist (NBQX, 10 μ M; Fig. 3B). IPSCs were also blocked by bicuculline (10 μ M; data not shown). LY379268 (100 nM) inhibited EPSCs and IPSCs, and coapplication of LY341495 (1 μ M) reversed the effect. Figure 3 shows individual examples (Fig. 3A, B) and summarizes data for the sample of neurons (Fig. 3C, EPSCs, $n = 5$; Fig. 3D, IPSCs, $n = 5$ neurons). The inhibitory effects of LY379268 and antagonism by LY341495 were significant ($P < 0.05$ - 0.01 , $F_{2,8} = 16.01$, EPSC; $F_{2,8} = 12.06$, IPSC; repeated measures ANOVA with Bonferroni posttests). Importantly, time course studies (Fig. 3E) show that the inhibitory effect of LY379268 on EPSCs ($n = 6$ neurons) preceded the effect on IPSCs ($n = 7$ neurons), resulting in significant differences at the early time points of drug application ($P < 0.05$, $F_{1,196} = 28.78$, two-way ANOVA with Bonferroni posttests). Note that these recordings were made in brain slices from arthritic rats since we were primarily interested in pain-related functions of group II mGluRs and did not detect a

difference in group II agonist effects between normal conditions and arthritis pain model in the synaptically evoked spiking assay (Fig. 1).

3.4 Presynaptic site of action of group II mGluR agonist on glutamatergic, not GABAergic, terminals

Quantal analysis of miniature EPSCs (mEPSCs, in the presence of TTX, 1 μ M) showed that LY379268 (100 nM) decreased frequency, but not amplitude, of mEPSCs ($n = 5$ neurons), which is consistent with a presynaptic site of action. Figure 4 shows representative examples of current traces of mEPSCs before (predrug), during application of LY379268 (100 nM) and during coapplication of LY379268 (100 nM) and LY341495 (1 μ M; Fig. 4A). Cumulative distribution analysis of mEPSC frequency (Fig. 4B) and amplitude (Fig. 4C) revealed a significant shift toward larger inter-event intervals ($P < 0.05$, Kolmogorov-Smirnov test, individual neuron) and a significant decrease of mean mEPSC frequency in the sample of neurons ($P < 0.001$, $F_{2, 15} = 17.93$, one-way ANOVA with Bonferroni posttests, $n = 5$ neurons), but had no significant effect on amplitude ($P > 0.05$, $F_{2, 15} = 0.06888$, one-way ANOVA). LY341495 (1 μ M) reversed the effect of LY379268. LY379268 had no effect on frequency and amplitude of mIPSCs ($P > 0.05$, Kolmogorov-Smirnov test for cumulative distribution in an individual neuron; $P > 0.05$, $F_{2, 15} = 0.2617$ and $F_{2, 15} = 0.1228$ for mean frequency and amplitude, respectively, in the sample of neurons, $n = 6$; one-way ANOVA; Fig. 4D-F), arguing against a direct action on GABAergic terminals. Recordings were performed in slices from arthritic animals as explained in the previous paragraph.

4. Discussion

This study provides novel information about the role of group II mGluRs in the prefrontal cortex related to pain. Our data show for the first time that activation of group II mGluRs with a selective agonist (LY379268) inhibits both excitatory and inhibitory synaptic inputs onto mPFC output cells. Inhibitory effects on monosynaptic excitatory transmission preceded the effects on glutamate-driven feedforward inhibition (Fig. 3) and so the overall consequence is decreased output (synaptically evoked spiking; Fig. 1). There was no difference between the pain model and normal conditions (Figs. 1 and 2), arguing against a significant change in activation, expression or function of these receptors. The site of action was presynaptic on glutamatergic but not GABAergic terminals (Fig. 4), which is consistent with dual effects on glutamatergic excitatory transmission and glutamate-driven feedforward inhibition of pyramidal cells in the mPFC. Data with a selective antagonist (LY341495) suggest endogenous activation of group II mGluRs in the mPFC.

The rationale for studying group II mGluR function is that they have been drug targets for anxiety disorders and depression as well as for cognitive deficits in neurodegenerative and neuropsychiatric disorders [12;22;28;36;37]. Pain is associated with affective and cognitive dysfunctions as well [3;30;34], and group II mGluR agonists have antinociceptive effects at different levels of the pain neuraxis, including nociceptors [7;32], spinal dorsal horn neurons [33;45] and amygdala [1;25]. Unexpectedly, we found evidence for endogenous activation of group II mGluRs in the mPFC under normal conditions and in the arthritis pain model because a selective antagonist had facilitatory effects on synaptically evoked spiking of

pyramidal cells. This is consistent with a previous report showing increased activity (c-Fos expression) in various brain areas, including mPFC, following systemic application of a group II mGluR antagonist [26].

The situation is more complex for other areas related to pain processing. A group II antagonist (EGLU) had pronociceptive effects on amygdala (central nucleus, CeA) neurons in an arthritic pain model but not under normal conditions in an in vivo study [25]. However, EGLU had no effects on synaptic transmission in CeA neurons in brain slices, suggesting that tonic input is required but may be lost in the reduced preparation [14]. In another study, LY341495 alone increased evoked EPSCs in CeA neurons in brain slices from formalin injected, but not control, mice, but this effect was not statistically significant [1]. A group II receptor antagonist (APICA) had pronociceptive effects on nociceptors in the capsaicin pain model but not under normal conditions [7]. EGLU alone had no effect on spontaneous EPSCs in spinal cord slices from neuropathic rats and sham controls [45]. In contrast to the largely pronociceptive effect of group II antagonists in other regions, the increase in synaptically evoked spiking of mPFC neurons observed here would seem to be desirable for counteracting pain-related cognitive deficits resulting from decreased mPFC output [19;21;42]. Beneficial effects of group II mGluR antagonists have also been reported in preclinical models of depression and in particular in behavioral tests of cognitive dysfunctions [6;12]. It is intriguing to speculate that these studies may suggest that group II antagonists can mitigate cognitive deficits whereas agonists target sensory and other aspects of pain (i.e., they are anti-nociceptive), and these distinct functions may be region-specific (cortical versus subcortical, spinal and peripheral).

We did not find evidence for a change of group II mGluR effects on mPFC output in the pain model. In contrast, a group II mGluR agonist (LY354740) had greater effects on excitatory transmission in amygdala (CeA) neurons in brain slices [13] and on the responsiveness of CeA neurons to mechanical stimuli in the anesthetized intact animal [25] in an arthritis pain model. Another study, however, found no change in inhibition of excitatory transmission by a group II mGluR agonist (SLx-3095-1) whereas effectiveness of an inhibitor of N-acetylaspartylglutamate (NAAG, endogenous group II activator) peptidase (ZJ43) was decreased 24 hours, but not 6 hours, after formalin-induced hindpaw inflammation [1], suggesting time-dependent changes. In the spinal cord, a group II agonist (DCG-IV) had greater inhibitory effects on polysynaptic and spontaneous EPSCs in slices from neuropathic rats compared to controls but the effect on monosynaptic EPSCs did not change [45]. Group II mGluR agonists (LCCG1 and LY379268) also reversed capsaicin-induced central sensitization of spinal neurons but had no effect on baseline activity [33]. Changes in group II mGluR function in prefrontal cortical areas were also reported for other disorders. Postmortem studies found decrease, increase or no change of mGluR2/3 expression in patients with schizophrenia [27]. Increased levels of mGluR2/3 protein were detected in the prefrontal cortex of patients with major depressive disorder [10]. Here we explored group II mGluR function at the acute stage of an arthritis pain model. It is entirely possible that changes in group II mGluR function occur at the persistent stage of pain and/or in different pain models such as nerve injury. Clearly, it is important to determine time-dependent and region- and disease-specific changes of group II mGluR functions, and so our study makes an important contribution.

Our analysis of evoked and miniature EPSCs and IPSCs suggests that group II mGluRs act presynaptically on glutamatergic terminals to inhibit direct excitatory transmission and glutamate-driven feedforward inhibition. The consequence of these synaptic effects was decreased pyramidal cell output, which would be consistent with the findings of an in vivo study showing that systemic application of a group II mGluR agonist (LY354740) decreased the firing rate of the majority of mPFC neurons [15]. Pharmacological activation of group II mGluRs in the mPFC produces long-term depression (LTD) of excitatory transmission [16;17;40]. LTD in mPFC neurons can be driven from the amygdala (basolateral nucleus, BLA) and involves group II mGluRs because a group II mGluR antagonist (LY341495) blocked long lasting suppression of mPFC responses evoked by BLA stimulation [39]. We stimulated afferent fibers in layer IV of the infralimbic cortex where we previously identified anterogradely labelled afferents from the BLA [21]. Recent evidence suggests that LTD in the mPFC is mediated through postsynaptic mGluR3 but not mGluR2. An mGluR3 negative allosteric modulator blocked LTD induced by LY379268 but not acute synaptic inhibition by LY379268 [43]. We did not measure LTD but our data on acute inhibitory effects of LY379268 would be consistent with the involvement of mGluR2 rather than mGluR3. Results from a recent autoradiography study using [³H]LY459477 identified the mPFC as a region enriched in mGluR2 compared to mGluR3, although this was primarily true for the prelimbic rather than the infralimbic region [44]. Therefore our data on infralimbic pyramidal cells do not rule out a modulatory role of mGluR3.

The novel finding of our study is that despite inhibitory effects both on excitatory and inhibitory transmission a group II mGluR agonist decreased synaptically evoked output of pyramidal cells whereas a selective antagonist increased output, and these effects did not change in a pain model.

Conclusions

Group II mGluRs act presynaptically to inhibit glutamatergic transmission onto medial prefrontal cortical pyramidal cells and interneurons (feedforward inhibition). The overall consequence on pyramidal cell output is inhibition as assessed with synaptically evoked spiking. No difference was found between normal conditions and a pain model. A group II mGluR antagonist by itself increased pyramidal cell output suggesting perhaps tonic control of mPFC output. The facilitatory effects of the antagonist suggest that group II mGluRs are endogenously activated and inhibit cortical output, which is not desirable under conditions that demand enhanced cortical function. Removing tonic inhibition with the antagonist would have beneficial disinhibitory effects on cortical output, which is consistent with the improvement of cognitive functions by group II antagonists observed in other studies.

Acknowledgments

This work was supported by National Institute of Neurological Disorders and Stroke (NIH/NINDS) Grants NS081121 and NS038261.

Reference List

1. Adedoyin MO, Vicini S, Neale JH. Endogenous N-acetylaspartylglutamate (NAAG) inhibits synaptic plasticity/transmission in the amygdala in a mouse inflammatory pain model. *Mol Pain*. 2010; 6:60–77. [PubMed: 20860833]
2. Ago Y, Yano K, Araki R, Hiramatsu N, Kita Y, Kawasaki T, Onoe H, Chaki S, Nakazato A, Hashimoto H, Baba A, Takuma K, Matsuda T. Metabotropic glutamate 2/3 receptor antagonists improve behavioral and prefrontal dopaminergic alterations in the chronic corticosterone-induced depression model in mice. *Neuropharmacology*. 2013; 65:29–38. [PubMed: 23022081]
3. Apkarian AV, Neugebauer V, Koob G, Edwards S, Levine JD, Ferrari L, Egli M, Regunathan S. Neural mechanisms of pain and alcohol dependence. *Pharmacol Biochem Behav*. 2013; 112C:34–41. [PubMed: 24095683]
4. Apkarian AV, Sosa Y, Krauss BR, Thomas PS, Fredrickson BE, Levy RE, Harden RN, Chialvo DR. Chronic pain patients are impaired on an emotional decision-making task. *Pain*. 2004; 108:129–136. [PubMed: 15109516]
5. Bond A, Jones NM, Hicks CA, Whiffin GM, Ward MA, O'Neill MF, Kingston AE, Monn JA, Ornstein PL, Schoepp DD, Lodge D, O'Neill MJ. Neuroprotective effects of LY379268, a selective mGlu2/3 receptor agonist: investigations into possible mechanism of action in vivo. *J Pharmacol Exp Ther*. 2000; 294:800–809. [PubMed: 10945827]
6. Campo B, Kalinichev M, Lambeng N, El YM, Royer-Urios I, Schneider M, Legrand C, Parron D, Girard F, Bessif A, Poli S, Vaugois JM, Le PE, Celanire S. Characterization of an mGluR2/3 negative allosteric modulator in rodent models of depression. *J Neurogenet*. 2011; 25:152–166. [PubMed: 22091727]
7. Carlton SM, Zhou S, Govea R, Du J. Group II/III metabotropic glutamate receptors exert endogenous activity-dependent modulation of TRPV1 receptors on peripheral nociceptors. *J Neurosci*. 2011; 31:12727–12737. [PubMed: 21900552]
8. Collado I, Pedregal C, Mazon A, Espinosa JF, Blanco-Urgoiti J, Schoepp DD, Wright RA, Johnson BG, Kingston AE. (2S,1'S,2'S,3'R)-2-(2'-carboxy-3'-methylcyclopropyl) glycine is a potent and selective metabotropic group 2 receptor agonist with anxiolytic properties. *J Med Chem*. 2002; 45:3619–3629. [PubMed: 12166935]
9. Fell MJ, Witkin JM, Falcone JF, Katner JS, Perry KW, Hart J, Rorick-Kehn L, Overshiner CD, Rasmussen K, Chaney SF, Benvenista MJ, Li X, Marlow DL, Thompson LK, Luecke SK, Wafford KA, Seidel WF, Edgar DM, Quets AT, Felder CC, Wang X, Heinz BA, Nikolayev A, Kuo MS, Mayhugh D, Khilevich A, Zhang D, Ebert PJ, Eckstein JA, Ackermann BL, Swanson SP, Catlow JT, Dean RA, Jackson K, Tauscher-Wisniewski S, Marek GJ, Schkeryantz JM, Svensson KA. N-(4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant properties: in vivo profiling suggests a link between behavioral and central nervous system neurochemical changes. *J Pharmacol Exp Ther*. 2011; 336:165–177. [PubMed: 20947638]
10. Feyissa AM, Woolverton WL, Miguel-Hidalgo JJ, Wang Z, Kyle PB, Hasler G, Stockmeier CA, Iyo AH, Karolewicz B. Elevated level of metabotropic glutamate receptor 2/3 in the prefrontal cortex in major depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010; 34:279–283. [PubMed: 19945495]
11. Fitzjohn SM, Bortolotto ZA, Palmer MJ, Doherty AJ, Ornstein PL, Schoepp DD, Kingston AE, Lodge D, Collingridge GL. The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. *Neuropharmacology*. 1998; 37:1445–1458. [PubMed: 9886667]
12. Goeldner C, Ballard TM, Knoflach F, Wichmann J, Gatti S, Umbricht D. Cognitive impairment in major depression and the mGlu2 receptor as a therapeutic target. *Neuropharmacology*. 2013; 64:337–346. [PubMed: 22992331]
13. Han JS, Bird GC, Neugebauer V. Enhanced group III mGluR-mediated inhibition of pain-related synaptic plasticity in the amygdala. *Neuropharmacology*. 2004; 46:918–926. [PubMed: 15081788]
14. Han JS, Fu Y, Bird GC, Neugebauer V. Enhanced group II mGluR-mediated inhibition of pain-related synaptic plasticity in the amygdala. *Mol Pain*. 2006; 2:18–29. [PubMed: 16681859]

15. Homayoun H, Jackson ME, Moghaddam B. Activation of metabotropic glutamate 2/3 receptors reverses the effects of NMDA receptor hypofunction on prefrontal cortex unit activity in awake rats. *J Neurophysiol.* 2005; 93:1989–2001. [PubMed: 15590730]
16. Huang CC, Hsu KS. The role of NMDA receptors in regulating group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex. *Neuropharmacology.* 2008; 54:1071–1078. [PubMed: 18378263]
17. Huang CC, Yang PC, Lin HJ, Hsu KS. Repeated cocaine administration impairs group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex. *J Neurosci.* 2007; 27:2958–2968. [PubMed: 17360919]
18. Ji G, Neugebauer V. Pain-related deactivation of medial prefrontal cortical neurons involves mGluR1 and GABA(A) receptors. *J Neurophysiol.* 2011; 106:2642–2652. [PubMed: 21880942]
19. Ji G, Neugebauer V. Pain-related deactivation of medial prefrontal cortical neurons involves mGluR1 and GABAA receptors. *J Neurophysiol.* 2011; 106:2642–2652. [PubMed: 21880942]
20. Ji G, Neugebauer V. CB1 augments mGluR5 function in medial prefrontal cortical neurons to inhibit amygdala hyperactivity in an arthritis pain model. *Eur J Neurosci.* 2014; 39:455–466. [PubMed: 24494685]
21. Ji G, Sun H, Fu Y, Li Z, Pais-Vieira M, Galhardo V, Neugebauer V. Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation. *J Neurosci.* 2010; 30:5451–5464. [PubMed: 20392966]
22. Kim SH, Steele JW, Lee SW, Clemenson GD, Carter TA, Treuner K, Gadiant R, Wedel P, Glabe C, Barlow C, Ehrlich ME, Gage FH, Gandy S. Proneurogenic Group II mGluR antagonist improves learning and reduces anxiety in Alzheimer Abeta oligomer mouse. *Mol Psychiatry.* 2014; 19:1235–1242. [PubMed: 25113378]
23. Kingston AE, Ornstein PL, Wright RA, Johnson BG, Mayne NG, Burnett JP, Belagaje R, Wu S, Schoepp DD. LY341495 is a nanomolar potent and selective antagonist of group II metabotropic glutamate receptors. *Neuropharmacology.* 1998; 37:1–12. [PubMed: 9680254]
24. Kiritoshi T, Sun H, Ren W, Stauffer SR, Lindsley CW, Conn PJ, Neugebauer V. Modulation of pyramidal cell output in the medial prefrontal cortex by mGluR5 interacting with CB1. *Neuropharmacology.* 2013; 66:170–178. [PubMed: 22521499]
25. Li W, Neugebauer V. Differential changes of group II and group III mGluR function in central amygdala neurons in a model of arthritic pain. *J Neurophysiol.* 2006; 96:1803–1815. [PubMed: 16760343]
26. Linden AM, Bergeron M, Schoepp DD. Comparison of c-Fos induction in the brain by the mGlu2/3 receptor antagonist LY341495 and agonist LY354740: Evidence for widespread endogenous tone at brain mGlu2/3 receptors in vivo. *Neuropharmacology.* 2005; 49:120–134. [PubMed: 16023151]
27. Marek GJ. Metabotropic glutamate2/3 (mGlu2/3) receptors, schizophrenia and cognition. *Eur J Pharmacol.* 2010; 639:81–90. [PubMed: 20371229]
28. Marek GJ. Metabotropic glutamate 2/3 receptors as drug targets. *Current Opinion in Pharmacology.* 2004; 4:18–22. [PubMed: 15018834]
29. Metz AE, Yau HJ, Centeno MV, Apkarian AV, Martina M. Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. *Proc Natl Acad Sci U S A.* 2009
30. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. *Prog Neurobiol.* 2011; 93:385–404. [PubMed: 21216272]
31. Neugebauer V. Glutamate receptor ligands. *Handb Exp Pharmacol.* 2007; 177:217–249. [PubMed: 17087125]
32. Neugebauer V, Carlton SM. Peripheral metabotropic glutamate receptors as drug targets for pain relief. *Exp Opin Therap Targets.* 2002; 6:349–361.
33. Neugebauer V, Chen PS, Willis WD. Groups II and III metabotropic glutamate receptors differentially modulate brief and prolonged nociception in primate STT cells. *J Neurophysiol.* 2000; 84:2998–3009. [PubMed: 11110827]
34. Neugebauer V, Galhardo V, Maione S, Mackey SC. Forebrain pain mechanisms. *Brain Res Rev.* 2009; 60:226–242. [PubMed: 19162070]

35. Neugebauer V, Han JS, Adwanikar H, Fu Y, Ji G. Techniques for assessing knee joint pain in arthritis. *Mol Pain*. 2007; 3:8–20. [PubMed: 17391515]
36. Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT, Pin JP. Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology*. 2011; 60:1017–1041. [PubMed: 21036182]
37. Nicoletti F, Bruno V, Ngomba RT, Gradini R, Battaglia G. Metabotropic glutamate receptors as drug targets: what's new? *Curr Opin Pharmacol*. 2015; 20C:89–94. [PubMed: 25506748]
38. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol*. 2010; 50:295–322. [PubMed: 20055706]
39. Onozawa K, Yagasaki Y, Izawa Y, Abe H, Kawakami Y. Amygdala-prefrontal pathways and the dopamine system affect nociceptive responses in the prefrontal cortex. *BMC Neurosci*. 2011; 12:115. [PubMed: 22085449]
40. Otani S, Daniel H, Takita M, Crepel F. Long-Term Depression Induced by Postsynaptic Group II Metabotropic Glutamate Receptors Linked to Phospholipase C and Intracellular Calcium Rises in Rat Prefrontal Cortex. *J Neurosci*. 2002; 22:3434–3444. [PubMed: 11978820]
41. Pais-Vieira M, Mendes-Pinto MM, Lima D, Galhardo V. Cognitive impairment of prefrontal-dependent decision-making in rats after the onset of chronic pain. *Neuroscience*. 2009; 161:671–679. [PubMed: 19362121]
42. Sun H, Neugebauer V. mGluR1, but not mGluR5, activates feed-forward inhibition in the medial prefrontal cortex to impair decision making. *J Neurophysiol*. 2011; 106:960–973. [PubMed: 21613584]
43. Walker AG, Wenthur CJ, Xiang Z, Rook JM, Emmitte KA, Niswender CM, Lindsley CW, Conn PJ. Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction. *Proc Natl Acad Sci U S A*. 2015; 112:1196–1201. [PubMed: 25583490]
44. Wright RA, Johnson BG, Zhang C, Salhoff C, Kingston AE, Calligaro DO, Monn JA, Schoepp DD, Marek GJ. CNS distribution of metabotropic glutamate 2 and 3 receptors: transgenic mice and [(3)H]LY459477 autoradiography. *Neuropharmacology*. 2013; 66:89–98. [PubMed: 22313530]
45. Zhou HY, Chen SR, Chen H, Pan HL. Functional plasticity of group II metabotropic glutamate receptors in regulating spinal excitatory and inhibitory synaptic input in neuropathic pain. *J Pharmacol Exp Ther*. 2011; 336:254–264. [PubMed: 20923868]

Highlights

- A group II mGluR agonist (LY379268) decreased mPFC output under normal conditions and in a pain model.
- LY379268 inhibited the excitatory drive of mPFC pyramidal cells presynaptically.
- LY379268 also decreased glutamate-driven feedforward inhibition of mPFC pyramidal cells.
- LY379268 did not act directly on GABAergic terminals.
- Blockade of group II mGluRs with LY341495 increased pyramidal cell output.

Figure 1.

A group II agonist (LY379268) decreased synaptically evoked spiking under normal conditions and in the arthritis pain model. (**A, B**) Individual examples of a pyramidal cell in a brain slice from a normal rat (**A**) and another one in a brain slices from an arthritic rat (**B**) show that LY379268 (100 nM) decreased E-S coupling. Coapplication of a group II mGluR antagonist (LY341495, 1 μ M) reversed the effect of LY379268. Individual traces (10 each) show current-clamp recordings of excitatory postsynaptic potentials and action potentials (spikes) evoked from a holding potential of -60 mV. (**C**) Time course of effects of LY379268 and LY341495 in the sample of neurons recorded under normal conditions ($n =$

5) and in the pain model ($n = 5$). **,*** $P < 0.01-0.001$ (normal), #,## $P < 0.05-0.01$ (arthritis), compared to predrug values at 0 min, repeated measures ANOVA with Bonferroni posttests. Inhibitory effects of LY379268 persisted in another 5 neurons when no antagonist (“no antag.”) was applied; +, ++, +++ $P < 0.05-0.001$, compared to predrug values at 0 min. **(D)** Concentration-response curves for LY379268 under normal conditions ($n = 3-6$ neurons) and in the arthritis pain model ($n = 3-6$ neurons). Each symbols shows the mean (and SEM) normalized to predrug controls (set to 100%). Probability of synaptically evoked spikes was calculated as follows: (number of trials with evoked spikes)/(number of trials).

Figure 2.

A group II mGluR antagonist (LY341495, 1 μ M) by itself increased synaptically evoked spiking under normal conditions (A, C) and in the arthritis pain model (5-6 h postinduction; B, D). (A, B) Individual examples of a pyramidal cell in a brain slice from a normal rat (A) and another one in a brain slice from an arthritic rat (B) show that LY341495 (1 μ M) increased E-S coupling. Individual traces (10 each) show current-clamp recordings of excitatory postsynaptic potentials and action potentials (spikes) evoked from a holding potential of -60 mV. (C, D) Summary of LY341495 effects. Bar histograms show mean (and SEM) normalized to predrug control values (set to 100%). LY341495 increased E-S coupling in brain slices from normal ($n = 5$ neurons, C) and arthritic rats ($n = 5$ neurons, D) significantly. * $P < 0.05$ compared to predrug (paired t-test).

Figure 3.

Differential time course of the inhibitory effect of LY379268 on evoked EPSCs versus IPSCs in the arthritis pain model. (**A**, **B**) Individual examples of EPSCs (**A**) and IPSCs (**B**) show the inhibitory effects of LY379268 (100 nM) that were reversed by coapplication of LY341495 (1 μ M). EPSCs were blocked by AP5 (50 μ M) and CNQX (20 μ M). IPSCs were blocked by NBQX (10 μ M). Individual traces recorded in voltage-clamp show EPSCs or IPSCs (average of 8–10) evoked with a stimulation intensity of 0.9 mA. (**C**, **D**) Summary of LY379268 effects and blockade by LY341495 (**C**, EPSCs, $n = 5$; **D**, IPSCs, $n = 5$ neurons). Bar histograms show mean (and SEM) of peak amplitudes. *,** $P < 0.05$, 0.01, repeated measures ANOVA with Bonferroni posttests. (**E**) Time course data show that the effect of LY379268 on IPSCs ($n = 7$ neurons) was slower than that on EPSCs ($n = 6$ neurons).

Symbols show means (and SEM) normalized to predrug controls (set to 100%). * $P < 0.05$, IPSCs compared to EPSCs, Bonferroni posttests.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

LY379268 acts on presynaptically on glutamatergic rather than GABAergic terminals in the arthritis pain model. Analysis of miniature EPSCs (mEPSCs, A-C) and IPSCs (mIPSCs, D-F) recorded in TTX (1 μ M) over periods of 5 min. (A) Representative current traces of mEPSCs recorded in voltage-clamp at -70 mV before (predrug) and during application of LY379268 (100 nM) alone and in combination with LY341495 (1 μ M). (B, C) Cumulative distribution analysis of mEPSC frequency (B) and amplitude (C). LY379268 caused a significant shift toward larger inter-event intervals ($P < 0.05$, Kolmogorov-Smirnov test,

individual neuron) and decreased mean mEPSC frequency in the sample of neurons significantly ($P < 0.001$, Bonferroni posttests, $n = 5$ neurons) but had no significant effect on mEPSC amplitude. LY341495 ($1 \mu\text{M}$) reversed the effect of LY379268. **(D)** Representative current traces of mIPSCs recorded in voltage-clamp at 0 mV before (predrug) and during application of LY379268 (100 nM) alone or in combination with LY341495 ($1 \mu\text{M}$). **(E, F)** Cumulative distribution analysis of mIPSC frequency (E) and amplitude (F). LY379268 had no significant effect ($P > 0.05$, Kolmogorov-Smirnov test for cumulative distribution in an individual neuron; $P > 0.05$, Bonferroni posttest for mean frequency and amplitude in sample of neurons, $n = 6$ neurons). *** $P < 0.001$ (Bonferroni posttests).