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Spinal neuropeptide expression and neuropathic behavior in the acute and chronic phases after spinal cord injury: Effects of progesterone administration

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ABSTRACT

Patients with spinal cord injury (SCI) develop chronic pain that severely compromises their quality of life. We have previously reported that progesterone (PG), a neuroprotective steroid, could offer a promising therapeutic strategy for neuropathic pain. In the present study, we explored temporal changes in the expression of the neuropeptides galanin and tyrosine (NPY) and their receptors (GalR1 and GalR2; Y1R and Y2R, respectively) in the injured spinal cord and evaluated the impact of PG administration on both neuropeptide systems and neuropathic behavior. Male rats were subjected to spinal cord hemisection at T13 level, received daily subcutaneous injections of PG or vehicle, and were evaluated for signs of mechanical and thermal allodynia. Real time PCR was used to determine relative mRNA levels of neuropeptides and receptors, both in the acute (1 day) and chronic (28 days) phases after injury. A significant increase in Y1R and Y2R expression, as well as a significant downregulation in GalR2 mRNA levels, was observed 1 day after SCI. Interestingly, PG early treatment prevented Y1R upregulation and resulted in lower NPY, Y2R and GalR1 mRNA levels. In the chronic phase, injured rats showed well-established mechanical and cold allodynia and significant increases in galanin, NPY, GalR1 and Y1R mRNAs, while maintaining reduced GalR2 expression. Animals receiving PG treatment showed basal expression levels of galanin, NPY, GalR1 and Y1R, and reduced Y2R mRNA levels. Also, and in line with previously published observations, PG-treated animals did not develop mechanical allodynia and showed reduced sensitivity to cold stimulation. Altogether, we show that SCI leads to considerable changes in the spinal expression of galanin, NPY and their associated receptors, and that early and sustained PG administration prevents them. Moreover, our data suggest the participation of galaninergic and NPYergic systems in the plastic changes associated with SCI-induced neuropathic pain, and further supports the therapeutic potential of PG- or neuropeptide-based therapies to prevent and/or treat chronic pain after central injuries.

1. Introduction

Neuropathic pain develops in 40–60% of patients with spinal cord injury (SCI), severely affecting their quality of life [1,2]. Unfortunately, currently available pharmacotherapy has limited efficacy and serious adverse side effects [3]. Recently, progesterone (PG), a neuroprotective steroid, has emerged as an attractive potential drug for preventing persistent pain conditions [for a recent review please see [4]]. In fact, we have recently shown that PG administration prevents the development of both mechanical and thermal allodynia after SCI [5–7].

Previous studies also show that neuropeptides such as galanin [8,9] and tyrosine, also known as neuropeptide Y (NPY) [10,11] are proven modulators of neuropathic pain induced by peripheral nerve injury. Therefore, they are currently addressed as promising candidates in the search of novel analgesic agents [12,13]. These neuropeptides and their receptors are expressed by primary afferent and spinal cord neurons [8,10]. Galanin and NPY have several

Abbreviations: CGRP, Calcitonin gene related peptide; CycB, cyclophilin B; CTL, control animals; DRGs, dorsal root ganglia; HX, hemisected animals; X+P, Ghemisected animals treated with progesterone; NPY, neuropeptide Y; PCR, polymerase chain reaction; PG, progesterone; SCI, spinal cord injury.

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different receptors (GalR1-3, Y1-5R) and, in both cases, type 1 and 2 receptors (GalR1-2, Y1-2R) seem to be the most relevant in pain neurotransmission, with GalR1, Y1R and Y2R mediating galanin and NPY analgesic actions at the spinal cord level [14,8,10,15,11].

At the dorsal horn, profuse galanin [16,17] and NPY [18,19] immunoreactive neuropils can be detected, corresponding to both primary afferent endings and local interneurons, the latter mainly located in laminae I–III. Regarding galanin and NPY receptors, GalR1 is expressed by numerous dorsal horn interneurons [20], while Y1R is found both in local interneurons and projection neurons [21]. In contrast, GalR2 and Y2R show a more restricted distribution, respectively confined to a small subpopulation of dorsal horn neurons [20] and primary afferent endings [22,23].

Research over the last 30 years has resulted in abundant data showing that both neuropeptides and their associated receptors suffer major changes in their expression patterns in dorsal root ganglia (DRGs), and to some extent also in the spinal cord, after various types of peripheral nerve injuries or tissue inflammation [see [8,10,15,9]]. In contrast, such analysis after SCI remains to be explored.

Using an experimental model of SCI, we have recently shown that PG administration prevents neuropathic pain associated behaviors [5–7], modulates the expression of NMDA receptor subunits [5] and attenuates the injury-induced pro-inflammatory cascade involving pro-inflammatory enzymes [6] and cytokines [7], all key players in neuropathic pain generation, probably by reducing NF-κB transactivation potential [6]. Interestingly, it has been shown that NF-κB-dependent pro-inflammatory mediators such as interleukin 6 (IL-6) [24,25] and tumor necrosis factor alpha (TNFα) [26] can modulate the expression of neuropeptides such as galanin and NPY. Furthermore, the levels of expression of galanin, NPY and their receptors have been shown to be influenced by circulating gonadal steroids, both during fluctuations due to the estrous cycle and after hormone administration [27–30]. Therefore, either directly or indirectly, PG could influence peptidergic expression after SCI.

In this study, we focused on the analysis of temporal changes in the spinal expression of the neuropeptides galanin and NPY and their associated receptors after SCI, and evaluated the impact of PG administration on such expression during the development of neuropathic pain-associated behaviors.

2. Materials and methods

2.1. Spinal cord injury

All experimental procedures were reviewed and approved by the local Animal Care and Use Committee (Assurance Certificate N° A5072-01) and the Ethical Committee from Instituto de Biología y Medicina Experimental (CE 004/2015), 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 g) bred at the colony of 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 thoracic T13 level [5–7], as originally described by Christensen et al. [31]. In sham-operated animals the spinal cord was exposed but not lesioned. Post-operative care included control of body temperature and prophylactic antibiotic administration (cephalexine 20 mg/kg/day) during 5 days.

2.2. PG administration

Injured animals received daily subcutaneous injections of bioidentical PG (Sigma, Saint Louis, MO, USA; P8783, 16 mg/kg/day; HX + PG) or vehicle (Vegetable oil, Ewe, Sanitas, Buenos Aires, Argentina; HX) [5–7]. PG was administered immediately after performing the lesion and once a day thereafter until the animals were euthanized (1 or 28 days after injury). We have previously tested this dose of PG in several animal models of nervous system injury [32,6,33]. In particular, this dose of PG has been shown to prevent mechanical and thermal allodynia after spinal cord [5] and sciatic nerve [32] injuries. Sham-operated animals receiving oil were used as control animals (CTL).

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 7, 14, 21 and 28) after SCI or sham-operation, as previously described [5–7]. Only rats showing normal responses to mechanical and thermal stimulation before surgery were included in the experiments. Eight animals were included in each experimental group. They were placed in transparent testing chambers and allowed to acclimate for 15 min.

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 filament was delivered three times with 5 s intervals. The lowest force at which application elicited at least two withdrawal responses (brisk paw withdrawal together with a nocifensive behavior such as attack to the stimulus, escape or vocalization) was taken as the mechanical response threshold. A paw withdrawal reflex obtained with 6 g or less was considered an allodynic response [5–7]. 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 [5–7].

2.3.2. Cold allodynia

Cold sensitivity of the hindpaw to acetone (Choi test) was quantified by paw withdrawal frequency [34]. Thus, a bubble 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 intervals of at least 5 min. The number of brisk foot withdrawals accompanied by nocifensive behaviors (mentioned in the previous section) was recorded. If paw withdrawal was observed at least two times after acetone exposure, it was considered an allodynic response [5–7]. 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 [5,6].

2.4. Tissue preparation for real time polymerase chain reaction (PCR)

Either 1 or 28 days after SCI animals receiving PG or vehicle, as well as CTL animals, were killed by decapitation after being deeply anesthetized with chloral hydrate (800 mg/kg, i.p.). Spinal lumbar segments caudal to the injury site (L4-5) and equivalent regions from CTL animals were immediately removed and the dorsal spinal regions were dissected [5–7]. Tissues were frozen and stored at −70 °C until expression studies were performed. Samples from the different experimental groups were run at the same time.

2.5. Real time PCR

RNA was extracted using Trizol (Invitrogen, USA), as previously described [5–7]. Nucleotide sequences of forward and reverse