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Re-innervation patterns by peptidergic Substance-P, non-peptidergic P2X3, and myelinated NF-200 nerve fibers in epidermis and dermis of rats with neuropathic pain ☆

Liron S. Duraku [a,b,*], Mehdi Hossaini [a], Barthold N. Schüttenhelm [a,b], Joan C. Holstege [a], Martijn Baas [a,b], Tom J.H. Ruigrok [a], Erik T. Walbeehm [b]

[a] Department of Neuroscience, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
[b] Department of Plastic, Reconstructive and Hand Surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

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ABSTRACT

Nerve endings in the epidermis, termed nociceptors, conduct information on noxious stimuli to the central nervous system. The precise role of epidermal nerve fibers in neuropathic pain is however still controversial. Here, we have investigated the re-innervation patterns of epidermal and dermal nerve fibers in a rat neuropathic pain model. After applying the spared nerve injury (SNI) model, we determined the mechanical and thermal withdrawal thresholds in the uninjured lateral (sural) and medial (saphenous) areas of the affected hind paw and investigated the innervations patterns of Substance P (SubP), Neurofilament-200 (NF-200) and P2X3-immunoreactive (IR) nerve fibers in the epidermis and dermis. We found a significant loss in the density of peptidergic (Sub P and NF-200) and non-peptidergic (P2X3) nerve fibers in the center area of the foot sole at 2 weeks postoperatively (PO). The densities of Sub P-IR fibers in the epidermis and upper dermis, and the density of P2X3-IR fibers in the upper dermis were significantly increased at 10 weeks PO as compared to 2 weeks PO, but were still significantly lower than the densities in controls. However, the density of NF-200-IR fibers in the center area reached control levels at 10 weeks PO. No changes were found in the densities of any of the fibers in the medial and lateral parts of the foot sole. The present results suggest that after peripheral nerve injury, specific nerve fibers have different re-innervation patterns in the epidermis and dermis and that they might be involved in the development of neuropathic pain.

Introduction

There is increasing evidence for the involvement of the epidermis in the development and maintenance of neuropathic pain (Lauria et al., 2009, 2011; Oaklander and Siegel, 2005). Changes in density of nerve fibers in the epidermis are often used as a diagnostic tool in patients suffering from neuropathic pain (Hsieh, 2006). However, an unequivocal correlation between the density of epidermal nerve fibers and the severity of experienced neuropathic pain has not yet been established (Lauria and Devigili, 2007).

Epidermal nerve fibers are divided in slow conducting unmyelinated C-fibers and fast conducting, thinly myelinated A-δ, mainly for detecting noxious stimuli (Baron, 2006). Unmyelinated C-fibers are either peptidergic and contain the neuropeptides calcitonin gene-related peptide (CGRP) and/or substance P (Sub P) (Ruscheweyh et al., 2007) or are non-peptidergic and are identified by their expression of purinergic receptor P2X3 and RET (Taylor et al., 2009). A-δ fibers are exclusively peptidergic and contain CGRP but can be distinguished from unmyelinated CGRP fibers by their co-expression of the marker neurofilament 200 (NF-200).

Previously, we have shown that the density of CGRP-immunoreactive (IR) fibers in the epidermis adjacent to the innervation area of the lesioned fibers is increased and correlates with neuropathic pain behavior (Duraku et al., 2012). The re-innervation found in the epidermal innervation area of the injured and degenerated fibers originated from the adjacent uninjured nerve fibers. It has been shown before, using electrophysiological and behavioral studies, that after nerve transection, the adjacent skin innervated by undamaged nerve fibers becomes sensitized to mechanical and thermal stimuli (Ji et al., 2007, 2008; Wu et al., 2001, 2002). But by using the SNI model the delineation between the injured epidermal nerve fibers and the adjacent uninjured nerve fibers was very clear and the uninjured fibers are likely to play a considerable role in the development of neuropathic pain in animal models (Campbell and Meyer, 2006).

Other epidermal cells involved in the pathogenesis of neuropathic pain are the epidermal Langerhans cells (LCs) (Lindenlaub and

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* Corresponding author at: Department of Plastic, Reconstructive and Hand Surgery, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands, Room EE.15.91. Fax: +31 10 7044685.
E-mail address: l.duraku@erasmusmc.nl (L.S. Duraku).

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Sommer, 2002). These bone marrow-derived cells are important in presenting antigens to the T- and B-cells of the immune system (Boulais and Misery, 2008). LCs produce the axonal marker protein gene product 9.5 (PGP9.5), and become intensely immunoreactive to PGP9.5 after peripheral nerve injury, possibly due to a rise in PGP9.5 synthesis (Lauria et al., 2005). Increased numbers of PGP9.5-IR LCs in epidermis have been found in patients with neuropathic pain (Calder et al., 1998; Rodriguez and Villamizar, 1992; Springall et al., 1991) and in neuropathic pain animal models (Ma and Eisenach, 2002; Siau et al., 2006). However, the distribution pattern of LCs in the epidermal innervation areas of the injured and uninjured nerve fibers, which might contribute to neuropathic pain behavior, is not known.

In the present study, we describe the normal and pathological changes in distribution of sensory subgroups of epidermal and dermal nerve fibers (Substance P, P2X3 and NF-200) and of epidermal Langerhans cells, after various post-lesion time intervals.

Methods

Animals

In the present study we used a total of 32 male Wistar rats weighing between 250 and 300 g. All experiments were approved by the Dutch Ethical Committee on Animal Welfare (DEC) and all procedures were in adherence to the European guidelines for the care and use of laboratory animals (Council Directive 86/6009/EEC).

The spared nerve injury (SNI) model

The SNI procedure was applied as described previously (see Decosterd and Woolf). In short, the three branches of the sciatic nerve i.e. the sural, common peroneal and tibial nerves were exposed under general anesthesia (2% isoflurane). The tibial and common peroneal nerves were ligated together with 5.0 silk, and cut approximately 2 mm distal to the ligation while leaving the sural nerve intact (Fig. 1). The muscles and skin were closed using a 3.0 silk. In the sham-treated rats the sciatic branches were only exposed. This provides a model with clear delineation between innervated and denervated areas. Furthermore, since the ligation suture around the proximal stump has prevented regeneration, all fibers found in the central denervated area have to originate from the fibers of the uninjured saphenous and sural nerves.

Mechanical withdrawal threshold

The mechanical thresholds of the affected hind paws were measured using Von Frey monofilaments, ranging from 0.6 to 300 g in a set of 14 filaments, at 2, 5 and 10 weeks postoperatively (PO). In this experiment, the rats were placed in a plastic box with a mesh floor in which they were able to move freely. For determining the withdrawal threshold, we started with the thinnest monofilament for producing the lowest force and increased the applied force in gradual steps. Each Von Frey hair was applied for 2 s at 5 s intervals, and the response threshold was set at 3 responses, in a set of maximum of 5 applications. The withdrawal thresholds were determined in the medial and lateral sides of the foot sole at the transition points from glabrous to hairy skin, and in the center of the foot sole at the plantar midpoint.

Hot and cold plate test

The hot and cold plate tests were performed on an aluminum plate (21×21 cm) encaged by see-through Plexiglas. The aluminum plate contained spiraling channels to provide a quick and homogenous adjustment of the plate temperatures. These channels are filled with water that is regulated by a water bath (HAAKE K20), with a temperature range from 0 to 50 °C, a maximum pressure of 300 mbar and a maximum flow rate of 12.5 L/min. The water bath is connected with PVC tubes to the channels in the aluminium plate. The plate temperatures were measured with thermocouples (J-type: Fe/Cu-Ni) at the center of each plate. The plate temperatures corresponded with the water bath temperatures with a maximum discrepancy of ± 0.5 °C. The rats were placed on the hot or cold plates, and subsequently the paw withdrawal latency, defined as latency starting from the first contact of the hind paw with the plate until a positive response, was measured. A positive response was defined as a quick flutter or flinch of the affected hind paw.

Tissue preparation

After survival periods of 2, 5 and 10 weeks, the animals received an overdose of pentobarbital (100 mg/kg) and immediately thereafter the glabrous skin of the affected hind limb was dissected in a distal to proximal strip of tissue. The dissected skin was immersion-fixed in 2% paraformaldehyde-lysine-periodate (PLP) for 24 h at 4 °C, and were thereafter embedded in gelatin blocks together with the

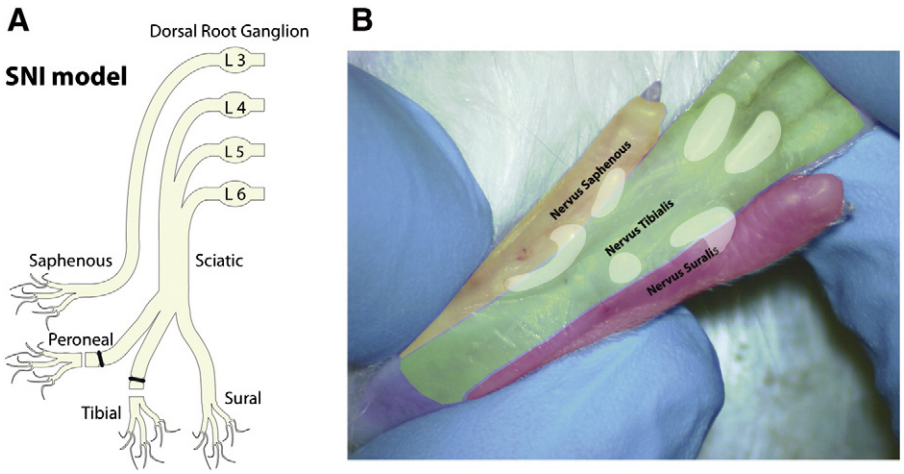

Fig. 1. SNI model. Drawing and micrograph illustrating the spared nerve injury (SNI) model procedure. In this experiment, the tibial and common peroneal branches of the sciatic nerve were ligated and transected, while the sural branch, and the saphenous nerve, respectively at the medial and lateral side of the foot sole, were left intact (A). This method results in complete denervation of the tibial nerve area (center), without affecting the medial and lateral sides of the hind paw glabrous skin (B). The highlighted six areas on the foot sole represent the footpads (B).