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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.