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Effects of decompression on neuropathic pain behaviors and skin reinnervation in chronic constriction injury

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Abstract

Decompression is an important therapeutic strategy to relieve neuropathic pain clinically; there is, however, lack of animal models to study its temporal course of neuropathic pain behaviors and its influence on nerve regeneration to sensory targets. To address these issues, we established a model of decompression on rats with chronic constriction injury (CCI) and investigated the effect on skin reinnervation. Animals were divided into a decompression group, in which the ligatures were removed, and a CCI group, in which the ligatures remained at postoperative week 4 (POW 4). At this time point, the skin innervation indexes of protein gene product 9.5 (PGP 9.5), substance P (SP), and calcitonin gene-related peptide (CGRP) were reduced in both groups to similar degrees. Beginning from POW 6, the decompression group exhibited significant reductions of thermal hyperalgesia and mechanical allodynia compared to the CCI group ($p<0.001$). At POW 8, neuropathic pain behaviors had completely disappeared in the decompression group, and the decompression group had a higher skin innervation index of SP than the CCI group (0.45 ± 0.05 vs. 0.16 ± 0.03, $p<0.001$). These indexes were similar in both groups for PGP 9.5 (0.32 ± 0.09 vs. 0.14 ± 0.04, $p=0.11$) and CGRP (0.38 ± 0.06 vs. 0.21 ± 0.07, $p=0.09$). These findings demonstrate the temporal changes in the disappearance of neuropathic pain behaviors after decompression and suggest that decompression causes different patterns of skin reinnervation for different markers of skin innervation.

Keywords: Nerve injury; Skin innervation; Decompression; Substance P; Neuropathic pain; Nerve regeneration; Calcitonin gene-related peptide; Neuropeptides; Painful neuropathy; Chronic constriction injury

Introduction

Nerve injury from compression is the foundation for establishing animal models of neuropathic pain, including chronic constriction injury (CCI), partial sciatic nerve ligation, and spinal nerve ligation (Bennett and Xie, 1988; Kim and Chung, 1992; Seltzer et al., 1990). Surgical decompression is frequently used in clinical practice to relieve symptoms of neuropathic pain, e.g., carpal tunnel syndrome (Steinberg, 2002; Thoma et al., 2004). Theoretically, several potential mechanisms underlie the disappearance or reduction of neuropathic pain after surgical decompression; these include regeneration of nerve fibers to reestablish contacts with the targets of cutaneous nerves, changes in the local environment of the previously injured nerves, and synaptic reorganization in the central nervous system, particularly, the dorsal horn of the spinal cord (George et al., 2000; Suzuki and Dickenson, 2005; Woolf et al., 1998; Woolf, 2000; Woolf, 2004; Woolf and Salter, 2006).

The assessment of skin innervation by examining sensory nerve terminals in the epidermis is a well-established approach for investigating the integrity of nociceptive nerves in both humans and animals (Hsieh et al., 2000; Kennedy, 2004; McCarthy et al., 1995). Several groups including our own have previously demonstrated that partial denervation of the skin in the territory of the injured nerve is a prerequisite for establishing animal models of neuropathic pain and painful neuropathies in humans (Chiang et al., 2005; Lin et al., 2001; Lindenlaub and Sommer, 2002; Ma and Bisby, 2000; Periquet et al., 1999). Traditionally, skin innervation is evaluated by immunohisto-

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chemistry with a general neuronal marker, protein gene product 9.5 (PGP 9.5) (Kennedy, 2004; McCarthy et al., 1995). Some epidermal nerves are also immunoreactive for sensory peptides: calcitonin gene-related peptide (CGRP) and substance P (SP) (Chiang et al., 2005; Ma and Bisby, 2000). Epidermal nerves of PGP 9.5 are the most abundant ones compared with those of other phenotypes (Chiang et al., 2005). It is not clear whether the reduction of epidermal nerves is different among different markers in neuropathic pain. These findings also raise several issues regarding the relationship between neuropathic pain and skin reinnervation. For example, does the epidermis become fully reinnervated upon decompression, does the skin reinnervation parallel the disappearance of neuropathic pain, are the patterns of skin reinnervation similar for epidermal nerves of different phenotypes after decompression, and which phenotype of epidermal nerves reflects the states of neuropathic pain?

To address these issues, we established a model of decompression by removing all ligatures of CCI 4 weeks after neuropathic pain behaviors had well developed. Specifically, we investigated the temporal changes in neuropathic pain behaviors and the pattern of skin reinnervation after decompression.

Materials and methods

Study design and surgical procedures

Adult male Sprague–Dawley rats, weighing 250–300 g, were used in these experiments. Three animals were housed together in plastic cages with soft sawdust as bedding to avoid mechanical damage to the hindpaw skin. These animals were placed in a temperature- and humidity-controlled room with a 12-h light/dark cycle. Food and water were available *ad libitum.* All procedures were conducted in accordance with ethical guidelines set up by the International Association for the Study of Pain (IASP) on the use of laboratory animals in experimental research (IASP Committee, 1980; Zimmermann, 1983).

CCI was induced in animals following established surgical procedures (Bennett and Xie, 1988; Lin et al., 2001). Briefly, under chloral hydrate anesthesia (400 mg/kg, i.p., Sigma, St. Louis, MO), the right sciatic nerve was exposed at the mid-thigh level by freeing the adhering fascia between the gluteus and biceps femoris muscles. Four ligatures (of 4/0 chromic gut) were loosely tied around the sciatic nerve at 1-mm intervals above the nerve's trifurcation. Ligatures constricted only about 1/3∼1/4 of the diameter of the nerve and produced a small, brief twitch in the muscle around the exposure. The circulation through the superficial epineural vasculature was blocked between the ligatures. This side was defined as the operated side; the contralateral side was used for comparison to normalize individual variations of different animals.

To examine the effect of decompression on neuropathic pain and skin reinnervation, animals were randomly assigned to two groups. In one group, all four ligatures were carefully removed without destroying the surrounding vessels at postoperative week 4 (POW 4); this group was designated the decompression group hereafter. The other group was designated the CCI group, in which ligatures remained throughout the experimental period. Examiners were blinded to the grouping information, and this information was only decoded during the final analyses.

Thermal hyperalgesia

We evaluated thermal hyperalgesia with a Hargreaves-type analgesiometer (Ugo Basile, Comerio-Varese, Italy) by measuring the paw withdrawal latency upon heat stimulation. Rats were individually placed in one of three separate Plexiglas containers (22 × 17 × 14 cm) located on an elevated floor of a clear glass plate (3 mm thick) and allowed 30 min to habituate to the apparatus. A radiant heat source was placed directly beneath the plantar surface of the hindpaw. The withdrawal latency was automatically measured as the time elapsed from the onset of radiant heat stimulation to the withdrawal of the hindpaw. A maximal time of 20 s for the thermal stimulus was imposed to avoid possible tissue damage. Each hindpaw was alternatively tested seven times with a minimal interval of 5 min between measurements, and readings were recorded to the nearest 0.1 s. Values of the last five consecutive measurements were used for the analysis (Chiang et al., 2005).

Mechanical allodynia

Mechanosensitivity was determined by measuring the withdrawal thresholds to a series of calibrated von Frey filaments (Somedic, Sweden) according to the up-and-down method (Chiang et al., 2005). Rats were individually placed in one of three separate Plexiglas containers on a wire mesh floor and allowed to acclimate for 10 min. The examiner touched the plantar surface of the hindpaw with a filament until a brisk withdrawal or paw flinching was noted, which was considered a positive response. Five stimuli using the selected hair were applied at 5-s intervals. If there was no withdrawal response to the initially selected hair with these five stimuli, a stronger stimulus was applied. If the animal withdrew its hindpaw in response to any of the five stimuli, the next weaker stimulus was chosen. The mechanical threshold was defined as the minimal force (g) initiating a withdrawal response.

Immunohistochemistry of footpads

Animals were sacrificed by an intracardiac perfusion of 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at pH 7.4. Footpads were fixed for another 6 h and then changed to 0.1 M PB for storage. After a thorough rinsing in PB, samples were cryoprotected with 30% sucrose in 0.1 M PB overnight. Sections of 30 μm perpendicular to the dermis were cut on a sliding microtome, labeled sequentially, and stored at −20 °C. To ensure adequate and systematic sampling, every fourth section of the skin was immunostained (Lin et al., 2001). Sections were treated with 0.5% Triton X-100 in 0.5 M Tris buffer (Tris), pH 7.6, for 30 min and processed for immunostaining. Briefly, sections were quenched with 1% H_2O_2 in methanol and blocked with 5% normal goat serum in 0.5% nonfat dry milk/Tris. Sections were incubated with primary antiserum overnight. These included protein gene product 9.5 (PGP 9.5, 1:1000, UltraClone, Isle of