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.

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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

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
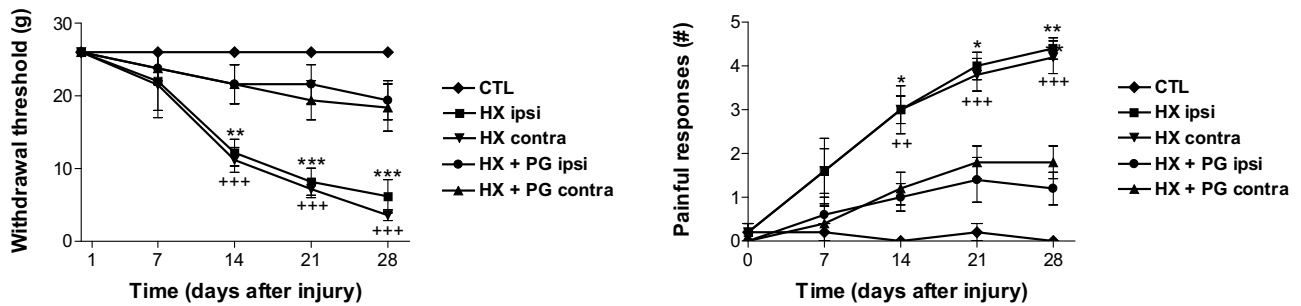
Fig. 1. Animals with spinal cord injury developed mechanical (a) and thermal (b) allodynia in both the ipsilateral and contralateral hindpaws. PG administration was able to prevent these pain-related behaviors (a,b). CTL: control animals, HX: injured animals receiving oil, HX + PG: injured animals treated with PG, ipsi: ipsilateral hindpaw, contra: contralateral hindpaw. 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.
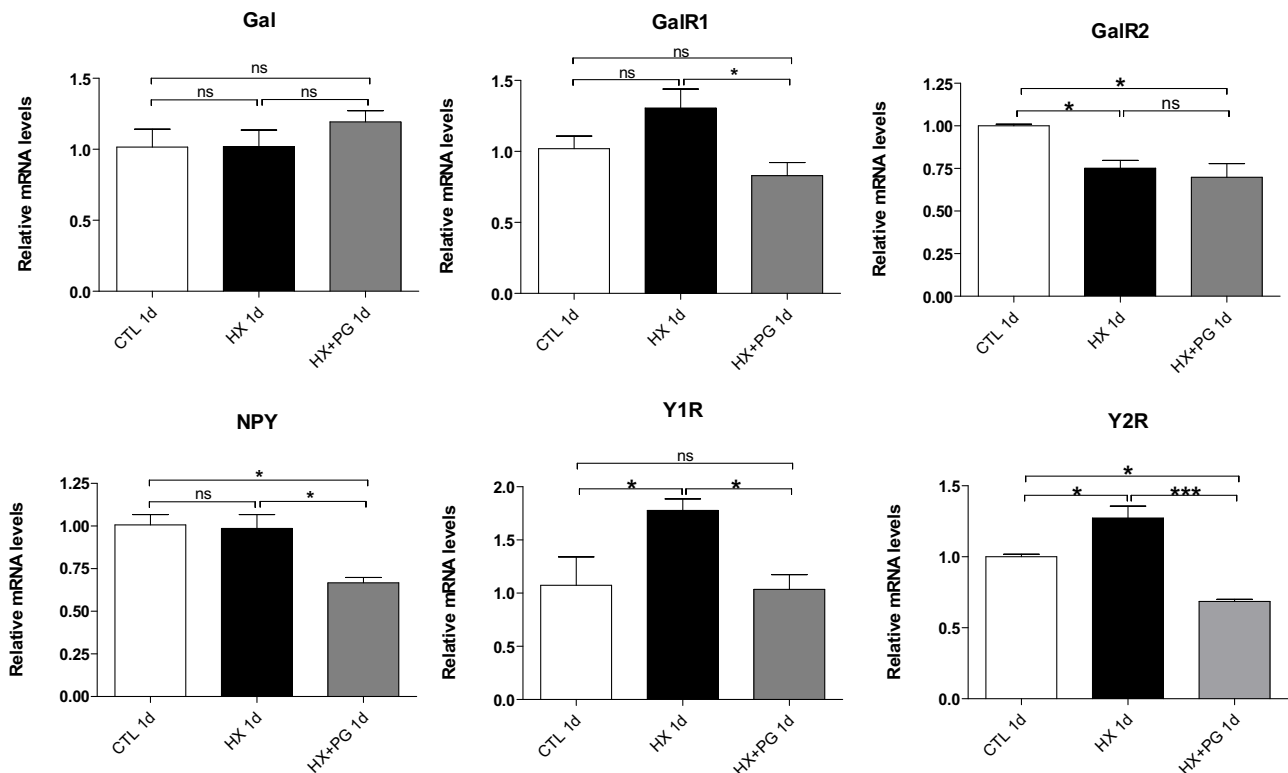

Fig. 2. Relative mRNA levels corresponding to galanin (Gal), neuropeptide Y (NPY) and their associated receptors (GalR1-2 and Y1-2R) detected in the lumbar dorsal spinal cord 1 day after spinal cord injury. Results are expressed as fold increase relative to control levels. Values correspond to the mean ± SEM and were analyzed by applying One Way ANOVA and Tukey Multiple Comparison Post Test. The following symbols were used to represent p values: ns p > 0.05, * p < 0.05, ** p < 0.01 and *** p < 0.001. CTL: control animals, HX: injured animals receiving oil, HX + PG: injured animals treated with PG.

primers used for amplification are listed in Table 1. Cyclophilin B (CycB) was chosen as housekeeping gene. The change in the target mRNA was calculated using the method proposed by Pffafl [35] and expressed as fold-increase relative to control values. Eight animals were included in each experimental group and samples were run in triplicate. Data shown in Figs. 2 and 3 correspond to the mean ± SEM of mRNA levels relative to control values (CTL: sham-operated animals receiving oil). Statistical analysis was performed by applying One-way Analysis of Variance (ANOVA) and Tukey Post-Test.

3. Results

3.1. Behavioral evaluation of mechanical and cold allodynia after spinal cord injury and PG administration

In agreement with previous findings, animals subjected to spinal cord hemisection showed guarding behaviors and changes in the posture such as plantar flexion and toe-clenching, and developed both mechanical and cold allodynia [31,5–7]. A progressive decrease in mechanical withdrawal threshold was observed in both the ipsilateral and contralateral hindpaws of injured animals, and allodynic values were detected 21 and 28 days after SCI (Fig. 1a, p < 0.001 vs CTL at both time points). When cold sensitivity was assessed, a similar behavioral pattern was obtained: there was a gradual and clear increase in the number of positive nociceptive responses in both hindpaws starting 14 days after injury (Fig. 1b, p < 0.01 vs CTL), with the highest number of allodynic responses detected at days 21 and 28 (Fig. 1b, p < 0.01 vs CTL at both time points).

It should be noted that in both tests, paw withdrawals were accompanied by active attention to the stimulus, abrupt head turning and attack, vocalization, and/or body reposituring. These aversive behaviors indicate that noxious stimuli were detected supraspinally [31].

Table 1
Forward and reverse primers sequences.

Gene	Primer sequence	Reference
Galanin	F: 5′ TGCAACCCTGTCAGCCACTC 3′	[9]
	R: 5′ TGTCGCTAAATGATCTGTGGTTGTC 3′	
GalR1	F: 5′ AGGCTTACGTGGTGTGCACTTTC 3′	[9]
	R: 5′ GCCATGATATGCCAAATACCACAA 3′	
GalR2	F: 5′ CATCGTGGCGGTGCTTTT 3′	[9]
	R: 5′ AGCGGGAAGCGACCAAAC 3′	
NPY	F: 5′ GGCCAGATACTACTCCGCTCTGCG 3′	Chottová
	R: 5′ TTCACAGGATGAGATGAGATGTG 3′	Dvoráková et al., 2008
Y1R	F: 5′ GCTGTGGAACGTCATCAGCTA 3′	Chottová
	R: 5′ TTGATAGATCACGAAGGGCAG 3′	Dvoráková et al., 2008
Y2R	F: 5′ CCCGGATCTGGAGTAAGCTAAA 3′	Chottová
	R: 5′ GTGGAGCACATCGCAATAATGT 3′	Dvoráková et al., 2008
CyCB	F: 5′ GTGGCAAGATCGAAGTGGAGAAAC 3′	Gen Bank
	R: 5′ TAAAAATCAGGCCTGTGGAATGTG 3′	Accession Number NM_022536

Injured animals receiving PG presented behavioral responses to both mechanical and thermal stimuli that were similar to those of control animals ($p > 0.05$ vs CTL at all time points evaluated, Fig. 1a, b). PG-treated animals did not develop mechanical allodynia (Fig. 1a, $p < 0.001$ vs HX at days 21 and 28) and showed reduced sensitivity to cold stimulation (Fig. 1b, $p < 0.05$ vs HX at days 14 and 21, $p < 0.01$ vs HX at day 28), which was consistent with our previous observations [5–7].

3.2. Effect of spinal cord injury and PG administration on galanin, GalR1, GalR2, NPY, Y1R and Y2R mRNA levels in the acute phase after injury

One day after spinal cord injury, we found a significant increase in Y1R and Y2R expression in the dorsal spinal cord (Fig. 2, $p < 0.05$ vs CTL in both cases). In addition, a significant decrease in GalR2 mRNA levels was detected (Fig. 2, $p < 0.05$ vs CTL). On the contrary, no changes were observed in galanin, GalR1 and NPY mRNA expression levels (Fig. 2, $p > 0.05$ vs CTL in all cases).

At this time point, animals receiving PG showed lower levels expression of Y1R and Y2R (Fig. 2, Y1R: $p < 0.05$ vs HX, $p > 0.05$ vs CTL; Y2R: $p < 0.001$ vs HX, $p < 0.05$ vs CTL). GalR2 mRNA levels remained similar to those observed in vehicle-treated injured animals (Fig. 2, $p > 0.05$ vs HX, $p < 0.05$ vs CTL). Furthermore, PG administration induced NPY downregulation (Fig. 2, $p < 0.05$ vs CTL and HX), while leaving galanin mRNA levels comparable to control values (Fig. 2, $p > 0.05$ vs CTL and HX).

3.3. Effect of spinal cord injury and PG administration on galanin, GalR1, GalR2, NPY, Y1R and Y2R mRNA levels in the chronic phase after injury

In contrast to the acute phase, a significant increase in galanin and NPY mRNA levels was detected 28 days after injury (Fig. 3, galanin: $p < 0.05$ vs CTL; NPY: $p < 0.01$ vs CTL). The spinal cord lesion also induced a late increase in GalR1 expression, while maintaining the elevated Y1R mRNA levels already detected 1 day after injury (Fig. 3, $p < 0.01$ vs CTL in both cases). Similar to findings in the acute phase, at this time point injured animals showed reduced GalR2 mRNA levels (Fig. 3, $p < 0.05$ vs CTL). No changes were detected in Y2R expression (Fig. 3, $p > 0.05$ vs CTL).

Animals receiving PG treatment did not exhibit Gal and NPY upregulation, maintaining expression levels similar to those observed in CTL animals at this chronic time-point (Fig. 3, Gal: $p > 0.05$ vs CTL, $p < 0.05$ vs HX; NPY: $p > 0.05$ vs CTL, $p < 0.01$ vs HX). In addition, basal levels of expression of both GalR1 and Y1R were detected (Fig. 3, GalR1: $p > 0.05$ vs CTL, $p < 0.001$ vs HX; Y1R: $p > 0.05$ vs CTL, $p < 0.01$ vs HX). PG administration did not modify GalR2 mRNA levels (Fig. 3, $p > 0.05$ vs HX), but maintained Y2R downregulation (Fig. 3, $p < 0.05$ vs CTL and HX).

4. Discussion

Two major findings arise from the present study: 1) SCI leads to considerable changes in the expression of galanin, NPY and their receptors; such changes are observed both in the acute and chronic phases after injury, becoming more widespread in the latter; 2) systemically administered PG modulates the observed changes in galaninergic and NPYergic systems, as observed at both time points analyzed. It also confirms our previous observation that early and sustained PG administration reduces SCI-induced thermal and mechanical allodynia [5–7].

To our knowledge, this is the first report showing temporal changes in the spinal expression of both neuropeptides and their receptors after SCI-induced neuropathic pain. As already mentioned, it has long been known that injury to peripheral nerves induces considerable changes in the expression of galanin, NPY and their associated receptors in primary afferent neurons [8,10,15,9]. Concerning the peptides, their upregulated synthesis in DRG neurons results in increased axonal transport and enhanced like-immunoreactivity in afferent nerve endings in the dorsal horn [36,37]. In contrast, most evidence suggests that galanin and NPY expression levels remain unaffected in spinal cord neurons after peripheral nerve injury [8,10], with the exception of upregulated galanin mRNA levels in the rat dorsal horn after sciatic nerve crush [38]. In contrast, increases in galanin [39] and NPY [40] transcript levels have previously been described in local dorsal horn neurons after inflammation of the hindpaw [40,39]. Here, we show that these neuropeptide systems also suffer plastic changes in the dorsal horn following SCI, resulting in considerable galanin and NPY upregulation in the chronic phase after injury. In accordance, an increase in both galanin and NPY expression levels has been shown in different brain areas in experimental models of nervous system injuries or diseases [41–43].

The present study also reveals that SCI results in significant changes in the expression of galanin and NPY receptors. Thus, 1 day after injury, Y1R and Y2R are upregulated, while lower GalR2 mRNA levels are observed. In the chronic phase, early induced-changes in GalR2 and Y1R expression are maintained, while Y2R mRNA returns to normal levels and GalR1 shows a significant upregulation. Changes in the expression of galanin and NPY receptors have been reported in local dorsal horn neurons also after peripheral tissue insult. Thus, an increase in Y1R transcript levels has been reported in the dorsal horn after hindpaw inflammation [40]. In contrast, GalR1 and GalR2 mRNA levels in local dorsal horn neurons appear unaltered after sciatic nerve axotomy [20]. Because our real time PCR analysis relates to local spinal changes, it remains to be established if SCI does not also affect the expression of galanin, NPY and their associated receptors in DRGs and their central nerve endings in the spinal cord. Furthermore, it would be interesting to evaluate the spinal expression of other related peptides such spexin, an endogenous ligand of GalR2 [44], after injury and PG administration. In fact, this novel neuropeptide, closely related to galanin, has been detected in the rat peripheral and central nervous system [45] and has been suggested to be involved in memory, learning, anxiety disorders and pain [44].

Real time PCR allowed detection of Y2R transcripts in the dorsal horn. This was an unexpected observation, since previous analysis in the mouse showed that Y2R is present exclusively in primary afferent nerve endings [23]. This discrepancy remains to be clarified, although it could be speculated that local dorsal horn neurons
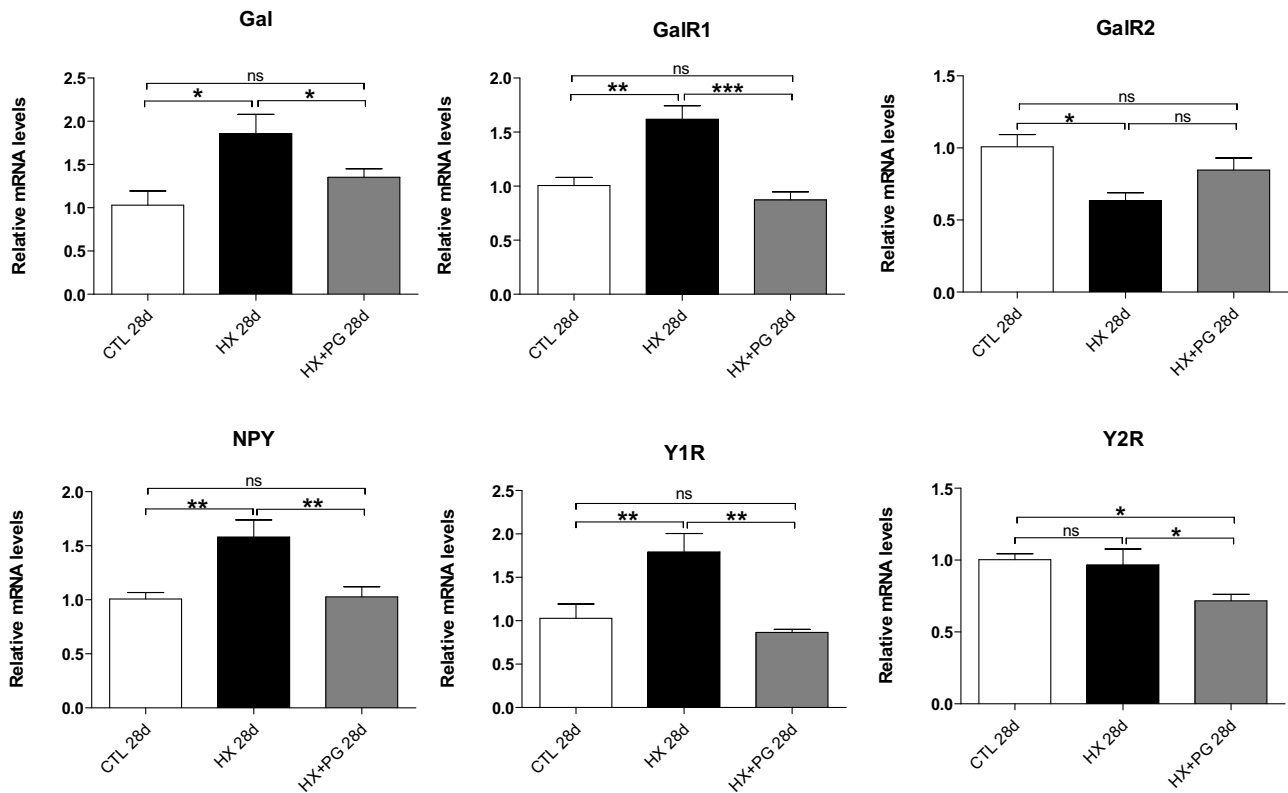
Fig. 3. Relative mRNA levels corresponding to galanin (Gal), neuropeptide Y (NPY) and their associated receptors (GalR1-2 and Y1-2R) detected in the lumbar dorsal spinal cord 28 days after spinal cord injury. Results are expressed as fold increase relative to control levels. Values correspond to the mean ± SEM and were analyzed by applying One Way ANOVA and Tukey Multiple Comparison Post Test. The following symbols were used to represent p values: ns p > 0.05, * p < 0.05, ** p < 0.01 and *** p < 0.001. CTL: control animals, HX: injured animals receiving oil, HX + PG: injured animals treated with PG.

in the rat are capable of synthesizing Y2R. Concerning GalR1, GalR2 and Y1R expression, our findings showing presence of their transcripts in normal rat dorsal horn are in agreement with previous descriptions of abundant GalR1- [20,17] and Y1R-expressing [40,46,21,47] dorsal horn neurons; in the case of GalR2, albeit modestly expressed in a subpopulation of rat dorsal horn neurons [20], its presence has been further supported electrophysiologically [48].

In addition to the certain participation of neurons, glial cells could also be contributing to the changes observed here, since evidence suggesting the presence of galanin and NPY in central nervous system glial cells is emerging [49,50]. In addition, both galanin [51] and NPY [52,53] functional binding sites have been described in cultured spinal cord astrocytes, while the presence of some of these receptors has been clearly evidenced in other central nervous system glial cells such as hippocampal astrocytes [54], retinal astrocytes and microglia [55,56], and olfactory ensheathing cells [57]. Therefore, the increased expression levels of galanin, NPY and their receptors detected in this study may also be related to changes in the number of activated astrocytes and microglial cells observed at the dorsal horn level after SCI [6]. Further single-cell studies using real time RT-PCR, immunohistochemistry or *in situ* hybridization may help to reveal the cellular populations involved in the expression changes observed after spinal cord injury.

The widespread changes in galaninergic and NPYergic systems in the dorsal spinal cord described here suggest their active participation in plastic changes associated with SCI-induced neuropathic pain. Since galanin and NPY are mostly expressed by inhibitory dorsal horn interneurons [18,58], and most cells expressing GalR1 or Y1R are considered to be excitatory interneurons [21,47] with the addition of a number of Y1R-expressing projection neurons [21], it could be speculated that the chronic phase SCI-induced increase in galanin, NPY, GalR1 and Y1R expression would serve

antinociceptive purposes. Furthermore, in addition to their local action on dorsal horn neurons, upregulated galanin and NPY could also contribute to reduce the release of various pronociceptive neurotransmitters from primary afferent nerve endings, acting on presynaptic receptors [8,36,59–61]. Moreover, a reduction of NMDA and an enhancement of GABA$_A$ receptor-mediated postsynaptic currents have been shown in the basolateral amygdala after activation of Y1R [62], suggesting a functional interaction between these receptors with key roles in pain neurotransmission and neuropeptide-mediated activity. Thus, both peptidergic systems could be involved in a protective response activated after injury that, albeit not sufficient for endogenous prevention of pain, could become a target for exogenous manipulation during painful conditions. Interestingly, both galanin and NPY have been shown to exert neuroprotective functions, mediating trophic support and reducing excitotoxicity and neuroinflammation [41,12,42,63] and thus have been proposed as potential therapeutic agents for neurodegenerative diseases [42,63]. In agreement, our studies support the therapeutic value of GalR1 and Y1R specific agonists for the alleviation of central neuropathic pain.

PG administration may also provide an effective therapeutic strategy to prevent the development of neuropathic pain since it has been found to prevent allodynia after sciatic nerve [32,64], trigeminal nerve root [65] or spinal cord [5] injuries, and to block allodynic and hyperalgesic symptoms in animals subjected to chemotherapy induced peripheral neuropathy [66]. In fact, as confirmed here, early and sustained PG treatment prevents both mechanical and thermal allodynia in animals subjected to a spinal cord hemisection.

In addition to pain alleviation, here we show that PG administration modulates the expression of galanin, NPY and their associated receptors. Our results agree with previous studies show-

ing hormone-dependent regulation of different components of galaninergic and NPYergic systems in other nervous system structures. Thus, Whitelaw and cols have recently shown oestrous cycle-induced changes in the mRNA levels of both galanin and GalRs in the hypothalamus [30]. In line with these findings, galanin levels have been shown to be regulated by estradiol [67] and GalR1 by estradiol and PG [68,69,28] in different brain areas in the rat. Finally, estradiol appears to control NPY levels in the arcuate nucleus of the hypothalamus [70], while PG and/or its metabolite allopregnanolone, also known as $3\alpha,5\alpha$-tetrahydroprogesterone, have been reported to modulate NPY and Y1R expression in the amygdala [27,71]. In the context of the spinal cord lesion, we expected that PG, indeed able to prevent allodynia, would enhance neuropeptide-mediated endogenous protective responses, by inducing the upregulation of galanin, NPY, GalR1 and Y1R transcripts. Instead, we observed an attenuation of injury-induced changes in both peptidergic systems in animals receiving the steroid, suggesting that chronic PG administration overrides the natural endogenous efforts against neuropathic pain involving neuropeptides.

Finally, different factors could be contributing to the observed effects of PG on neuropeptidergic expression including the lower number of activated glial cells [6], the reduced levels of IL-6 and TNFα expression [7] and the reduced NF-κB transactivation potential [6] detected in the dorsal horn of injured animals treated with PG. Interestingly, in a recent study, transgenic selective inhibition of glial NF-κB expression has been reported to result in reduced galanin and calcitonin gene related peptide (CGRP) expression levels in animals subjected to a sciatic nerve chronic constriction injury [72]. Thus, altered expression/activity of glial NF-κB at the spinal cord level may also potentially affect the local expression of galaninergic and NPYergic systems. Nevertheless, the mechanisms contributing to the attenuation of changes in spinal peptidergic expression during PG treatment remain to be established. These could relate to direct actions of PG on spinal cord neurons or glial cells through the activation of both intracellular and membrane receptors [73–75].

In conclusion, the results here obtained are in line with previous reports showing that PG treatment is able to prevent spinal NMDA receptor subunits upregulation and phosphorylation [5], as well as glial cell activation and production of pro-inflammatory mediators [6,7], thus suggesting that PG creates a molecular microenvironment that makes unnecessary the triggering of spinal neuropeptide-associate protective mechanisms. Moreover, they support PG- or neuropeptide-based therapies as potentially attractive to prevent and/or treat chronic pain after central injuries.

Acknowledgements

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