Neuropharmacology 66 (2013) 317–329

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Role of metabotropic glutamate receptor 1 in the basolateral amygdala-driven prefrontal cortical deactivation in inflammatory pain in the rat

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ARTICLE INFO

Article history:
Received 7 December 2011
Received in revised form
25 April 2012
Accepted 28 May 2012

Keywords:
mGluRs
Prefrontal cortex
Basolateral amygdala
Inflammatory pain
Rat

ABSTRACT

Plastic changes in the amygdala and limbic cortex networks have been widely shown in chronic pain. We have here investigated the role of group I metabotropic glutamate receptors (mGluRs) in the basolateral amygdala (BLA) pre-infra-limbic (PL-IL) divisions of the medial prefrontal cortex (mPFC) neuron connections after carrageenan-induced inflammatory pain in the rat. Intra-plantar injection of carrageenan decreased either spontaneous or mechanically/electrically evoked activity of PL cortex pyramidal neurons which responded with excitation in a way prevented by CPCOOEt, a selective mGluR1 antagonist, though not by MPEP, a selective mGluR5 antagonist. Accordingly, intra-BLA microinjection of DHPG, a group I mGluR agonist, caused PL cortex neuron activity depression, antagonized by CPCCOEt. CPCOOEt, but not MPEP, reduced also carrageenan-induced mechanical allodynia. The PL cortex cell deactivation in inflammatory pain condition was associated with increased GABA (conversely glutamate was decreased) in the PL/IL cortex. The local application of bicuculline, a $GABA_A$ receptor selective antagonist, reduced mechanical allodynia. An over-expression of mGluR1, but not mGluR5, have been observed in the PL-IL cortex after inflammatory pain suggesting an increased mGluR1-dependent cross-talk among BLA and IL-PL cortex neurons in inflammatory pain conditions.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

The medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) are anatomically and functionally interconnected (Aggleton, 2000; Neugebauer et al., 2003) and cooperate in the processing and integration of sensory inputs (Garcia et al., 1999; Milad et al., 2004). Evidence suggests that the pain-related unpleasantness and affective component are critically driven by amygdala and mPFC connections (Garcia et al., 1999; Roozendaal et al., 2009). BLA-mPFC pathway represents the major source of input from the amygdala to the mPFC (Kita and Kitai, 1990; Bacon et al., 1996; Gabbott et al., 2006) whose prelimbic and infralimbic (PL/IL) divisions are involved in the affective component of pain (Lorenz et al., 2003). Frontal cortex functional reorganization, or even atrophy, which can be correlated to the cognitive impairment, has been reported in chronic pain conditions (Apkarian et al., 2004a,b; 2006; Baliki et al., 2006; Metz et al., 2009; de Novellis et al., 2011). In the arthritis pain model, input to and output from central nucleus of the amygdala (CeA) or BLA proved to be enhanced, reflecting an increased excitability of the same neurons that respond to group I metabotropic glutamate receptor (mGluR) stimulation (Neugebauer and Li, 2002; Neugebauer et al., 2003). Those findings support the idea that group I mGluRs in the amygdala may play a critical role in the processing of the affective aspect of pain. Based on recent evidence that pain-related plasticity in the BLA might affect the function of pyramidal neurons in the PL division of mPFC (Ji et al., 2010; de Novellis et al., 2011), mGluR1 and mGluR5 could represent a target for further investigating the mechanisms leading to central neural remodelling in inflammatory pain conditions. Consistently with the hyperactivity of the BLA and depression of mPFC in arthritis inflammation (Ji et al., 2010), we hypothesized here that carrageenan-induced inflammation may also inhibit the activity of mPFC neurons. Although the majority of the pyramidal neurons encountered were those responding with an inhibition according to the literature (Floresco and Tse, 2007;

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0028-3908/$ – see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.neuropharm.2012.05.047

Pérez-Jaranay and Vives, 1991; de Novellis et al., 2011), we focused our investigation on mPFC pyramidal neurons responding with an excitation to mechanical and/or BLA electrical stimulation since a) their deactivation can be easily quantified b) the firing of neurons responding with an inhibition was completely erased after carrageenan injection and the electrophysiological parameters were, in turn, not properly measurable c) no previous studies have been performed on these neurons in carrageenan-induced inflammatory pain *in vivo*.

Therefore, based on BLA plasticity occurring after prolonged pain stimuli (i.e. neural sensitization and increased excitatory outflow towards cortex) and the mGluRs capability to modulate nocifensive and anxiety-like behaviour in inflammatory pain states (Ji et al., 2010; Palazzo et al., 2011), we have hypothesized that intra-BLA mGluR1/5 ligands affect nociceptive behaviour and induce phenotypic changes of the excitatory responding PL cortex neurons after inflammatory pain (de Novellis et al., 2011; Giordano et al., in press). Furthermore, we have hypothesized that carrageenan-induced inflammatory pain could be associated with changes in BLA mGluR1 or mGluR5 expression and with an increased GABA release in the mPFC which may in turn induce neural deactivation following intra-paw carrageenan. Finally, pilot experiments were performed to assess possible phenotypic changes in the BLA-mPFC pathway after intra-paw formalin injection.

2. Methods

2.1. Animals

Male Wistar rats (250–300 g) were housed 3 per cage under controlled illumination (12:12 h light:dark cycle; light on 06.00 h) and standard environmental conditions (ambient temperature 20–22 °C, humidity 55–60%) for at least 1 week before the commencement of experiments. Rat chow and tap water were available *ad libitum*. The experimental procedures were approved by the Animal Ethics Committee of the Second University of Naples. Animal care was in compliance with Italian Legislative Decree (D.L. 116/92) and European Commission Directive (O.J. of E.C. L358/1, 18/12/86) regulations on the protection of laboratory animals. All efforts were made to minimise animal suffering and the number of animals used.

2.2. The carrageenan pain model

Peripheral inflammatory pain was induced by a single subcutaneous injection of λ-carrageenan (150 μl of 1% solution) in the plantar surface of the right hind paw using a 30-gauge needle in accordance with a previous study (Palazzo et al., 2011).

The formalin pain model was performed by injecting formalin in the paw (100 μl of 5% solution).

2.3. Treatments

A) For *in vivo* extracellular recordings groups of rats ($n = 10$ for each treatment) received the intra-BLA microinjection (200 nl) of vehicle or drug solution as follows:
   1. dimethyl sulfoxide (DMSO) 0.05% in artificial cerebrospinal fluid (ACSF, composition in mM: NaCl, 125; KCl, 2.5; $MgCl_2$, 1.18 and $CaCl_2$, 1.26), CPCCOEt (10 nmol) or MPEP (50 nmol) 30 min after the intraplantar injection of saline or carrageenan into the plantar surface of the rat's hind paw;
   2. DHPG (25 nmol) alone or in combination with CPCOOEt (10 nmol) or MPEP (50 nmol), administered 30 min after DHPG;

B) For *in vitro* RT-PCR, western blot and immunohistochemical analysis, groups of rats ($n = 5$) were sacrificed and BLA was dissected out 4 h after intraplantar injection of saline or carrageenan;

C) For *in vivo* microdialysis experiments, groups of rats ($n = 7$) were used 4 h after intraplantar injection of saline or carrageenan;

D) For *in vivo* behavioural experiments, groups of rats ($n = 7$ for each treatment) received the microinjection of 200 nl of vehicle or drug solutions as follows:
   1. intra-BLA CPCOOEt (10 nmol) or MPEP (50 nmol), both administered 1.5 h after the intraplantar injection of carrageenan;
   2. the intra-IL mPFC microinjection of bicuculline (0.5 nmol), a selective $GABA_A$ antagonist, 1 h before than intraplantar injection of carrageenan.

2.4. In vivo single unit extracellular recording

For electrophysiological recordings, rats were anaesthetised with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic device (David Kopf Instruments, Tujunga, CA, USA). Body temperature was maintained at 37 °C with a temperature-controlled heating pad. In all surgical preparations, the scalp was incised and holes were drilled into the skull overlying the site of recording, the II–III layers of mPFC (AP: +3.8–2.7 mm from bregma, L: 0.5–0.8 from the midline and V: 2.2–3.4 mm from cortical surface), electrical stimulation BLA (AP: −9 mm from bregma, L:4.2 from the midline at anteroposterior angle of 30°, and V: 8.8 mm from cortical surface) and drug administration or single-unit recordings, BLA (AP: −2.5–3.1 mm from bregma, L:4.5–5.0 from the midline and V: 7.2–9 mm from cortical surface) according to the coordinates from Paxinos and Watson (1986) and contralaterally with respect of the injection of saline/carrageenan into the hind paw. Concentric bipolar electrical stimulating electrodes (SNE-100; David Kopf Instruments, Tujunga, CA) were implanted into the caudal region of the BLA. This region was selected for two reasons, the first being that most of the projections from the BLA to the mPFC originate in the more caudal regions of this nucleus (Kita and Kitai, 1990; McDonald, 1991; Conde et al., 1995). Second, projections originating from the mPFC terminate in the more rostral regions of the BLA (Sesack et al., 1989; McDonald et al., 1996). Thus, by placing our electrodes in the more caudal BLA, we would minimize the possibility that BLA-evoked changes in mPFC neural firing were attributable to antidromic activation of recurrent axon collaterals originating in the mPFC. Anaesthesia was maintained with a constant, continuous infusion of propofol (5–10 mg/kg/h, i.v.) and a bipolar concentric electrode (NEX-100; Rhodes Medical Instruments Inc., Summerland, CA) connected to A320 stimulator (World Precision Instruments, England) was lowered into the caudal region of the BLA according to Floresco and Tse (2007). After the lowering of the stimulating electrode into the BLA, a glass-insulated tungsten filament electrode (3–5 MΩ) (FHC Frederick Haer & Co., ME, USA) was stereotaxically lowered into the mPFC. The recorded signals were amplified and displayed on a digital storage oscilloscope to ensure that the unit under study was unambiguously discriminated throughout the experiment. Signals were also processed by an interface CED 1401 (Cambridge Electronic Design Ltd., UK) connected to a Pentium III PC. Spike2 software (CED, version 4) was used to create peristimulus rate histograms on-line and to store and analyse digital records of single unit activity off-line. Configuration, shape and height of the recorded action potentials were monitored and recorded continuously using a window discriminator and Spike2 software for on-line and off-line analysis. This study only included neurons with a regular spiking pattern and a spontaneous firing rate of between 0.4 and 3.8 Hz, which were classified as pyramidal neurons (Floresco and Tse, 2007). Once a neuron was encountered in mPFC, the position of the microelectrode was adjusted to maximize the spike amplitude against background noise. We then delivered electrical stimuli into the BLA (700 μA) at 2 s intervals. At least 50 single pulses were delivered to generate peristimulus time histograms (PSTHs). Once the cell was identified, mechanical stimuli were applied to the hind paw (contralateral to the mPFC) by using home-made spring-operated forceps that closed with a force (>600 g/10 $mm^2$and <800 g/10 mm^2) calibrated with a tension spring balance, delivered for 5 s (Hoheisel et al., 2007). By using electrical (BLA) or mechanical (hind paw) stimuli, we were able to determine whether each individual neuron was inhibited, excited, or else showed no response to stimulation. We did not record data from neurons displaying no change in firing in response to stimulation and continued the cell-searching procedure. At the end of the experiment, the electrically stimulated BLA area was histologically verified. The recording site was marked with a 20 Ma DC current for 20 s. After fixation by embedding in 10% formalin, the recording sites were identified. Electrical stimulation outside of the BLA nucleus of amygdala was excluded from the study. All the electrophysiological evaluations have been performed in the right mPFC.

2.5. Intracerebral drug injection

A guide stainless steel cannula (26 gauge) was stereotaxically lowered 2 mm above the BLA (coordinates AP: −2.5–3.1 mm from bregma, L:4.5–5.0 from the midline and V: 7.2–9 mm from cortical surface) or the intra-IL mPFC. The cannula was fixed into the skull using dental screw and cement. A vehicle/drug solution prefilled inner cannula (33 gauge stainless steel tubing) was inserted and driven 2 mm below the tip of the guide. The microinjection was made using a 2 μl Hamilton syringe that was connected to the injection cannula by a length of polyethylene (PE-10) tubing. Volumes of 0.2 μl of vehicle or drug solutions were microinjected into the BLA or IL mPFC over a period of 60 s and the injection cannula was gently removed 2 min later. At the completion of the experiment, the microinjection sites were histologically verified and plotted on a standardized section derived from the stereotaxic atlas of Paxinos and Watson (1986). At the completion of the experiment, a volume of 200 nl of neutral red (0.1%) was also injected into the BLA or IL mPFC 30–40 min prior to killing the rat. Rats were then intra-cardially perfused with 20 ml phosphate buffer solution (PBS) followed by 200 ml 10% formalin solution in PBS. The brains were removed and immersed in a saturated formalin solution for 2 days. The injection sites were ascertained by using 2 consecutive sections (40 μm), one stained with neutral red to identify nuclei and the other unstained in order to determine dye spreading.