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Research report

The lesion of dorsolateral funiculus changes the antiallodynic effect of the intrathecal muscimol and baclofen in distinct phases of neuropathic pain induced by spinal nerve ligation in rats

Quintino Moura Dias[a], Wiliam A. Prado[b,*]

[a] Oswaldo Cruz Foundation (FIOCRUZ), Porto Velho, Rondônia, Brazil
[b] Department of Pharmacology, Ribeirao Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

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ABSTRACT

The abnormal firing of damaged primary afferents and the changes in the central nervous system (CNS) play important role in the initiation and maintenance phases of neuropathic pain. These phases of neuropathic pain involve changes in the GABAergic control of descending pathways that travel through the dorsolateral funiculus (DLF). The present study shows that unilateral DLF lesion increased the antiallodynic effect of muscimol (0.2 μg/5 μL) (a $GABA_A$ receptor agonist) in the initiation, but not maintenance phase of the mechanical allodynia induced by a spinal nerve ligation (SNL) of the ipsilateral hindpaw of rats. The unilateral DLF lesion increased the antiallodynic effect of baclofen (0.8 μg/5 μL) (a $GABA_B$ receptor agonist) in the initiation phase and reduced your effect in the maintenance phase of the mechanical allodynia induced by a spinal nerve ligation (SNL) of the ipsilateral paw of rats. The unilateral DLF lesion significantly reduced the proallodynic effect of an intrathecal injection of phaclofen (30 μg/5 μL) (a $GABA_B$ receptor antagonist), but not bicuculline (0.3 μg/5 μL) (a $GABA_A$ receptor antagonist). The effect of DLF lesion on the proallodynic effect of phaclofen was observed in the maintenance, but not in the initiation phase of the mechanical allodynia induced by SNL. We than conclude that the spinal GABAergic neurotransmission is negatively modulated by DLF using $GABA_A$ and $GABA_B$ receptors, in the initiation phase of mechanical allodynia induced by SNL. In addition, the integrity of DLF is necessary for the effectiveness of GABAergic transmission that occurs via spinal $GABA_B$, but not $GABA_A$ receptors, in the maintenance phase of mechanical allodynia induced by SNL.

1. Introduction

Both descending pathways and intrinsic spinal inhibitory GABAergic neurons critically contribute to the modulation of spinal nociceptive inputs (Sivilotti and Nistri, 1991; Takehana et al., 2016). The antinociception produced by μ-opioid activation In the central nervous system is at least in part due to the inhibition of pre- and post-synaptic potentials in the periaqueductal gray (PAG; Chieng and Christie, 1994) or spinal trigeminal nucleus caudalis (Takehana et al., 2016). Blockade of μ-opioid receptors increases the activity in the PAG via a GABAergic disinhibition, which can activate serotoninergic (5-HT) neurons in nucleus raphe magnus via a PAG-nucleus raphe magnus-trigeminal pathway (Gebhart 2004; Takeda et al., 2002). Some substances, such as polyphenol reveserol, produce antinociceptive effect probably by suppression of the excitatory synaptic transmission of spinal trigeminal nucleus caudalis via activation of $5\text{-}HT_3$ receptor-mediated GABAergic inhibition and/or via activation of endogenous opioidergic mechanisms (Takehana et al., 2016). The intrathecal injection of GABAergic agonists in rats reduces pain behaviors produced by intraplantar formalin (Kaneko and Hammond, 1997), intraarticular Complete Freund's Adjuvant (Castro et al., 2006), surgical incision (Reichl et al., 2012), or spinal nerve injury (Eaton et al., 1999; Hwang and Yaksh, 1997; Malan et al., 2002). Accordingly, pharmacological blockade of spinal $GABA_A$ or $GABA_B$ receptors in rats increases the animals' sensitivity to external stimuli that results in various pain conditions (Gwak et al., 2006; Malan et al., 2002; Reichl et al., 2012; Yaksh, 1989).

GABA is an inhibitory neurotransmitter widely distributed in interneurons of substantia gelatinosa in the spinal dorsal horn (Carlton et al., 1992; Polgár et al., 2003; Todd and Sullivan, 1990). The spinal GABAergic inhibition may occur via control of

* Corresponding author at: Department of Pharmacology, Ribeirao Preto Medical School, Av. Bandeirantes 3900, CEP 14049-900, Ribeirão Preto, SP, Brazil.
E-mail address: wadprado@fmrp.usp.br (W.A. Prado).

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central nerve terminals of sensory fibers involving depolarizing mechanism (Rudomin and Schmidt, 1999) or post-synaptic hyperpolarization of dorsal horn cells (Coull et al., 2003; Hwang and Yaksh, 1997; Janssen et al., 2011). GABA released in the spinal cord may interact with $GABA_A$ and $GABA_B$ receptors. The $GABA_A$ receptor is a ligand-gated chloride channel receptor that mediates fast synaptic inhibition and is present on both neurons and glial cells throughout the spinal cord gray matter. Activation of $GABA_A$ receptor increases the permeability of chloride ions and hyperpolarizes postsynaptic neurons, which increases the cell resting membrane conductance (Gwak and Hulsebosch, 2011; Jensen et al., 2002; Sieghart and Sperk, 2002). The $GABA_B$ receptor is a metabotropic receptor coupled to G-protein. The activation of $GABA_B$ receptor inhibits the synaptic transmission at primary afferent terminals in the spinal cord via reduction of calcium entry at the presynatic terminal and hyperpolarization at the postsynaptic terminal via increased conductance of potassium ions (Bowery et al., 1980; Gwak and Hulsebosch, 2011). This receptor is concentrated in neurons and glial cell in the superficial layers of the spinal cord horn (Albrecht et al., 1986; Bowery et al., 1980; Charles et al., 2003a,b).

The importance of spinal GABAergic transmission is confirmed by the behavioral signs of tactile allodynia induced by the administration of the $GABA_A$ receptor antagonist bicuculline into the rat spinal cord (Yaksh, 1989).

Reduction of the inhibitory control at the spinal cord has been proposed as a key factor in chronic pain syndromes (Zeilhofer et al., 2009). Despite this notion, the importance of the integrity of GABAergic transmission in the induction and maintenance of painful peripheral neuropathies is still controversial. Loss of spinal inhibitory GABAergic mechanisms accounts for the sustained cell hyperexcitability that is usually found in persistent pain states (Hwang and Yaksh, 1997; Ibuki et al., 1997; Malan et al., 2002; Patel et al., 2001). In support for this idea, a significant reduction in GABA-immunoreactivity of spinal neurons was found after peripheral nerve lesion, and the peak severity of mechanical allodynia occurs when the loss of GABA-immunoreactivity is maximal (Ibuki et al., 1997). In contrast, some studies reported no significant change in the GABA content of synaptosome preparations (Somers and Clemente, 2002) or found reduction of the number of GABAergic neurons in the superficial laminae of the ipsilateral dorsal horn in a rat model of neuropathic pain (Polgár et al., 2003; Polgár et al., 2005; Polgár and Todd, 2008). Satoh and Omote (1996) reported significant increase of the GABA concentrations in the ipsilateral spinal cord in a rat model of neuropathic pain, while Kontinen et al. (2001) provided evidences for an increase in GABAergic inhibition of dorsal horn neurons in the same model. Evidences also indicate that the inhibitory influences of GABAergic neurotransmission over central terminals of sensory fibers is shifted to disinhibition following peripheral nerve injury (Coull et al., 2003; Janssen et al., 2011). Nevertheless, it is evident the anti-allodynic effect of intrathecal injection of $GABA_A$- and $GABA_B$-receptor agonists (Hwang and Yaksh, 1997; Malan et al., 2002).

The injury of the sciatic nerve in rats produces tactile hypersensitivity to mechanical stimulation that begins two days after injury (characterized as initial phase), and becomes progressively more intense 5 days later and then reaches a plateau level (characterized as maintenance phase) that is maintained until at least the post injury day 14 (Burgess et al., 2002). The neuropathic pain is associated with several neuroanatomical and neurochemical changes including activation of an excitatory system that descends from supraspinal structures to the spinal cord via the dorsolateral funiculus (DLF) (Bian et al., 1998; Burgess et al., 2002; Dias et al., 2012; Millan, 2002) and mood change, such as anxiety- and depression-like behaviors (Rácz et al., 2015). A progressive increase of excitatory mechanisms descending through the DLF that can be blocked by DLF lesion is evidenced during the maintenance phase of neuropathies (Burgess et al., 2002; Ossipov et al., 2000). The affective changes are observed 4–8 weeks, but not 2–4 weeks, after peripheral nerve injury, demonstrating that affective changes induced by neuropathic pain appear much later then the sensory hypersensibility (Rácz et al., 2015). Moreover, the systems involved in the affective aspects, such as endocannabinoid system, appear to be independents of the sensorial aspects of neuropathic pain.

The present study therefore examines whether the effects of intrathecal agonist (muscimol) or antagonist (bicuculline) of $GABA_A$ receptors and agonist (baclofen) or antagonist (phaclofen) of $GABA_B$ receptors depend on the integrity of pain inhibitory pathways that descend to the spinal cord via the DLF in a spinal nerve ligation (SNL) model of neuropathic pain in rats.

2. Material and methods

2.1. Subjects

The experiments were conducted on male Wistar rats (140–160 g) from the main animal house of the University of São Paulo (USP; Campus of Ribeirão Preto). Before surgical procedures, each animal was randomly assigned to a particular experimental group. The study was conducted using five rats in each group. The animals were housed two to a cage with free access to food and water and maintained at a controlled temperature ($23 \pm 1\,^{\circ}C$) with a 12-h light-dark cycle before and after surgery. For itch The experiments were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Protocol number 211/2005). The proposals of the Committee for Research and Ethical Issues of IASP were followed throughout the experiments (Zimmermann, 1983).

2.2. Surgical procedures

SNL and DLF lesioning were sequentially conducted in rats anesthetized with tribromoethanol (250 mg/kg, i.p.), as described elsewhere (Dias et al., 2012). First, the right L5 and L6 spinal nerves were isolated and tightly ligated with chromic catgut 5-0 suture as described elsewhere (Kim and Chung, 1992). The incision was then closed by planes with silk sutures. Next, the spinal cord was subsequently exposed by laminectomy at the T8 level, the dura mater was slit and a lesion was unilaterally made by cutting a portion of the right dorsolateral quadrant of the spinal cord with a sharp knife, avoiding damage to the major blood vessels supplying the cord. Finally, hemostasis was confirmed, the wound was packed with gelfoam and closed, and the animal was allowed to recover for 2 or 7 days before the experiment. Sham-lesion rats were submitted to similar procedures except that there was no nerve ligation and/or DLF lesion. Rats that exhibited motor deficiency or a lack of increased sensitivity to innocuous mechanical stimulation were excluded from additional testing.

2.3. Intrathecal injection

Two or 7 days after surgery, each rat was anesthetized with isoflurane via a loose-fitting, cone-shaped mask, and catheterization of the spinal subarachnoid space was performed, as described elsewhere (Prado, 2003). Briefly, a 20-gauge Weiss needle was introduced through the skin into the L5-L6 intervertebral space. The correct positioning of the needle was assured by a typical flick of the tail or hind paws. A 12-mm length of polyethylene tubing (PE tubing, o.d. = 0.4 mm, dead space = 10 μL) was then introduced through the needle to protrude 2.0 cm into the subarachnoid space in a cranial direction. The needle was then carefully removed, the tubing was anchored to the back skin with a cotton thread suture, and anesthesia was discontinued.