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Research report

Non-pharmacological treatment affects neuropeptide expression in neuropathic pain model

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ABSTRACT

Chronic constriction injury (CCI) of the sciatic nerve elicits changes in neuropeptide expression on the dorsal root ganglia (DRG). The neural mobilization (NM) technique is a noninvasive method that has been proven clinically effective in reducing pain. The aim of this study was to analyze the expression of substance P, transient receptor potential vanilloid 1 (TRPV1) and opioid receptors in the DRG of rats with chronic constriction injury and to compare it to animals that received NM treatment. CCI was performed on adult male rats. Each animal was submitted to 10 sessions of neural mobilization every other day, starting 14 days after the CCI injury. At the end of the sessions, the DRG (L4-L6) were analyzed using Western blot assays for substance P, TRPV1 and opioid receptors (μ-opioid receptor, δ-opioid receptor and κ-opioid receptor). We observed a decreased substance P and TRPV1 expression (48% and 35%, respectively) and an important increase of μ-opioid receptor expression (200%) in the DRG after NM treatment compared to control animals. The data provide evidence that NM promotes substantial changes in neuropeptide expression in the DRG; these results may provide new options for treating neuropathic pain.

1. Introduction

Many studies have shown that changes in the molecules or receptors expressed in the DRG are responsible for pain-related behaviors, and that the DRG spinal neurons are responsible for transmission of nociceptive information (Kuo et al., 2011; LaCroix-Fralish et al., 2011; Sapunar et al., 2012). Nerve injury results in molecular changes, which are involved in the mechanism of neuropathic pain, and DRG neurons may become an important source of increased nociceptive signaling through increased neuronal excitability and generation of ectopic discharges (Sapunar et al., 2005; Xie et al., 2006). Many investigations have focused on the functional changes of receptors, proteins, peptides in both the spinal cord and DRG neurons following nerve injury (Narita et al., 2004; Obara et al., 2009; Svensson et al., 2006). It has been shown that inflammation and tissue injury increase expression of the TRPV1 in myelinated neurons, contributing to hyperalgesia (Ueda, 2006). TRPV1 upregulation contributes to mechanical allodynia and thermal hyperalgesia, while the administration of its antagonists can reverse the allodynia and hyperalgesia in a spinal nerve ligation model (Vilceanu et al., 2010). The activation of TRPV1 triggers the propagation of pain sensation and affects the release of some neurotransmitters, including substance P, that are released from activated nerve endings, resulting in a neurogenic inflammation (Pailleux et al., 2012).

Substance P is an 11-amino-acid peptide of the tachykinin family which is produced in the central and peripheral terminals of

Abbreviations: CCI, chronic constriction injury; DOR, δ-opioid receptor; DRG, dorsal root ganglion; KOR, κ-opioid receptor; MOR, μ-opioid receptor; NM, neural mobilization; TRPV1, transient receptor potential vanilloid 1.

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primary sensory neurons and released following noxious stimuli in the periphery (Chen et al., 2006). Substance P also plays an important role in the development of chronic pain (Gao et al., 2003; Steinhoff et al., 2003), acting on a G protein-coupled receptor known as a tachykinin receptor (NK1). Studies have demonstrated the involvement of the NK1 receptor in neuropathic pain induced by sciatic nerve constriction, showing that substance P is responsible for the development of hyperalgesia in rats (Hoot et al., 2011; Jang et al., 2004). In clinical practice, it has been extensively reported that neuropathic pain is difficult to treat, due to inadequate understanding of the cellular and molecular mechanisms involved in the development and maintenance of this type of pain (Sapunar et al., 2005; Xie et al., 2006). Opioid drugs are the most widely used to treat pain ranging from moderate to severe. More recently, studies showed that endomorphin-2, one of the endogenous ligands for the μ-opioid receptor (MOR) is co-localized with substance P in DRG neurons and in the spinal cord (Luo et al., 2014; Sanderson Nydahl et al., 2004; Wu et al., 2015). Opioid receptors are heterogeneously distributed in the neuronal nociceptive system, and all three types of opioid receptors are synthesized and expressed in the cell bodies of DRG neurons. Studies that evaluate the effects of opioids in different models of neuropathic and inflammatory pain have obtained different results in relation to different types of opioid receptors. There is no pattern of opioid receptor expression for inflammatory pain models or for neuropathic pain models (Porreca et al., 1998; Truong et al., 2003; Zhang et al., 2016).

The NM technique is a manual therapy method used by physiotherapists to treat patients with neural origin pain, such as the compression of the sciatic nerve. The technique aims to restore mobility and elasticity of the peripheral nervous system by strains that are imposed on the nerve trunks, roots, nerves, spinal cord and their epineurium and to decrease sensitivity (Santos et al., 2012). We have shown that NM treatment reverses pain symptoms in rats submitted to CCI and induces changes in glial cells and neurotrophins, besides improving the nerve regeneration after treatment (da Silva et al., 2015; Santos et al., 2012; Santos et al., 2014). Additionally, in this work we focused on evaluating whether the NM can influence TRPV1, substance P and opioid receptor expression on DRG neurons in animals under a neuropathic pain condition. This issue was evaluated by Western blotting assays in the DRG of adult neuropathic rats after treatment with NM.

2. Results

2.1. Effects of NM on substance P expression

Fig. 1 shows an increase of substance P protein levels (48%) after CCI in comparison to naive rats ($p \leq 0.05$), taken as a control. After NM treatment (CCI-NM), we observed a decrease of substance P expression of approximately 65% when compared to CCI animals ($p \leq 0.001$) (Fig. 1). No significant differences in substance P expression were observed between sham and naive rats or between sham and sham-NM animals (data not shown).

2.2. Effects of NM on TRPV1 expression

We have evaluated the protein expression of TRPV1 in the DRG to assess the possible effects of CCI and NM treatment. Our results showed an increase of 35% in TRPV1 levels after CCI injury when compared to naive animals ($p \leq 0.05$), (Fig. 2). After NM treatment, we observed a decrease of 80% above the control in TRPV1 expression and a decrease of 110% when comparing the CCI-NM group to the CCI group ($p \leq 0.001$). No differences were observed between naive and sham rats or between sham and sham-NM rats (data not shown).

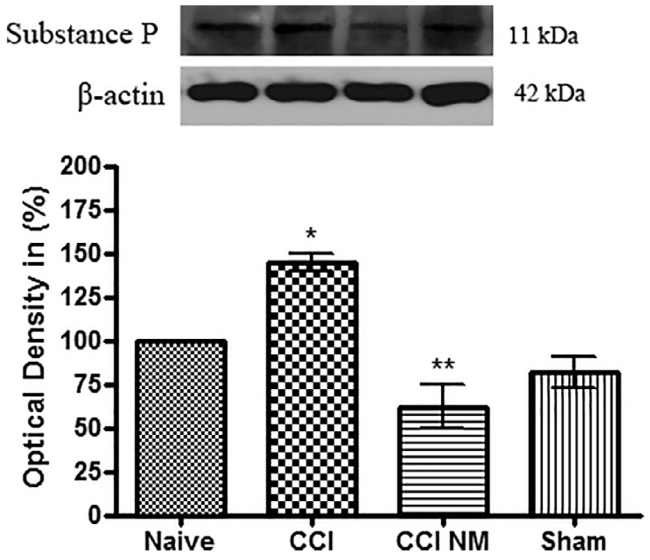

Fig. 1. Densitometric analysis of Substance P levels in the DRG. The normalized average between sham and experimental groups (CCI and CCI-NM) is reported. Data for naive animals were taken as 100% and mean ± SEM of 6 animals per group. $^{*}p \leq 0.05$ compared CCI and naive groups.

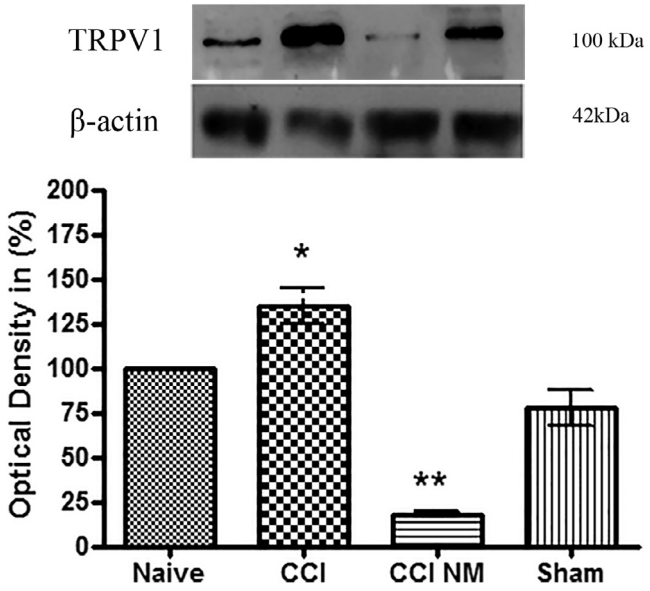

Fig. 2. Densitometric analysis of TRPV1 levels in the DRG. The normalized average between sham and experimental groups (CCI and CCI-NM) is reported. Data for naive animals were taken as 100% and mean ± SEM of 6 animals per group. $^{*}p \leq 0.05$ compared CCI and naive groups.

2.3. Effects of NM on opioid receptor expression

To evaluate how NM treatment can interfere with opioid receptors, we also analyzed MOR, DOR and KOR protein expression. Our results showed an increase of 110% ($p \leq 0.001$) in MOR levels after CCI injury when compared to naive animals (Fig. 3). After NM treatment, we observed an enhancement of MOR expression when compared to the control group (200% naive $p \leq 0.001$) and when compared with CCI injury animals (43% CCI $p \leq 0.01$). No difference was observed between naive and sham rats or between sham and sham-NM rats (data not show). Regarding the evaluation of DOR and KOR opioid receptors, it was not possible to observe any immunoreactivity of these receptors in our model (data not show).