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Research Report

Redistribution of voltage-gated sodium channels after nerve decompression contributes to relieve neuropathic pain in chronic constriction injury

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ABSTRACT

Nerve decompression is an important therapeutic strategy to relieve neuropathic pain and promote the peripheral nerve regeneration. To address these issues, we investigated the effects of nerve decompression on relief of neuropathic pain behaviors, redistribution of voltage-gated sodium channels (VGSCs), and skin reinnervation with chronic constriction injury (CCI). At post-operative week (POW) 4, animals were divided into a decompression group, in which the ligatures were removed, and a CCI group, in which the ligatures remained. Thermal hyperalgesia and mechanical allodynia at POW 8 had distinct reductions in decompression group compared to CCI group. At that time in CCI group, morphological evidence of pan VGSCs (Pan Nav) and isoforms of VGSCs (Nav1.6, Nav1.9, except for Nav1.8) were shown the widely distribution along the injured sciatic nerve. All of the VGSCs in decompression group became clustering around the node of Ranvier, similar to the pattern of control sciatic nerve at POW 8. Skin reinnervation was demonstrated by epidermal nerve density (END) for protein gene product 9.5 (PGP 9.5)-immunoreactive (IR) nerve fibers and a significant difference between groups only at POW 24 ($p=0.01$). Growth-associated protein 43 (GAP-43) is participated in the nerve fiber growth and sprouting, a difference in END for GAP-43-IR nerve fibers at POW 24 between groups were also significant ($p=0.02$). These observations demonstrated that nerve decompression was accompanied with the disappearance of neuropathic pain behaviors after CCI. Morphological studies provided the evidence that redistribution of VGSCs along the injured sciatic nerve but still with an incomplete skin reinnervation. These significant findings

Abbreviations: VGSCs, Voltage-gated sodium channels; CCI, chronic constriction injury; POW, post-operative week; END, epidermal nerve density; PGP 9.5, protein gene product 9.5; GAP-43, growth-associated protein 43; IR, immunoreactive; DRG, dorsal root ganglion

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demonstrated a role of VGSCs in the pathogenesis of neuropathic pain, and gave an approaching in pharmacological basis of therapeutics.

1. Introduction

Nerve injury by compression is a major principle for establishing animal models of neuropathic pain, including chronic constriction injury (CCI), partial sciatic nerve ligation (PSNL), and spinal nerve ligation (SNL) (Bennett and Xie, 1988; Seltzer et al., 1990; Kim and Chung, 1992). Surgical decompression is frequently used in clinical strategy to reduce symptoms of neuropathic pain, for example, carpal tunnel syndrome and spinal root compression (Steinberg, 2002; Thoma et al., 2004; Binder et al., 2002). Theoretically, two potential mechanisms after nerve decompression cause the disappearance or reduction of neuropathic pain behaviors, *i.e.*, changes in the distribution of ion channels along the injured sciatic nerve and regeneration of nerve fibers to reestablish contacts with the targets in the epidermis. There are, however, limited references which have provided direct evidence in these mechanisms.

Previous studies have elucidated the normal distribution of voltage-gated sodium channels (VGSCs) in dorsal root ganglia (DRG) neurons and axons from peripheral nociceptive receptors to the central nerve terminals (Dib-Hajj et al., 2010; Black et al., 2012). At restricted regions of myelinated axons, such as at the node of Ranvier, are expressed VGSCs and important in initiating the action potential to establish the nerve conduction velocity (Levinson et al., 2012; Wang et al., 2011). Recently, gene expression of VGSCs in DRG neurons has focused on the acute period of neuropathic pain after peripheral nerve injury (Devor, 2009; Waxman, 2006; Kim et al., 2001). Transcripts for different VGSCs in neuropathic pain are either upregulated or downregulated in DRG neurons (Dib-Hajj et al., 2009; Sleeper et al., 2000). The aggregated expression of VGSCs in neuromas has further been implicated as a source of ectopic discharges (Devor, 2006; Coward et al., 2000; Kretschmer et al., 2002). Thus, whether the morphological distribution of VGSCs correlates with neuropathic pain behaviors in CCI remains elusive, particularly after nerve decompression.

Skin innervation is a well-established approach for investigating nerve integrity at the terminal part of axons in the epidermis. Several studies including our laboratory data have demonstrated that partial denervation in the territory of skin is a requirement for establishing animal models of inflammatory and neuropathic pain (Hsieh et al., 2012; Tseng et al., 2007; Ma and Bisby, 2000). Moreover, there is indirect evidence implying that the previously denervated epidermis is not fully reinnervated in long-term period of CCI (Lindenlaub and Sommer, 2002). These findings raise several issues regarding the relationship on the temporal course between nerve fiber regeneration and neuropathic pain behaviors. For example, whether all the epidermal nerve fibers regenerate upon the nerve decompression and the skin reinnervation is corresponding to the disappearance of neuropathic pain behaviors.

In the current study, we produced CCI by applying 4 loose ligatures to compress the sciatic nerve of animals; all these ligatures were removed 4 weeks later, after neuropathic pain behaviors had been established. Notably, we indicated that the nerve decompression speeded up disappearance of neuropathic pain behaviors. And the morphological evidence further provided the redistribution of VGSCs along the injured sciatic nerve and still an incomplete skin reinnervation. These observations demonstrated the important distribution of VGSCs in the pathogenesis of neuropathic pain, and gave an approaching in pharmacological basis of therapeutics.

2. Results

Animals developed typical neuropathic pain behaviors within 2 week after CCI in CCI and decompression groups, such as everting and clenching the hind paw on the operated hind limb, and sudden licking of the operated hind paw. After stimulation, the operated hind limb was held away from the floor for a longer period of time than the control hind limb.

2.1. *Thermal hyperalgesia*

These neuropathic pain behaviors in both groups accompanied similar degrees of thermal hyperalgesia until postoperative week (POW) 4 (Fig. 1A). Before CCI, both groups had similar withdrawal latencies to the simulation of noxious radian heat on the operative side (9.13±0.77 s in decompression group, 9.24±0.78 s in CCI group, $p=0.61$). Between POW 2 and POW 4, both groups showed significant reductions of withdrawal latency, for example, 6.56±1.05 s *vs.* 6.74±0.65 s, $p=0.57$ at POW 4. The ligatures were removed at POW 4 in decompression group after a behavioral test was conducted. At POW 8, the withdrawal latencies became normalized in decompression group compared to CCI group (8.99±0.91 s *vs.* 7.24±0.62 s, $p<0.05$). The withdrawal latencies had also been returned to normal level in CCI group at POW 12 (8.95±0.46 s *vs.* 9.16±0.96 s in decompression group, $p=0.53$) and persisted through the end of experiments at POW 24 (9.11±0.61 s *vs.* 9.28±1.20 s in decompression group, $p=0.79$). There were no significant temporal changes on the control side in decompression group ($p=0.93$) and CCI group ($p=0.82$) through the entire experimental period.

2.2. *Mechanical allodynia*

Mechanical allodynia were measured as the mechanical thresholds and also disappeared after decompression with similar temporal changes as those for thermal hyperalgesia