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Sympathetic Fiber Sprouting in Chronically Compressed Dorsal Root Ganglia Without Peripheral Axotomy

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Abstract

Sympathetic axonal sprouting in axotomized dorsal root ganglia (DRG) has been shown to be a major phenomenon implicated in neuropathic pain. However, it is not known whether sympathetic sprouting can occur in pathologic ganglia without peripheral axotomy. We thus examined presence and density of sympathetic axonal sprouting within DRG of rats subjected to a persistent compressive injury by inserting a stainless steel metal rod into L₄ and L₅ lumbar intervertebral foramen. Sympathetic axons were identified by immunohistochemical staining with anti-tyrosine hydroxylase antibodies. Results indicate that progressive increase in sympathetic axonal sprouting occurred in the bilateral DRGs between postoperative days 2 and 28. The sympathetic fiber density was greater on the lesion side than the contralateral side. In conclusion, chronic compressive injury of the DRG results in sympathetic sprouting in the non-axotomized ganglion and may partially contribute to the development and maintenance of certain pathological pain states.

Keywords

Dorsal root ganglion; neuropathic pain; sympathetic sprouting

INTRODUCTION

Clinical observations and animal studies have shown that coupling of the activated sympathetic nervous system and the sensitized sensory nervous system is important for development of certain neuropathic pain states.^{6,7} Under normal physiological conditions, the afferent sensory nervous system and the efferent sympathetic nervous system are anatomically separated.⁷ There is evidence that an abnormally enhanced communication between these two systems can occur under a variety of neuropathies such as peripheral nerve injury. Additionally, chemical or surgical sympathectomy relieves allodynia and hyperalgesia, and improves chronic pain behavior^{2,9,10,16,20} in certain patients with sympathetically maintained pain. These observations suggest that increased activity of the sympathetic nervous system may play an important role in sensitization of sensory neurons towards development of neuropathic pain.

The dorsal root ganglion (DRG) has been identified as an important site for peripheral sympathetic-sensory coupling.¹⁷ Within the normal DRG, sympathetic axons are only found accompanying blood vessels.¹¹ Following peripheral nerve injury, sympathetic efferent fibers sprout extensively into both DRG and spinal nerves. Sprouting fibers sometime form

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distinctive tyrosine hydroxylase immunoreactive (TH-IR) basket-like webs (sympathetic basket) or rings wrapping preferentially around medium and large-sized neurons.^{15,12,17,18} Interestingly, sympathetic sprouting not only occurs in the axotomized DRG ipsilateral to the injury, but also develops in the intact DRGs both ipsilateral and contralateral to the injury although the sprouting is less extensive than that in axotomized DRG.^{12,17,19}

In this study, we sought to determine if sympathetic sprouting can be triggered in pathologic DRG without peripheral axotomy. We employed chronic compression of the DRG (CCD) model of neuropathic pain that has been shown to exhibit prolonged cutaneous hypersensitivity to mechanical and thermal stimuli.²¹ Unlike other animal models of neuropathic pain, in the CCD model, an intact peripheral innervation has been reserved.²¹ Using this model, we examined the presence and changes of enhanced TH-IR fibers in the DRG after a compressive injury.

METHODS

Nineteen male Sprague-Dawley rats weighing 150–300 g were housed in groups of 3 in plastic cages with soft bedding for at least 5 days before surgery and up to 28 days after surgery. All the surgical procedures were reviewed and approved by Institutional Animal Care and Use Committee (IACUC).

The surgical procedure for CCD has been described previously.²¹ Briefly, after induction of general anesthesia with intraperitoneal pentobarbital sodium (40 mg/kg) and separation of right paraspinal muscle from L₅ and L₆ transverse processes, the L₄–L₅ and L₅–L₆ intervertebral foramina were exposed. An L-shaped rod made of stainless steel (3.5 × 2 mm in length and 0.6 mm in diameter) was carefully inserted into each foramen at an angle of 30° to the midline.

Rats were sacrificed on different days after surgery and fixed by perfusing 200–300 ml of Zamboni's fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH = 7.4) through the left ventricle of heart. The bilateral L₄ and L₅ DRGs were removed, post-fixed in perfusion fixative for 1 hour, and kept in 15% sucrose at 4°C overnight. The ganglia were horizontally sectioned with a Cryostat at a thickness of 30 μm.

Tissue sections were incubated in antibodies to TH (raised from rabbit; obtained from Pel-Freez, Rogers, AR) at a dilution of 1:1,000 for 48 hours at 4°C, followed by reaction with biotinylated secondary antibody and, finally, with VectorABC reagent. Triton-X (0.3%) was used in all reaction solutions to enhance antibody penetration. Immunoreaction products were visualized by the diaminobenzidine method in the presence of H₂O₂ in 0.1 M phosphate buffer. Tissues were then mounted on gelatin-coated slides, air dried, dehydrated, and coverslipped for light-microscopic observation.

The numerical density of TH-IR fibers was estimated by measuring the length of TH-IR fibers within one-third of the 18–35 serial sections from each DRG sampled systemically with a random start (e.g., every 3rd section starting from section 2). Images from selected sections were captured under a light microscope (20x), equipped with a colored digital camera, and stored in a Pentium IV computer for measurements. The length of all TH-IR fibers in the cellular zone of the images was measured using Scion Image Analysis software (Scion Corporation, Frederick, MD). The numerical density of the TH-IR fibers within each DRG was obtained by dividing the total fiber length by the size of the measured area (area in mm²).

RESULTS

Immunohistochemical staining was performed on bilateral L₄ and L₅ DRGs in 3 health control rats without any surgery and 19 CCD rats at different times after surgery (day 2: n = 2, days

8, 15, 22, and 28; n = 3 for each day). In DRGs from normal rats, scattered “dark” cells with TH-IR axons were found throughout the section (Figure 1A, 1B). These dark cells represent a subpopulation of dopaminergic DRG neurons.⁵ TH-IR-positive sympathetic fibers formed varicose plexuses around vascular processes (Figure 1B), as reported previously.¹⁷

In CCD rats, extensive sympathetic axonal sprouting was found bilaterally in both L₄ and L₅ DRGs (Figure 1C). Sprouting fibers distributed in entire DRG in both fiber tracts and cellular region with more sprouting at fiber region. The sprouting axons were frequently extensions of TH-IR fibers from the tracts or perivascular TH-IR plexuses. Some neurons were surrounded by TH-IR fibers and formed distinct basket-like structures (Figure 1D), as previously reported in DRGs with peripheral axotomy. Sympathetic sprouting was progressive on both sides from postoperative day 2 to day 28. The density of TH-IR sympathetic fibers reached its maximum by day 14 postoperatively and maintained at a similar level through day 28 (Figure 2). TH-IR sprouting in the contralateral DRG followed a similar time course as the ipsilateral ones, but the fiber density was lower compared to the ipsilateral DRG.

DISCUSSION

This study demonstrated that chronic compression of DRG caused extensive sprouting of noradrenergic sympathetic fibers in the DRGs ipsilateral and, to a lesser degree, contralateral to the injury. Sympathetic sprouting in the DRG has been demonstrated in all animal models with partial or complete peripheral axotomy. Sympathetic sprouting is detected within 4 days after a loose ligation of the sciatic nerve^{12,18} and 5–7 days following a partial tight ligation of the sciatic nerve. In rats with spinal nerve ligation, sympathetic sprouting was observed as early as 2 days after nerve injury.^{4,5,18} The onset of sympathetic fiber sprouting was slower, typically 1–2 weeks after surgery, in rats with a complete transection of the sciatic nerve.^{17,19} In the present study, for the first time, sympathetic sprouting is demonstrated in compressed DRGs with intact peripheral innervation.

The mechanism for sympathetic sprouting in the DRG is not fully understood. Evidence suggests that nerve growth factor (NGF) and its homologue (e.g., neurotrophin-3 [NT-3]) are contributing factors for sympathetic sprouting. After nerve injury, NGF protein levels and NGF-IR in the DRG are dramatically increased as early as 2 days after surgery,¹³ which is parallel to the occurrence of sympathetic sprouting. A recent study found that NGF and NT-3 synthesis is up-regulated in glial cells surrounding neurons in axotomized DRG. Sympathetic sprouting around the axotomized neurons was associated with p75-IR glial cells.²³ These results implicate glial-cell-derived neurotrophins in the induction of sympathetic sprouting. Other possible triggering factors for sympathetic sprouting include inflammatory cytokines, such as interleukin-6, tumor necrosis factor- α , and leukemia inhibitory factor.^{14,19}

Sympathetic sprouting has been observed previously in intact ganglia ipsilateral and contralateral to the injury in rats with peripheral nerve injury,³ and is confirmed in compressed DRGs without axotomy in the present study. This has led us to believe that axotomy or nerve injury may not be the only factors that trigger sympathetic sprouting. As observed in our present study, which is consistent with previous reports, sprouting sympathetic fibers forms basket structures preferentially around large- and medium-sized neurons in pathologic DRGs. These large- and medium-sized neurons often present with high-frequency abnormal discharges as demonstrated in virtually all neuropathic animal models.^{1,22} Further, it is rare to find basket formation in association with small-sized neurons, which often exhibit low-incidence and low-frequency, if any, discharges. Therefore, it is possible that sympathetic sprouting is related to high-frequency discharge-induced neurotrophins releases in the neuropathic DRGs.¹⁵

There are conflicting reports with respect to the role of sympathetic sprouting in the development of neuropathic pain. Chung et al. demonstrated that sympathectomy performed shortly after surgical ligation of L₅ and L₆ spinal nerves abolished sympathetic sprouting and neuropathic pain behaviors.⁵ However, lack of correlation between sympathetic sprouting and neuropathic behaviors has also been reported in animal model with spinal nerve injury at S₁ and S₂ levels.⁸ It is likely that sympathetic sprouting may partially contribute to the development of neuropathic behavior in CCD rats.

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FIGURE 1.

FIGURE 2.