European Journal of Pharmacology 749 (2015) 115–123

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and analgesia

The influence of microglia activation on the efficacy of amitriptyline, doxepin, milnacipran, venlafaxine and fluoxetine in a rat model of neuropathic pain

Magdalena Zychowska, Ewelina Rojewska, Wioletta Makuch, Barbara Przewlocka, Joanna Mika*

Department of Pain Pharmacology, Institute of Pharmacology, Krakow, Poland

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form
12 November 2014
Accepted 17 November 2014
Available online 25 November 2014

Keywords:
Antidepressants
Astroglia
Microglia
Neuropathic pain

ABSTRACT

The analgesic properties of antidepressants are often used in the treatment of neuropathy; however their influence on glial cells in maintaining neuropathic pain is unknown. Our studies examined the neuropathic pain-relieving properties after intraperitoneal injection of amitriptyline, doxepin, milnacipran, venlafaxine and fluoxetine 7 days after sciatic nerve injury (CCI) in rats and its influence on microglia/macrophages (IBA-1) and astroglia (GFAP) activation in the spinal cord and dorsal root ganglia (DRG) using Western blot. All tested antidepressants significantly reduced CCI-induced allodynia but hyperalgesia was only antagonised by fluoxetine, doxepine and venlafaxine. The strongest analgesia was observed after fluoxetine administration. Western blot analysis showed the upregulation of the IBA-1 in the lumbar spinal cord and DRG after amitriptyline or milnacipran administration in CCI-exposed rats, whereas after fluoxetine the downregulation was observed. The administration of doxepin did not change the IBA-1 protein level in both studied structures; however venlafaxine decreased the IBA-1 only in the DRG. No changes in the GFAP level in both structures were observed after any of listed above antidepressants administration. Chronic minocycline treatment enhanced amitriptyline and milnacipran, but did not fluoxetine analgesia under neuropathic pain in rats. Our results suggest that nerve injury-induced pain is related with the activation of microglia, which is diminished by fluoxetine treatment in the neuropathic pain model.

1. Introduction

Neuropathic pain is a chronic condition that affects 6–8% of the general population and reduces quality of life (Sindrup et al., 2005). The current therapies for neuropathic pain involve the use of analgesics, such as anticonvulsants, antidepressants and opioids. Attention has focused on a better understanding of the mechanisms of action of antidepressant drugs that relieve neuropathy (Benbouzid et al., 2008; Coluzzi and Mattia, 2005; Mika et al., 2013b; Zychowska et al., 2013b) and the correlation between depression and pain development (Bravo et al., 2012; Fava, 2003; Suzuki et al., 2004). Various animal models have shown that chronic pain is associated with the serotonergic, dopaminergic and noradrenergic systems (Fishbain, 2000). The neurotransmitters of these systems enhance endogenous analgesic mechanisms through participation in inhibitory pain pathways in the central nervous system (Millan, 2002). The most effective analgesics belong to the tricyclic antidepressants (TCA), which block the non-selective re-uptake of serotonin and/or noradrenaline. Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs) increase the extracellular concentration of 5-HT and NA in the synaptic gap, and Selective Serotonin Reuptake Inhibitors (SSRIs) increase the extracellular level of 5-HT by inhibiting its reuptake into the presynaptic cell (Baldessarini, 2001). Recent studies strongly suggest that glia are active partners of neurons in the orchestration of the molecular signals that regulate the arrangement of neuronal circuits in the nervous system.

Some evidence suggests that glia are strongly activated in injury-induced neuropathic pain (Levin et al., 2008; Rodriguez Parkitna et al.,

Abbreviations: CCI, chronic constriction injury; DRG, dorsal root ganglia; *i.p.*, intraperitoneal; 5-HT, serotonin; NA, noradrenaline; TCA, tricyclic antidepressants; SNRIs, Serotonin and Norepinephrine Reuptake Inhibitors; SSRIs, Selective Serotonin Reuptake Inhibitors; IBA-1, ionised calcium-binding adapter molecule 1; GFAP, glial fibrillary acidic protein

* Correspondence to: Department of Pain Pharmacology Institute of Pharmacology Polish Academy of Sciences 12 Smetna Str. 31-343 Krakow, Poland.
Tel.: +48 12 6623240; fax: +48 12 6374500.
E-mail address: joamika@if-pan.krakow.pl (J. Mika).

http://dx.doi.org/10.1016/j.ejphar.2014.11.022
0014-2999/

2006) and in consequence may modulate the effects of different drugs used in chronic pain therapy. The role of glia in analgesic response to antidepressants is not known. Activated glia release a variety of neurotransmitters and neuromodulators (McMahon et al., 2005), cytokines (DeLeo et al., 2006; Mika et al., 2008) and complement components (Levin et al., 2008). An imbalanced proportion of neurons and glia activity may be an important factor in the pathology of diseases and, therefore, their co-occurrence and possibilities for therapy (Mika et al., 2013a; Watkins and Maier, 2003). Our studies have shown that minocycline, an inhibitor of microglial activation, attenuates the symptoms of neuropathic pain (Makuch et al., 2013; Mika et al., 2009; Zychowska et al., 2013a); however, the role of glia cell in antidepressant efficacy and their mechanisms of action in neuropathic pain require further evaluation.

The present study determined the effect of intraperitoneal injection of antidepressants of different classes, including amitriptyline and doxepin (TCA), fluoxetine (SSRIs) and milnacipran and venlafaxine (SNRIs), on the hyperalgesia and allodynia induced by chronic constriction injury (CCI) in rats. We also investigated with Western blot analysis the influence of these antidepressants on microglia/macrophages and astroglia/satellite cells activation in the spinal cord and/or DRG using IBA-1 (Ionized Calcium Binding Adaptor Molecule-1) and GFAP (glial fibrillary acidic protein) markers, respectively. We verified also the influence of minocycline on allodynia and hyperalgesia after amitriptyline, milnacipran and fluoxetine administration. The results of this study may improve current treatment regimens via the use of drugs that have a better profile of action on glia.

2. Materials and methods

2.1. Animals

Male Wistar rats (300–350 g) from Charles River (Sulzfeld, Germany) were housed in cages lined with sawdust under a standard 12/12 h light/dark cycle (lights on at 08.00 h) with food and water available ad libitum. All experiments were performed according to the recommendations of IASP (Zimmermann, 1983) and the NIH Guide for the Care and Use of Laboratory Animals, and the II Local Bioethics Committee branch of the National Ethics Committee for Experiments on Animals based at the Institute of Pharmacology, Polish Academy of Sciences (Cracow, Poland).

2.2. Surgical preparations

Chronic constriction injury (CCI) was produced according to Bennett and Xie (1988). The right sciatic nerve was exposed under sodium pentobarbital anaesthesia (60 mg/kg; *i.p.*). Four ligatures (4/0 silk) were made around the nerve distal to the sciatic notch with 1-mm spacing until a brief twitch in the respective hind limb was observed. The rats developed long-lasting allodynia and hyperalgesia after CCI.

2.3. Drug administration

The following chemicals and their sources were used: amitriptyline hydrochloride (AMT; Sigma Aldrich), doxepin hydrochloride (DOX; Sigma Aldrich), fluoxetine hydrochloride (FLU; Sigma Aldrich), milnacipran hydrochloride (MIL; Sigma Aldrich), minocycline hydrochloride (MC; Sigma Aldrich) and venlafaxine hydrochloride (VEN; Sigma Aldrich). All drugs were dissolved in water for injection.

Antidepressants were administered in a single *i.p.* injection on day 7 after CCI at the following doses: AMT 10 mg/kg, DOX 10 mg/kg, FLU 20 mg/kg, MIL 20 mg/kg and VEN 10 mg/kg. The control groups received vehicle injections (water for injection) according to the same schedule. The behavioural tests were conducted on day 7 after CCI first 30 min before drug administration (pretest) and then 30 and 60 (von Frey) and 35 and 65 (cold plate) min after antidepressant injections (Fig. 1A).

In experiment with minocycline this drug (30 mg/kg; *i.p.*) or vehicle (water for injection) was preemptively administered 16 h and 1 h prior to CCI surgery, then twice daily for 7 days. Amitriptyline, fluoxetine and milnacipran were injected 60 min after last minocycline injections and behavioural tests were conducted 30 and 60 (von Frey) and 35 and 65 (cold plate) min (Fig. 3A).

2.4. Behavioural tests

2.4.1. Nociceptive threshold (tail-flick test)

The pain threshold to a thermal stimulus was assessed using tail-flick latency evoked by noxious thermal stimulation as determined with a tail-flick analgesic meter (Analgesia Meter; Ugo Basile, Comerio, Italy) as described previously (Makuch et al., 2013). The tail-flick test consisted of focusing a beam of light on the dorsal tail surface approximately 2 cm from the tip of the tail. The intensity of the light was adjusted so that the baseline tail-flick latencies were 1.4 s. The cut-off time for the tail-flick reaction was set to 9 s.

2.4.2. Tactile allodynia (von Frey test)

Allodynia was measured after application of a non-noxious touch stimulus using an automatic von Frey apparatus (Dynamic Plantar Aesthesiometer Cat. no. 37400, Ugo Basile, Italy). Animals were placed in plastic cages with wire mesh floors 5 min before the experiment. The von Frey filament was applied to the mid-plantar surface of the hind paw, and measurements were taken automatically, as described previously (Mika et al., 2010). We tested the ipsilateral paw twice in 3-min intervals, and the mean value was calculated. The strengths of the von Frey stimuli used in our experiments ranged from 0.5 to 26 g. There was almost no response in naïve animals to the highest strengths (26 g). Therefore, a cut-off was drawn at this value.

2.4.3. Thermal hyperalgesia (cold plate test)

Hyperalgesia was measured after the application of a noxious (low temperature) stimulus using the cold plate test (Cold/Hot Plate Analgesia Meter no. 05044, Columbus Instruments, USA) as described previously (Mika et al., 2010). The temperature of the cold plate was maintained at 5 °C, and the cut-off latency was 30 s. The animals were placed on the cold plate, and the time until a lifting of the hind paw occurred was recorded. The reaction of the first hind paw to be lifted was measured in the naïve rat group. The ipsilateral paw reacted the first in rats subjected to nerve injury.

2.5. Biochemical tests

2.5.1. Western blot

The influence of single antidepressant administration on protein level was studied using Western blot in the ipsi- and contralateral dorsal part of the lumbar spinal cord (L4–L6) and DRG (L4–L6) collected after 4 h of each antidepressant injection on day 7 after CCI.

Tissue samples were homogenised in RIPA buffer and cleared using centrifugation (10,000*g* for 10 min). Protein concentration in the supernatant was determined using the BCA Protein Assay Kit (Sigma). Samples extracted from DRG (L5–L6) or dorsal spinal cord containing 15 μg and 20 μg of protein, respectively, were hea;ted for 8 min at 99 °C in loading buffer (50 mM Tris–HCl, 2% SDS, 2%