Journal of Neuroimmunology 292 (2016) 85–92

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

# Progesterone modulates pro-inflammatory cytokine expression profile after spinal cord injury: Implications for neuropathic pain

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ARTICLE INFO

*Article history:*
Received 16 November 2015
Received in revised form 11 January 2016
Accepted 14 January 2016

*Keywords:*
Chronic pain
Neuroactive steroids
Spinal cord
Glial activation
NMDA receptor
Pro-inflammatory cytokines

ABSTRACT

Neuropathic pain is a frequent complication of spinal cord injury (SCI), still refractory to conventional treatment. Glial cell activation and cytokine production contribute to the pathology of central neuropathic syndromes. In this study we evaluated the effects of progesterone, a neuroactive steroid, on pain development and the spinal expression of IL-1β, its receptors (IL-1RI and IL-1RII) and antagonist (IL-1ra), IL-6 and TNFα, and NR1 subunit of NMDAR. Our results show that progesterone, by modulating the expression of pro-inflammatory cytokines and neuronal IL-1RI/NR1 colocalization, emerges as a promising agent to prevent chronic pain after SCI.



## 1. Introduction

Neuropathic pain, a frequent complication of spinal cord injury (SCI), is an important contributor to decreased quality of life (Finnerup, 2013). This chronic pain is characterized by the presence of both spontaneous and induced pain. Unfortunately, the incomplete understanding of the mechanisms involved in pain arising after SCI threatens the search of effective medical treatment.

While multiple mechanisms may contribute to neuropathic pain after central nervous system (CNS) lesions (Hulsebosch et al., 2009; Yezierski, 2009), central neuroinflammation is a critical driving force for the development and maintenance of chronic pain (Ji et al., 2013; Walters, 2014).

Spinal glial cells play a central role in the onset of the neuroinflammation. Among the glial mediators released within the CNS, special emphasis has been placed on pro-inflammatory cytokines like interleukin 1β (IL-1β) and tumor necrosis factor α (TNFα), possibly for their involvement in a plethora of CNS diseases and neurotoxic conditions (Burda and Sofroniew, 2014; Vezzani and Viviani, 2015). It has been demonstrated that these cytokines facilitate pain via neural–glial interactions (Kawasaki et al., 2008; Ji et al., 2013). In particular, IL-1β plays a pivotal role in pain mechanisms (Taves et al., 2013; Ren and Dubner, 2015). The actions of IL-1β are regulated by different mechanisms involving a functional receptor (IL-1RI), a decoy receptor (IL-1RII), and a specific endogenous antagonist (IL-1ra) (Dinarello, 1998).

Since IL-1RI is not only found in glial cells but also allocated in neurons (Ravizza and Vezzani, 2006; Gardoni et al., 2011), IL-1β may act directly on neurons to modulate their activity. In fact, IL-1β has been shown to enhance synaptic transmission and neuronal activity in the superficial dorsal horn (Kawasaki et al., 2008; Pedersen et al., 2010). Furthermore, IL-1RI acts as a coordinating factor for the functional interaction between IL-1β and N-methyl-D-aspartate receptor (NMDAR) (Fogal and Hewett, 2008; Zhang et al., 2008), a key player in pain transmission. Therefore, targeting these processes could reduce the excitability of dorsal horn neurons and prevent the development of chronic pain.

Progesterone, a neuroactive steroid, has multiple non-reproductive functions in the CNS, exerting neuroprotective and remyelinating

*Abbreviations:* (SCI), spinal cord injury; (CNS), central nervous system; (IL-1β), interleukin 1β; (TNFα), tumor necrosis factor α; (NMDAR), N-methyl-D-aspartate receptor; (IL-1RI), IL-1β functional receptor; (IL-1RII), IL-1β decoy receptor; (IL-1ra), IL-1β receptor antagonist; (GFAP), glial fibrillary acidic protein; (iNOS), inducible isoform of the nitric oxide synthase; (COX-2), cyclooxigenase 2; (PG), progesterone; (CTL), control animals; (PCR), polymerase chain reaction; (PBS), phosphate-buffered saline; (PGRMC1), progesterone receptor membrane component 1.

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http://dx.doi.org/10.1016/j.jneuroim.2016.01.011
0165-5728/

actions after experimental traumatic brain and spinal cord injuries (De Nicola et al., 2013; Garcia-Ovejero et al., 2014; Geddes et al., 2014; Schumacher et al., 2014). Furthermore, progesterone is emerging as an attractive potential drug for preventing persistent pain conditions (Milani et al., 2010; Coronel et al., 2011a, b, 2014; Dableh and Henry, 2011; Melcangi et al., 2014). In particular, we have recently shown that progesterone reduces the number of GFAP and OX-42 positive glial cells, regulates iNOS and COX-2 expression and modulates the expression and phosphorylation of NMDAR subunits at the dorsal horn level after SCI (Coronel et al., 2011b, 2014).

In the present study, we used molecular, immunohistochemical and behavioral studies to evaluate the impact of progesterone administration on the temporal expression of IL-1β, its receptors IL-1RI and IL-1RII and antagonist IL-1ra, IL-6, and TNFα in the injured spinal cord. These parameters were assessed in injured male rats treated with daily injections of progesterone or vehicle, as well as control animals. Since neuronal activity and pain sensitivity are controlled by these pro-inflammatory mediators, their modulation by progesterone could represent a crucial factor to regulate neuroinflammatory dynamics and prevent pain after SCI.

## 2. Methods

### 2.1. Spinal cord injury

All experimental procedures were reviewed and approved by the local Animal Care and Use Committee (Assurance Certificate No. A5072-01) and the Ethical Committee from Instituto de Biología y Medicina Experimental (Buenos Aires, Argentina), and followed the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). Care was taken to minimize animal discomfort and to limit the number of animals used. Male Sprague–Dawley rats (200–220 g), bred at the colony of the Instituto de Biología y Medicina Experimental, were deeply anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). In a group of rats, the spinal cord was exposed and unilaterally hemisected at the thoracic T13 level (Labombarda et al., 2008; Coronel et al., 2011b, 2014), as originally described by Christensen et al. (Christensen et al., 1996). In sham-operated animals the spinal cord was exposed but not lesioned. Post-operative care included control of body temperature using an electric heating pad, and antibiotic administration (cephalexin 20 mg/kg/day) for 5 days, starting immediately after surgery. Animals were monitored for eventual infections until the end of the experiment.

### 2.2. Progesterone administration

Injured animals received daily subcutaneous injections of natural progesterone (Sigma, Saint Louis, MO, USA; P8783, 16 mg/kg/day; HX+PG) or vehicle (Ricine oil, Ewe, Sanitas Argentina, Buenos Aires, Argentina; HX) (Coronel et al., 2011b, 2014). Progesterone was administered immediately after performing the lesion and once a day thereafter until the animals were euthanized (either 1, 14 or 28 days after injury). We have previously tested this dose of progesterone in several animal models of nervous system injury (Labombarda et al., 2009; Coronel et al., 2011a, b, 2014; Garcia-Ovejero et al., 2014). Sham-operated animals receiving oil were used as control animals (CTL). Therefore, the study included three experimental groups: animals subjected to the spinal cord injury receiving oil (HX group), injured animals treated with progesterone (HX+PG group) and sham-operated animals receiving oil (CTL group).

### 2.3. Assessment of pain behaviors

Behavioral testing was performed by a blinded observer. The animals were tested 1 day before surgery, in order to obtain normal baseline values, and at different time points (days 1, 7, 14, 21 and 28) after SCI or sham-operation, as previously described (Coronel et al., 2011b, 2014). Only rats showing normal responses to mechanical and thermal stimulation before surgery were included in the experiments. At least 13 animals were included in each experimental group. They were placed in transparent testing chambers and allowed to acclimate for 15 min before testing.

#### 2.3.1. Mechanical allodynia

Paw mechanical sensitivity was assessed by evaluating the response to normally innocuous mechanical stimuli using a series of 8 calibrated von Frey filaments (1, 2, 4, 6, 8, 10, 15, 26 g, Stoelting, Wood Dale, IL, USA). Each hair was delivered three times with 5 s intervals. The lowest force at which application elicited a brisk paw withdrawal was taken as the mechanical response threshold. A paw withdrawal reflex obtained with 6 g or less was considered as an allodynic response. Values shown in Fig. 1a correspond to the mean ± SEM. As previously reported, results were analyzed using the Friedman repeated measures of analysis of variance followed by multiple comparison test (Coronel et al., 2011b, 2014).

#### 2.3.2. Cold allodynia

Cold sensitivity of the hind paw to acetone (Choi test) was quantified by paw withdrawal frequency. Thus, 100 μl of acetone was applied to the plantar surface of the paw using a plastic tubule connected to a 1 ml syringe. Acetone was applied five times to each paw at an interval of at least 5 min. The number of brisk foot withdrawals was recorded. Values shown in Fig. 1b correspond to the mean ± SEM. As previously reported, results were analyzed using the Friedman repeated measures of analysis of variance followed by multiple comparison test (Coronel et al., 2011b, 2014).

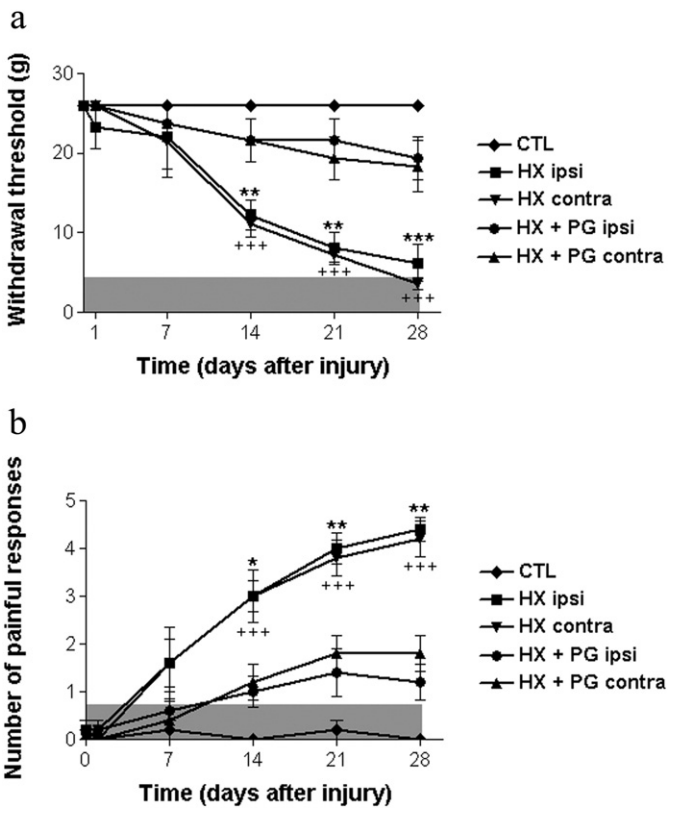


**Fig. 1.** Spinal cord injury induced the development of mechanical (a) and thermal (b) allodynia in both the ipsilateral and contralateral hind paws. Progesterone administration was able to prevent these pain-related behaviors (a, b). The following symbols were used to represent p values: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ when comparing HX vs HX+PG, and $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ when comparing HX vs CTL. Shaded areas in both graphs represent the period of progesterone administration.