Neuropharmacology 95 (2015) 388–394

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

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

Takaki Kiritoshi [a], Volker Neugebauer [a, b, *]

[a] Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center (TTUHSC), School of Medicine, 3601 4th Street, Mail Stop 6592, Lubbock, TX 79430-6592, USA
[b] Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center (TTUHSC), School of Medicine, 3601 4th Street, Mail Stop 6592, Lubbock, TX 79430-6592, USA

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form
26 March 2015
Accepted 2 April 2015
Available online 22 April 2015

Keywords:
Medial prefrontal cortex
Pain
Synaptic transmission
mGluR
Patch-clamp

ABSTRACT

The medial prefrontal cortex (mPFC) serves executive control functions that are impaired in neuropsychiatric disorders and pain. Therefore, restoring normal synaptic transmission and output is a desirable goal. Group II metabotropic glutamate receptors mGluR2 and mGluR3 are highly expressed in the mPFC, modulate synaptic transmission, and have been targeted for neuropsychiatric disorders. Their pain-related modulatory effects in the mPFC remain to be determined. Here we evaluated their ability to restore pyramidal output in an arthritis pain model.

Whole-cell patch-clamp recordings of layer V mPFC pyramidal cells show that a selective group II mGluR agonist (LY379268) decreased synaptically evoked spiking in brain slices from normal and arthritic rats. 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 pain 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 alone increased synaptically evoked spiking under normal conditions and in the pain model.

In conclusion, 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. Facilitatory effects of a group II antagonist suggest the system may be tonically active to control pyramidal output. Failure to release the inhibitory tone and enhance mPFC output could be a mechanism for the development or persistence of a disease state such as pain.

1. Introduction

The medial prefrontal cortex (mPFC) serves executive control functions such as decision-making and behavioral control, which are impaired in pain patients (Apkarian et al., 2004; Moriarty et al., 2011) and in animal models of pain (Ji et al., 2010; Pais-Vieira et al., 2009). Work from our group (Ji and Neugebauer, 2011, 2014; Ji et al., 2010) and others (Metz et al., 2009) 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 (Ji et al., 2010). Conversely, increasing pyramidal cell activity inhibited amygdala activity and pain behaviors (Ji and Neugebauer, 2014). 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 (Ji and Neugebauer, 2014; Kiritoshi et al., 2013).

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 (Nicoletti et al., 2011; Niswender and Conn, 2010). Group II mGluRs are highly expressed in the prefrontal cortex with

* Corresponding author. Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, School of Medicine, 3601 4th Street, Mail Stop 6592, Lubbock, TX 79430-6592, USA. Tel.: +1 806 743 3880; fax: +1 806 743 2744.
E-mail address: volker.neugebauer@ttuhsc.edu (V. Neugebauer).

http://dx.doi.org/10.1016/j.neuropharm.2015.04.003
0028-3908/

mGluR2 showing a bilaminar distribution (layer I and V), particularly in the mPFC, and mGluR3 being present on GABAergic axons and in pyramidal cells (Marek, 2010; Wright et al., 2013). Activation of group II mGluRs produces well-established antinociceptive, anxiolytic and antidepressant effects (Fell et al., 2011; Neugebauer, 2007; Nicoletti et al., 2011). Preclinical studies also suggested beneficial effects of group II mGluR agonists on positive and negative symptoms of schizophrenia but clinical trials failed (Nicoletti et al., 2015). Interestingly, group II mGluR antagonists and negative allosteric modulators also showed antidepressant effects and in particular on outcome measures of cognitive dysfunction (Ago et al., 2013; Campo et al., 2011; Goeldner et al., 2013; Nicoletti et al., 2015), possibly involving the prefrontal cortex (Ago et al., 2013). Group II mGluR antagonists had beneficial effects in an Alzheimer's Disease mouse model, improving cognitive functions and anxiety (Kim et al., 2014). However, mGluR3 negative allosteric modulators impaired extinction of conditioned fear (Walker et al., 2015). 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 (Marek, 2010). 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 (Walker et al., 2015). 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 h. 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 (Neugebauer et al., 2007). A kaolin suspension (4%, 100 μl) 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 μl) 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 (Neugebauer et al., 2007). 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 (Ji et al., 2010; Kiritoshi et al., 2013; Sun and Neugebauer, 2011). Coronal brain slices (300–500 μm) 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 ± 1 °C) superfusing 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. 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 (~700 μm lateral to the interhemispheric fissure) using infrared DIC-IR videomicroscopy as described previously (Ji et al., 2010; Kiritoshi et al., 2013; Sun and Neugebauer, 2011). Recording electrodes (3–5 MΩ tip resistance) were made from borosilicate glass and 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; 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 μs square-wave pulses; using an S88 stimulator; Grass Instruments) of afferent fibers in layer IV (500 μm from the medial surface of the slice) of the infralimbic cortex as described previously (Ji et al., 2010; Kiritoshi et al., 2013; Sun and Neugebauer, 2011). 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 (Ji et al., 2010; Sun and Neugebauer, 2011). 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 μA 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 (~50%). E–S coupling was measured as the number of spikes evoked in 10 trials.

Miniature (in TTX 1 μM) 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 (Ji et al., 2010; Kiritoshi et al., 2013; 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 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(6H)-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 (Bond et al., 2000; Collado et al., 2002) and LY341495 (Fitzjohn et al., 1998; Kingston et al., 1998).