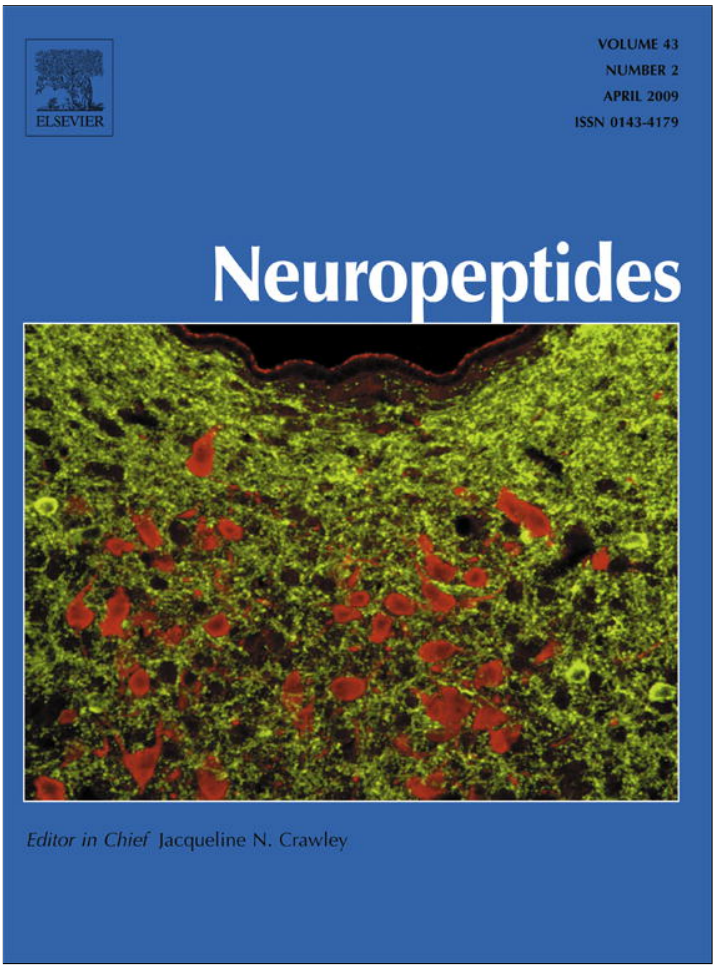

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Bone marrow stromal cells attenuate injury-induced changes in galanin, NPY and NPY Y_1-receptor expression after a sciatic nerve constriction ☆

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Abstract

Single ligature nerve constriction (SLNC) of the rat sciatic nerve triggers neuropathic pain-related behaviors and induces changes in neuropeptide expression in primary afferent neurons. Bone marrow stromal cells (MSCs) injected into the lumbar 4 (L4) dorsal root ganglia (DRGs) of animals subjected to a sciatic nerve SLNC selectively migrate to the other ipsilateral lumbar DRGs (L3, L5 and L6) and prevent mechanical and thermal allodynia. In this study, we have evaluated the effect of MSC administration on the expression of the neuropeptides galanin and NPY, as well as the NPY Y_1-receptor (Y_1R) in DRG neurons. Animals were subjected to a sciatic nerve SLNC either alone or followed by the administration of MSCs, phosphate-buffered saline (PBS) or bone marrow non-adherent mononuclear cells (BNMCs), directly into the ipsilateral L4 DRG. Seven days after injury, the ipsilateral and contralateral L4–5 DRGs were dissected out and processed for standard immunohistochemistry, using specific antibodies. As previously reported, SLNC induced an ipsilateral increase in the number of galanin and NPY immunoreactive neurons and a decrease in Y_1R-positive DRG neurons. The intraganglionic injection of PBS or BNMCs did not modify this pattern of expression. In contrast, MSC administration partially prevented the injury-induced changes in galanin, NPY and Y_1R expression. The large number of Y_1R-immunoreactive neurons together with high levels of NPY expression in animals injected with MSCs could explain, at least in part, the analgesic effects exerted by these cells. Our results support MSC participation in the modulation of neuropathic pain and give insight into one of the possible mechanisms involved.

Keywords: Neuropeptides; Pain modulation; Single ligature nerve constriction; Mesenchymal stem cells; Cellular therapy

1. Introduction

Single ligature nerve constriction (SLNC) is a newly developed animal model for the study of neuropathic pain (Brumovsky et al., 2004; Musolino et al., 2007; Coronel et al., 2008). Animals subjected to a sciatic nerve SLNC develop both mechanical (Brumovsky et al., 2004; Musolino et al., 2007) and thermal (Musolino et al., 2007) allodynia within 3 days of the lesion, and the allodynic responses are observed even 56 days after injury (Musolino et al., 2007). In this model, there are also dramatic phenotypic changes in primary afferent neurons, including changes in the expression of some neuropeptides involved in pain modulation (Brumovsky et al., 2004; Coronel et al., 2008).

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Thus, SLNC of the sciatic nerve results in a marked ipsilateral increase in neuropeptides galanin (Coronel et al., 2008) and tyrosine (NPY) (Brumovsky et al., 2004) like immunoreactivities (LIs) in lumbar dorsal root ganglia (DRGs), with a parallel decrease in the number of neurons expressing the NPY Y_1-receptor (Y_1R) (Brumovsky et al., 2004).

NPY and galanin have been shown to participate in the modulation of neuropathic pain, although the exact role they play has not yet been elucidated. Thus, proalgesic as well as analgesic actions have been described for both (Liu and Hökfelt, 2002; Holmes et al., 2005; Wiesenfeld-Hallin et al., 2005; Brumovsky et al., 2007; Gibbs et al., 2007; Smith et al., 2007). One factor that contributes to their complex action is the variety of receptors to which they respectively bind (Larhammar, 1996; Iismaa and Shine, 1999; Brain and Cox, 2006; Lang et al., 2007). In the case of NPY, the Y_1R appears to be the subtype through which this neuropeptide exerts its analgesic effects (Xu et al., 1999; Naveilhan et al., 2001; Gibbs et al., 2007; Smith et al., 2007), while Y_2R has been suggested to mediate NPY proalgesic actions (Brumovsky et al., 2007; Gibbs et al., 2007).

Bone marrow stromal cells (MSCs), also known as mesenchymal stem cells, have a well documented role in providing the appropiate microenvironment within the bone marrow which supports the tightly regulated process of hematopoiesis (Bianco et al., 2001; Short et al., 2003). It has recently been demonstrated that MSCs participate in the regeneration process that is activated following several types of lesion of the nervous system, contributing to the animals' functional recovery (Chen et al., 2001; Lu et al., 2001; Cuevas et al., 2004; Mahmood et al., 2004). Thus, local MSC implantation in the distal stump of the transected rat sciatic nerve promotes functional recovery assessed by the walking track test (Cuevas et al., 2004). Also, in an animal model of cerebral ischemia, the intravenous administration of MSCs results in the selective engraftment of the cells in the ischemic hemisphere and in a significant recovery of the somatosensory behavior (Chen et al., 2001). Finally, MSCs administered intravenously to rats subjected to a traumatic brain injury preferentially migrate into the injured hemisphere, where they increase the expression of growth factors (Mahmood et al., 2004) and improve functional recovery (Lu et al., 2001; Mahmood et al., 2004).

We have recently shown that when MSCs are injected into the ipsilateral lumbar 4 (L4) DRGs of animals subjected to a sciatic nerve SLNC, these cells selectively migrate to the other lumbar ganglia affected by the lesion (ipsilateral L3, L5 and L6) (Coronel et al., 2006). In the ganglia where homing occurs, MSCs acquire a striking perineuronal localization, resembling glial/satellite cells (Coronel et al., 2006). This characteristic distribution, acquired in an active and time-dependent fashion, suggests an association with a specific role in the injured nervous tissue. In fact, MSC administration prevents the generation of mechanical allodynia and reduces the number of allodynic responses to cold stimuli (Musolino et al., 2007).

In this work, we have investigated the potential mechanisms involved in the reduction of neuropathic pain-related behaviors observed after MSC administration. For this purpose, we have analyzed by immunohistochemistry the expression of galanin, NPY and the Y_1R in DRG neurons from animals subjected to a sciatic nerve SLNC and MSC intraganglionic administration.

2. Experimental procedures

2.1. Animals

Adult Sprague-Dawley male rats (200–300 g, Fucal, Buenos Aires, Argentina) were maintained in a 12 h light-cycle, with water and food *ad libitum*. All the experiments performed in this study were approved by the local Ethical Committee from the Department of Bioethics of the School of Biomedical Sciences from Austral University, and were carried out in accordance to the policy of the Society for Neuroscience and the International Association for the Study of Pain for the use of animals in pain research.

2.2. Isolation of MSCs and BNMCs

Rats were sacrificed using an overdose of chloral hydrate (1.5 g/kg, i.p.), and their tibiae and femurs were dissected out from attached muscle and connective tissue. The epiphyses of the bones were removed, and the marrow was extracted with 3 ml of DMEM (GIBCO, Maryland, USA) using a 15G needle and a syringe. Red cells were lysed with 0.15 M buffered ammonium chloride solution, and the remaining cells were washed twice with phosphate-buffered saline (PBS) and centrifuged through a density gradient (Ficoll-Paque Plus, 1.077 g/ml, Pharmacia Biotech, USA) for 30 min at 400*g*. The interface containing mononuclear cells was washed with PBS and centrifuged for 10 min at 250*g*. The cells were then suspended at a concentration of 1×10^6 cells/ml in DMEM, 10% fetal bovine serum (GIBCO), 50 μg/ml gentamicine, 2.5 μg/ml anfotericine B, and 5×10^6 cells were plated in 25 cm^2 cell culture flasks. After 3 days, the non-adherent cells (afterwards referred to as Bone marrow Non-adherent Mononuclear Cells, BNMCs) were removed by replacing the culture medium. Medium was then changed every 4–5 days until confluence was reached. The adherent cells (MSCs) were then harvested by incubation with 0.25% trypsin-1 mM EDTA (GIBCO), washed with PBS and suspended at a concentration of 50×10^6 cells/ml in PBS.

2.3. Transplantation of MSCs and nerve injury model

Experiments were performed as previously described (Coronel et al., 2006; Musolino et al., 2007). Briefly, the animals were anesthetized with chloral hydrate (350 mg/kg, i.p.) and their right L4 DRG was exposed using a micro bone rongeur after dissection of the aponeurotic and the paraspinal muscle group. For MSC transplantation a suspension of cells (2×10^5 cells in 4 μl PBS) was injected over a 60 s period of time via a drawn glass micropipete (70–100 μm tip diameter) using a micropump syringe injector. Immediately after the muscular-aponeurotic and skin individual suture, the sciatic nerve constriction was performed. The right sciatic nerve was exposed and dissected free from the surrounding tissue at the mid-thigh level. It was then wrapped with a thin square strip (5 mm) of polyethylene and constricted with a tie around the strip using 3.0 silk suture (Barbour Threads, Lisburn, Ireland) to a "medium" SLNC with a reduction of 40–80% of its original diameter (Brumovsky et al., 2004). The degree of constriction of each nerve was confirmed after dissection under a surgery microscope using a 10 mm ruler, and also by microscopical observation of 16 μm sections stained with neutral red. Control animals were subjected to a SLNC and an intraganglionic injection of either PBS (4 μl) or BNMCs (2×10^5 cells in 4 μl PBS). For control purposes, sections of the injected DRG stained with neutral red were microscopically analyzed. Another group of control animals was only subjected to a SLNC. Finally, sham animals were prepared, placing the strip of polyethylene and the silk suture around the sciatic nerve without constriction. Ten animals were evaluated in each of the studied groups.

2.4. Tissue preparation

The animals were allowed to survive for 7 days and were then deeply anesthetized using an overdose of chloral hydrate (1.5 g/kg, i.p.), and perfused through the heart with 60 ml of warm (37 °C) Tyrodés buffer (pH 7.4), followed by 60 ml of fixative (4% paraformaldehide and 0.2% picric acid in 0.16 M phosphate buffer, pH 7) (Zamboni and De Martino, 1967) at 37 °C and 300 ml of the same fixative at 4 °C. Immediately after perfusion, the ipsilateral and contralateral L4–5 DRGs were carefully dissected out and post fixed in the same fixative for 90 min at 4 °C. The tissues were then rinsed in 20% sucrose in phosphate buffer (pH 7.2) containing 0.1% sodium azide and stored in the same solution at 4 °C.

2.5. Immunohistochemistry

Tissues were embedded in OCT compound (Tissue Tek, Miles Laboratories, Elkhart, USA) and cut longitudinally at 14 μm thickness in a cryostat (Microm, Heildeberg, Germany). The sections were mounted onto chrome alume-gelatin coated slides, allowed to dry for at least 1 h, rinsed twice in PBS and dehydrated. Endogenous peroxidase was inactivated with 0.5% hydrogen peroxide (H_2O_2), followed by rehydration and rinses in PBS. Sections were incubated overnight in a humid chamber at 4 °C with anti-galanin (1/3000, rabbit, Peninsula), anti-NPY (1/4000, rabbit, Peninsula, California, USA) and anti-Y_1R (1/8000, rabbit) (Zhang et al., 1994a) antibodies, diluted in PBS containing 0.2% bovine serum albumin, 0.03% Triton X-100 and 0.1% sodium azide. The slides were rinsed twice in PBS and incubated at room temperature for 1 h with a biotinylated secondary antibody (1/200, Vector, California, USA), rinsed twice in PBS, and incubated according to the avidin-biotin complex ABC protocol (Vectastain Elite kit, Vector) for 30 min at room temperature. Peroxidase activity was demonstrated by reaction with 3,3'-diaminobenzidine using H_2O_2 and nickel salts for enhancement of the reaction product. After dehydration, the sections were coverslipped and mounted with Permount (Fisher Scientific Company, New Jersey, USA). Controls were done by incubating sections after adsorption of the primary antibodies with the corresponding antigenic peptide (10^{-5}, 10^{-6} M, Peninsula) or by omitting the primary or secondary antibodies.

2.6. Microscopy

All sections were examined under bright-field illumination with a Nikon Eclipse E-800 photomicroscope (Nikon, Tokyo, Japan). Photographs were taken using a Nikon DXM 1200 digital camera. Resolution, brightness and contrast of the digital images were optimized using the Adobe Photoshop software (Adobe Systems Inc., California, USA).

2.7. Quantification

The number of neurons exhibiting galanin-, NPY- or Y_1R-LI was determined in L4 and L5 DRGs by counting immunostained neuronal profiles under bright-field illumination using a 20× objective, in randomly, systematically sampled sections throughout the DRG (every 8th section, eight sections per ganglion). For each marker, the total number of immunoreactive cells counted was correlated to the sum of cross-sectional area of the corresponding sampled section (Brumovsky et al., 2004). The total sampled mean area was 944636 ± 50413.27 pixels^2. Area calculations were performed using a KS400 system (Kontron Elektronic, Zeiss, Germany) and a Hyper HAD Sony CCD IRIS black and white video camera mounted onto an Optiphot-2 Nikon microscope for image acquisition. Briefly, images were digitalized in a rectangular frame of 1000×840 pixels using the 10× objective in the photo

mode of illumination intensity. To adjust possible defects in the illumination of optical pathway, a low-pass image was produced for subtraction and background shading correction. Complete area of eight sections per ganglia were interactively delineated and measured, and the mean value ± S.E.M. was calculated for each ganglion.

2.8. Statistical analysis

Statistical analysis was carried out by applying One-way Analysis of Variance (ANOVA) and Newman–Keuls Multiple Comparison Post-Test. The number of immunoreactive neurons/area was expressed as mean ± S.E.M. p values are presented as following: ns $p > 0.05$; $^{*}0.05 > p > 0.01$; $^{**}0.01 > p > 0.001$ and $^{***}p<0.001$.

3. Results

Microscopical analysis (Fig. 1) and subsequent quantification (Fig. 2) of galanin-, NPY- and Y_1R-immunoreactive (IR) neuronal profiles showed extensive changes in the expression of these markers in the ipsilateral L4–5 DRGs from animals with a sciatic nerve constriction. The intraganglionic injection of PBS or BNMCs did not modify this pattern of expression. However, MSC administration partially prevented the injury-induced changes in galanin, NPY and Y_1R expression in both L4 and L5 DRGs.

3.1. Galanin-LI

In lumbar DRGs from sham animals (not shown), as well as in the contralateral ganglia (Figs. 1a and 2) from animals included in any of the experimental groups, galanin-LI remained restricted to a few small-sized neurons. Galanin pattern of expression was similar in animals only subjected to the sciatic nerve SLNC (not shown) and lesioned animals injected with either PBS (Figs. 1b and 2) or BNMCs (not shown). In these three groups, the sciatic nerve lesion induced a marked ipsilateral increase in the number and intensity of galanin-IR neurons 7 days after the lesion (Figs. 1b and 2). Different subpopulations of primary afferent neurons upregulated galanin, which could therefore be detected in small-, medium- and large-sized cells (Fig. 1b). MSC administration partially prevented the injury-induced galanin upregulation and resulted in a 33% reduction in the number of neuronal profiles expressing galanin, when compared to animals receiving PBS (Figs. 1c and 2).

3.2. NPY-LI

In lumbar DRGs from sham animals (not shown), as well as in the contralateral ganglia from lesioned rats (Figs. 1d and 2), only single NPY-IR neuronal profiles could be detected. Animals subjected to a sciatic nerve SLNC either alone (not shown) or followed by the intraganglionic injection of PBS (Figs. 1e and 2) or BNMCs (not shown) exhibited a significant increase in the number of NPY-IR medium- and large-sized neurons in the ipsilateral ganglia 7 days after the lesion. The signal varied from lightly stained cells with cytoplasmatic granules to neurons completely filled with NPY-LI (Fig. 1e). On the contrary, and as for galanin, MSC administration resulted in a 33% reduction in the number of NPY-IR neurons in L4–5 DRGs, when compared to animals injected with PBS (Figs. 1f and 2).

3.3. Y_1R-LI

Several Y_1R-IR neurons were detected in the contralateral ganglia from lesioned animals (Figs. 1g and 2). The immunostaining was mainly related to the plasmalemma of small- and some medium-sized primary afferent neurons (Fig. 1g). This pattern was also observed in L4–5 DRGs from sham animals (not shown). The sciatic nerve SLNC either alone (not shown) or followed by the intraganglionic injection of PBS (Figs. 1h and 2) or BNMCs (not shown) induced an ipsilateral decrease in the number of Y_1R-IR neuronal profiles 7 days after the injury. On the contrary, MSC administration counteracted the injury-induced decrease in Y_1R expression (Figs. 1i and 2). Thus, high numbers of Y_1R-IR neurons remained in these animals, similar to those observed in control rats.

3.4. Controls

None of the staining patterns described above was observed after preadsorption of each antiserum with the corresponding antigenic peptide, or after omitting either the primary or secondary antibodies (data not shown).

4. Discussion

These results show that the changes observed in galanin, NPY and Y_1R expression in primary afferent neurons after a sciatic nerve SLNC can be partially prevented by MSC administration. This effect cannot be reproduced after PBS or BNMC administration.

It is well known, that peripheral nerve injury induces drastic phenotypic changes in primary afferent neurons, involving a great variety of molecules among which are the neurotransmitters galanin and NPY. In normal conditions, only a few galanin (Villar et al., 1989, 1991) and NPY (Wakisaka et al., 1991, 1992; Nahin et al., 1994) IR neuronal profiles are observed in the DRGs, while several small-sized neurons express Y_1R-LI (Zhang

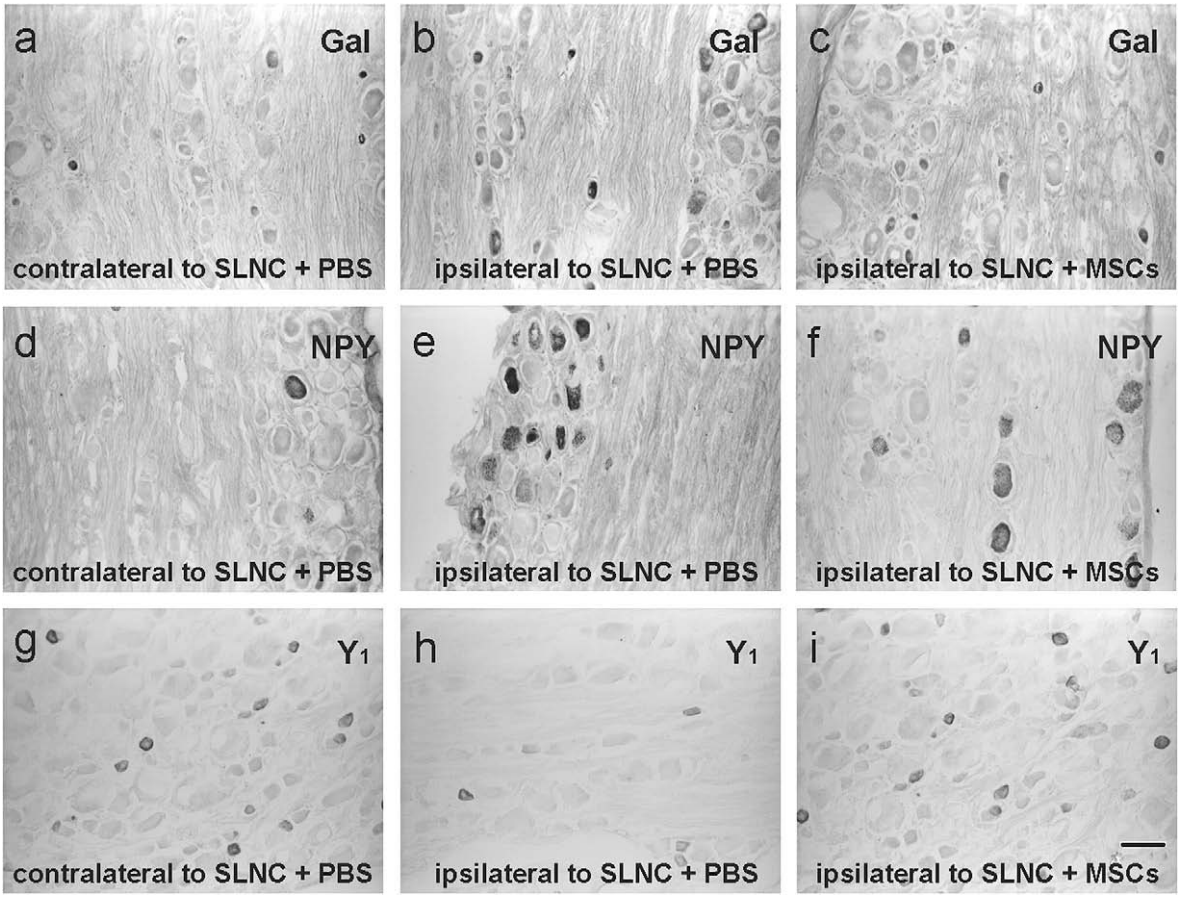

Fig. 1. Photomicrographs showing galanin (Gal, a–c), NPY (d–f) and Y_1R (g–i) like immunoreactivities in L4–5 DRGs from animals subjected to a sciatic nerve SLNC. Contralateral (a, d and g) and ipsilateral (b, e and h) ganglia from animals with a sciatic nerve constriction and PBS intraganglionic administration are shown. In (c, f and i), ipsilateral DRGs from animals subjected to a SLNC and an intraganglionic MSC injection, can be observed. Note the low number of galanin and NPY-IR neuronal profiles in contralateral ganglia (a and d, respectively), and the great increase in the number of immunoreactive neurons ipsilaterally to the lesion (b and e, respectively). In contrast, there are several Y_1R-IR neurons in contralateral ganglia (g) and SLNC induces an ipsilateral decrease in the receptor like immunoreactivity (h). Finally, MSC administration drastically prevents the changes in galanin (c), NPY (f) and Y_1R (i) expression induced by the sciatic nerve lesion, with a 33% reduction in the number of galanin and NPY-IR neuronal profiles (c and f, respectively) and the maintenance of the high number of Y_1R-IR neurons (i) observed in control DRGs (g). Calibration bar: 100 μm.

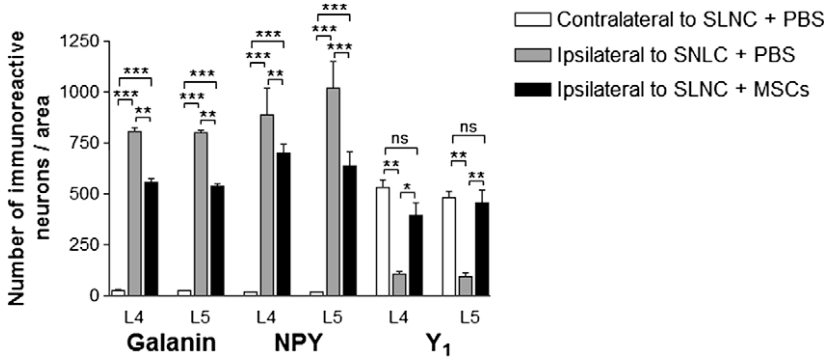

Fig. 2. Number of neuronal profiles expressing galanin-, NPY- or Y_1R-LIs in L4 and L5 DRGs from animals with a sciatic nerve SLNC and a simultaneous intraganglionic injection of PBS or MSCs. Values show mean ± S.E.M. and represent the total number of immunoreactive cells per area (total sampled mean area 944.636 ± 50413.27 pixels^2). p values are presented as following: ns $p > 0.05$; $^{*}0.05 > p > 0.01$; $^{**}0.01 > p > 0.001$ and $^{***}p < 0.001$. Since no statistically significant differences were observed when comparing neuropeptide/receptor expression in L4 vs. L5 DRGs, the corresponding p values were not included in the graph.

et al., 1994a,b, 1999). Following a peripheral nerve injury there is a dramatic upregulation of both galanin (Hökfelt et al., 1987; Villar et al., 1989, 1991; Nahin et al., 1994; Ma and Bisby, 1997; Shi et al., 1999; Coronel et al., 2008) and NPY (Wakisaka et al., 1991, 1992; Nahin et al., 1994; Shi et al., 1999; Brumovsky et al., 2004). The main effect on Y_1R expression is a marked downregulation in small DRG neurons (Zhang et al., 1994a, 1995; Brumovsky et al., 2004).

We have previously shown that when MSCs are injected into the ipsilateral L4 DRG of animals subjected to a sciatic nerve SLNC, these cells selectively migrate to the other lumbar ganglia affected by the sciatic nerve constriction (Coronel et al., 2006). MSC migration and homing probably involve chemotactic factors expressed by DRG neurons or glia cells in response to the injury. Preliminary data from our lab suggest that stromal cell-derived factor-1 (SDF-1) and monocyte chemoattractant protein-1α (MCP-1α) may be at least some of the molecules involved in this process. Both factors are strongly upregulated in the ipsilateral DRGs after the nerve lesion, SDF-1 in satellite glia cells and MCP-1α in small- and medium-sized primary afferent neurons (unpublished data). Importantly, MSCs express the corresponding chemokine receptors, CXCR4 and CCR2, respectively (Ji et al., 2004).

Once in the lesioned DRG, MSCs acquire a characteristic perineuronal localization (Coronel et al., 2006) resembling satellite cells and suggesting that MSCs could be behaving in a glial cell fashion in the injured ganglia: producing trophic factors that protect sensory neurons and modify their phenotypic response to injury. In fact, it has been shown that MSC administration increases the expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in animals with traumatic brain (Mahmood et al., 2004) or spinal cord (Neuhuber et al., 2005) injuries. Supporting the hypothesis of an activation of neurotrophin signaling, we have recently observed an increase in the expression of the phosphorylated (activated) form of the neurotrophins-binding Trk receptors in lumbar DRGs of animals with a sciatic nerve SLNC and MSC intraganglionic injection (unpublished data).

In bone marrow, their natural home, MSCs constitutively secrete a diverse spectrum of interleukines, growth factors and chemokines that modulate the survival, proliferation and differentiation of hematopoietic stem cells (Eaves et al., 1991; Majumdar et al., 2000; Bianco et al., 2001). After migration into the lesioned DRGs, MSCs could behave in a similar way, modulating primary sensory neurons response to injury and thus influencing pain behavior. In fact, we have observed an attenuation of the injury-induced changes in galanin, NPY and Y_1R expression in primary afferent neurons (this study) and a reduction in the number and intensity of the allodynic responses to both mechanical and thermal stimuli (Musolino et al., 2007) after MSC administration.

NPY and galanin participate in nociception and have been attributed both anti- and pronociceptive roles (Liu et al., 2001; Liu and Hökfelt, 2002; Holmes et al., 2005; Wiesenfeld-Hallin et al., 2005; Brumovsky et al., 2007; Gibbs et al., 2007; Smith et al., 2007). Such disparate actions are probably related to their binding to different subtypes of receptors (Larhammar, 1996; Iismaa and Shine, 1999). With regard to NPY, there is some evidence that the Y_1R may be important for the antinociceptive effect (Xu et al., 1999; Naveilhan et al., 2001; Gibbs et al., 2007; Smith et al., 2007), while Y_2R probably mediates NPY proalgesic actions (Brumovsky et al., 2007; Gibbs et al., 2007). Thus, it has been postulated that the Y_1R normally expressed in the somatic membrane of small-sized primary afferent neurons could contribute to stress-induced analgesia (Bodnar, 1990), when bound by NPY released from sympathetic nerve endings (Lundberg, 1982) around blood vessels and from chromaffin cells in the adrenal medulla (Cavadas, 2006). The Y_1R could also be targeted by NPY released from large DRG neurons, mediating an intraganglionic chemical "cross-talk" between A- and C-neurons (Abdulla and Smith, 1999; Abdulla et al., 2001). However, the low levels of expression of this neuropeptide in DRG neurons under normal circumstances and the decrease in the number of Y_1R-IR neurons that occurs in parallel to NPY upregulation after different types of sciatic nerve injury, suggests less significance for this mechanism. Instead, Y_2R which is strongly upregulated after nerve injury (Zhang et al., 1997; Landry et al., 2000) is more likely involved in somatic "cross-talk", thus mediating small- and medium-sized DRG neurons excitation (Abdulla and Smith, 1999; Abdulla et al., 2001).

In this study, we show that SLNC of the sciatic nerve results in a marked increase in galanin and NPY expression in the ipsilateral DRGs, with a parallel decrease in the number of neurons expressing the Y_1R. MSC administration resulted in a 33% reduction in the number of galanin- and NPY-IR neuronal profiles and counteracted the lesion-induced decrease in Y_1R expression, maintaining the high number of Y_1R-IR neuronal profiles observed in control DRGs. These effects were not observed after the administration of PBS or BNMCs, another cell fraction obtained from bone marrow. The latter suggests that the capacity to modify the reaction of primary afferent neurons to the lesion was inherent to the presence of MSCs and not due to unspecific factors such us mechanical damage or pathological changes of DRG neurons due to the intraganglionic injection. Furthermore, similar levels of peptide/receptor expression were detected in injected (L4) and non-injected (L5) ganglia, suggesting a "saturation" of DRG neurons response to MSC stimulation. Finally, the elevated lev-

els of NPY together with the maintenance of almost normal levels of Y_1R expression in the DRGs from MSC-treated animals may create the appropriate scenario for intraganglionic "cross-talk" between A- and C-neurons, resulting in a reduction in C-neurons excitability. Alternatively, conserved levels of Y_1R in primary afferent terminals in the dorsal horn may also enhance the NPYergic modulation of spinal transmission, thus potentially alleviating the pain-related behaviors observed in animals subjected to a sciatic nerve SLNC. However, parallel mechanisms involving other molecules related to pain transmission, such as galanin, should also be considered.

Peripheral nerve injury triggers neuropathic pain, a devastating condition of chronic and severe pain. The limited success of currently available strategies for the treatment of neuropathic pain suggests the need for new therapeutic approaches. Our results show that MSCs modulate primary afferent neurons response to injury and thus influence pain behavior. Data here provided, based on an animal pain model, indicate that MSCs could be used as modulators of neuropathic pain.

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