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Nociceptive responses and spinal plastic changes of afferent C-fibers in three neuropathic pain models induced by sciatic nerve injury in the rat

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ABSTRACT

Peripheral nerve injuries induce plastic changes on primary afferent fibers and on the spinal circuitry, which are related to the emergence of neuropathic pain. In this study we compared three models of sciatic nerve injury in the rat with different degrees of damage and impact on regeneration capability: crush nerve injury, chronic constriction injury (CCI) and spared nerve injury (SNI). All three models were characterized by means of nerve histology, in order to describe the degenerative and regenerative process of injured axons. Nociceptive responses were evaluated by mechanical and thermal algesimetry tests. Crush animals displayed higher withdrawal thresholds on the ipsilateral paw compared to the contralateral during the time of denervation, while CCI and SNI animals showed mechanical and thermal hyperalgesia. Central plasticity was evaluated by immunohistochemical labeling of non-peptidergic (IB4-positive) and peptidergic (substance P-positive) nociceptive C-fibers on L4–L6 spinal cord sections. After crush nerve injury and SNI, we observed progressive and sustained reduction of IB4 and SP immunolabeling at the sciatic projection territory in the superficial laminae of the dorsal horn, which affected only the tibial and peroneal nerves projection areas in the case of SNI. After CCI, changes on SP-immunoreactivity were not observed, and IB4-immunoreactive area decreased initially but recovered to normal levels on the second week post-injury. Thus, nociceptive responses depend on the type of injury, and the immunoreactivity pattern of afferent fibers at the spinal cord display changes less pronounced after partial than complete sciatic nerve injury. Although signs of neuropathic pain appear in all three lesion models, nociceptive responses and central plasticity patterns differ between them.

Introduction

Peripheral nerve injuries often induce the development of neuropathic pain, which is characterized by spontaneous pain, hyperalgesia and allodynia. A variety of mechanisms affecting the peripheral nerve itself as well as the central nervous system may trigger the emergence of pain after nerve injuries. These mechanisms include sensitization of pain receptors, spontaneous and ectopic firing of nociceptive neurons, and changes in gene expression of ion channels and receptors in nociceptive axons and dorsal root ganglia (DRG) neurons. At the spinal level, the main mechanisms related to pain after peripheral nerve injuries consist in central sensitization of second order neurons, changes in the expression of neurotransmitters and neuropeptides, disregulation of inhibitory neurons and modulatory descending pathways, and the anatomical and functional reorganization of primary afferent projections in the dorsal horn (Sah et al., 2003; Campbell and Meyer, 2006; Navarro et al., 2007).

It is accepted that the lesion leading to pain must directly involve the nociceptive pathways (Boivie et al., 1989). Functional features, as the nociceptive stimuli transmission, and their particular sensitivity to nerve injury, point to the phenotypic and morphological alterations of C nociceptive fibers and neurons as main participants in the emergence of neuropathic pain. Of interest, two different populations of primary afferent nociceptors convey pain-related information, which in fact engage two parallel, potentially independent, ascending pathways (Hunt and Rossi, 1985; Braz et al., 2005). One of these C-fiber populations contains the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), and is NGF-dependent, whereas the other population is represented by non-peptidergic C-fibers, which display high affinity for the isolectin B4 from *Griffonia simplicifolia* (IB4) and are GDNF-dependent (Eriksson et al., 1997; Bennett et al., 2000). The central projections of peptidergic fibers end at laminae I and IIo of the dorsal horn, where second order neurons express the NK1 receptor, while non-peptidergic fibers terminate at lamina IIi that contains interneurons expressing PKC-γ (Ribeiro-da-Silva et al., 1989; Malmberg, 1997; Hunt and Mantyh, 2001; Alvarez and Fyffe, 2000).

Experimental evidences suggest that both populations are functionally different from each other. It has been described that the

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peptidergic one participates in the signaling of highly noxious pain and the central maintenance of hyperalgesia, since its selective destruction results in loss of the increased sensitivity to stimulation that appears after the induction of inflammatory and neuropathic pain (Nichols et al., 1999). Furthermore, nerve injury results in substantial changes in neuropeptide expression by unmyelinated C fibers. Particularly, SP and CGRP are markedly down-regulated after axotomy (Jessel et al., 1979; McGregor et al., 1984; Villar et al., 1991), a phenomenon that is dependent upon interruption of target-derived NGF to the neuronal body (Eriksson et al., 1997). However, SP expression has been reported to increase in A large neurons and large myelinated peripheral nerve fibers (Noguchi et al., 1995), which suggested its contribution to hyperalgesia and allodynia, although this finding has not been corroborated in other studies (Hughes et al., 2007).

On the contrary, the role of the non-peptidergic nociceptors is still poorly understood, although electrophysiological and behavioral studies shed light on this issue. In DRG cultures, IB4 positive neurons display robust inward currents after capsaicin application (Liu et al., 2004), and compared to IB4-negative neurons, they generate longer-duration action potentials, show higher densities of TTX-resistant sodium currents and smaller noxious heat-activated currents (Stucky and Lewin, 1999). Sciatic nerve transection, crush or constriction injuries have been associated to a depletion of IB4-labeling in the superficial dorsal horn, although its expression was restored in weeks after crush or partial injuries (Molander et al., 1996; Bailey and Ribeiro-da-Silva, 2006).

Different animal models of peripheral nerve injury have been used to analyze the contribution of nociceptive C-fibers to mechanical and thermal hyperalgesia and the development of neuropathic pain. Despite reported discrepancies, it can be summarized that different types of peripheral nerve injuries induce changes in the expression of neurotransmitters and neuropeptides and the reorganization of afferent projections in the dorsal horn of the spinal cord. In turn, the assessment of the nociceptive responses among these injury models has demonstrated that all of them alter the sensitivity to thermal and mechanical stimuli, and indeed induce behaviors attributed to the experience of spontaneous pain (Sah et al., 2003; Dowdall et al., 2004; Campbell and Meyer, 2006). As C-fiber plasticity is suggested to be one of the mechanisms involved in the development of neuropathic pain, in the present study we analyzed the changes in the central projections of both populations of nociceptive fibers in three sciatic nerve injury models of different severity: crush nerve injury, chronic constriction injury (CCI; Bennett and Xie, 1988), and spared nerve injury with preservation of the sural branch (SNI; Decosterd and Woolf, 2000). While in the crush nerve injury all the axons are severed and allowed to regenerate, after the CCI only a proportion of the axons are injured, and the SNI promotes the degeneration of axons from the tibial and peroneal nerves, which form a neuroma at the level of the injury and are prevented from regenerating. We have comparatively characterized the three nerve injury models in terms of nociceptive responses and histologically regarding the degree of nerve degeneration and regeneration that they induce, and studied the central plasticity of nociceptive C-fibers by analyzing SP and IB4 immunoreactivity in the dorsal horn.

Materials and methods

Animals and surgery

Adult female Sprague-Dawley rats (260 ± 6 g) were used in the study. All rats were kept on standard laboratory food and tap water ad libitum with a light–dark cycle of 12 h. All experimental procedures were approved by the Ethics Committee of our institution, and followed the European Communities Council Directive 86/609/EEC.

Surgery was performed under pentobarbital anesthesia (40 mg/kg i.p.). The right sciatic nerve was exposed at the mid-thigh level and freed of adhering tissues. In the Crush group ($n = 16$) the sciatic nerve was crushed during 30 s in three different orientations by means of a fine forceps (Dumont no. 5). A 10-0 suture was placed in a neighbor muscle at the level of the crush for later recognition of the injury site. In a second group of rats ($n = 16$), a CCI was induced by placing 4 loose ligatures of chromic gut (5-0) around the nerve as described previously (Bennett and Xie, 1988). In the SNI group ($n = 16$) the three terminal branches of the sciatic nerve were gently separated proximal to the popliteal fossa, the tibial and the peroneal nerves were tightly ligated with 7-0 silk and sectioned distal to the ligature, removing about 2 mm of the distal stump. Care was taken to avoid damage of the sural nerve. The wound was closed in two layers. The rats were kept in a warm environment until their complete recovery from anesthesia. An additional group of unoperated rats ($n = 8$) was used as a control for behavioral responses and the histological studies.

Electrophysiological assessment

Nerve conduction tests were used to assess the degree of denervation or reinnervation in the injured hindlimb. At 28 days postoperation (dpo) with the rats under pentobarbital anesthesia, the sciatic nerve was stimulated with single electrical pulses (100 μs duration and supramaximal intensity) delivered by monopolar needles percutaneously placed at the sciatic notch, proximal to the injury (Navarro et al., 1994b; Valero-Cabré and Navarro, 2002), and the compound muscle action potentials (CMAP) of the plantar muscles were recorded by means of monopolar needles (28G) and displayed in an oscilloscope (Sapphyre 4M, Medelec Vickers). The active recording electrode was placed at the 3rd metatarsal space and the reference at the tip of the fourth toe. The latency to the onset and the maximal baseline to peak amplitude of the evoked M wave were measured. The same measurements were made for the H wave recorded in the motor conduction tests; the H wave is produced by electrical activation of the spinal stretch reflex arc. Sensory compound nerve action potentials (CNAPs) were similarly recorded by electrodes placed near the tibial nerve at the ankle and near the digital nerves of the toes. For normalization of the data, values obtained in the operated hindlimb were expressed as percentage of values of the contralateral limb, tested at the same time for each animal.

Nociceptive behavioral testing

The rats were observed daily for behavioral signs indicative of pain, including signs of autotomy of the injured limb. The threshold responses to painful mechanical and thermal stimuli were evaluated on both hindpaws at 7, 14, 21 and 28 days after injury by means of algesimetry tests. Depending on the injury model different areas of the plantar surface of the hindpaw were tested: in the Crush group the lateral (normally innervated by tibial and sural nerves) and medial (normally innervated by tibial and saphenous nerves) regions, in the SNI the lateral region, and in the CCI the central area (innervated by the tibial nerve).

Painful mechanical sensibility was assessed by means of an electronic Von Frey algesimeter (Bioseb, Chaville, France). Rats were placed on a wire mesh platform in a plastic enclosure. Testing consisted on the application on the plantar surface of each hindpaw of a 0.8 mm diameter spring wire connected to a force sensor, and the pressure gradually increased until the rat withdrew its paw. Paw withdrawal pressure was recorded in triplicate, with 15 min interval between stimuli, for both hindpaws at each testing day, and the mean of the three values was used for calculating the percentage of the injured *vs* the contralateral intact hindpaw.