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ScienceDirect
Experimental Neurology 206 (2007) 17–23
Experimental Neurology
www.elsevier.com/locate/yexnr

Reversal of ERK activation in the dorsal horn after decompression in chronic constriction injury

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Received 1 February 2007; revised 28 March 2007; accepted 13 April 2007
Available online 4 May 2007

Abstract

Injury-induced neuropathic pain is related to changes in the central terminals of dorsal root ganglia neurons, i.e., dorsal horn plasticity. We investigated the influences of decompression by removing ligatures producing chronic constriction injury (CCI) in Sprague–Dawley rats at postoperative week (POW) 4, the decompression group; for comparison, all ligatures remained through the experimental period in the CCI group. The effect was evaluated with extracellular signal-regulated kinase (ERK) activation in the dorsal horn, i.e., number of phosphorylated ERK (+) cells in the dorsal horn. At POW 1, the dorsal horn indexes had increased to a similar degree in both groups (2.40 ± 0.58 vs. 2.27 ± 0.36, $p = 0.73$). At POW 8, thermal hyperalgesia and mechanical allodynia had completely disappeared with a normalization of dorsal horn index (1.17 ± 0.11 vs. 1.02 ± 0.12 at POW 0, $p = 0.07$) in the decompression group; in contrast, the dorsal horn index remained elevated in the CCI group (2.48 ± 0.30, $p < 0.001$) with persistent neuropathic pain behaviors at POW 8. This report suggests that ERK activation in the dorsal horn is correlated with neuropathic pain behaviors and its normalization reflects the reversal of neuropathic pain behaviors after decompression.

Keywords: Nerve injury; Surgical decompression; Neuropathic pain; Painful neuropathy; Chronic constriction injury; Dorsal horn; Extracellular signal-regulated kinase

Introduction

The dorsal horn is the first relay center which processes sensory and nociceptive information, and is a potential site of central sensitization in generating neuropathic pain behaviors (Balasubramanyan et al., 2006; Urban et al., 2003; Urban and Gebhart, 1999). For example, chronic constriction injury (CCI) increases the excitatory postsynaptic currents in dorsal horn neurons, which provides a foundation for central hypersensitivity after CCI (Balasubramanyan et al., 2006). In addition to physiological changes in dorsal horn neurons, molecular expression profiles also change in dorsal horn neurons after noxious stimulation or injury-induced neuropathic pain, in particular, the phosphorylation of extracellular signal-regulated kinase (ERK). The expression of ERK was upregulated in the dorsal horn after CCI as analyzed by subtractive hybridization (Ciruela et al., 2003). The expression of the phosphorylated ERK (pERK) in the dorsal horn has been implicated in the generation of neuropathic pain behaviors after CCI and inflammatory pain models (Song et al., 2005). These findings provide molecular mechanisms underlying excitatory physiological changes, and raise an intriguing question: do these molecular profiles parallel neuropathic pain behaviors?

Surgical decompression is an important procedure for relieving neuropathic pain and resuming neural clinical functions (Ducic et al., 2006; Huisstede et al., 2006; Siemionow et al., 2006; Steinberg, 2002; Thoma et al., 2004). Several mechanisms may underlie the attenuation of neuropathic pain by surgical decompression, including a reduction of central sensitization particularly in the dorsal horn and reinnervation of injured nerves and their sensory targets. Our previous study indicated that decompression by removing the ligatures of CCI speeded up the alleviation of neuropathic pain behaviors but

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0014-4886/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.expneurol.2007.04.006

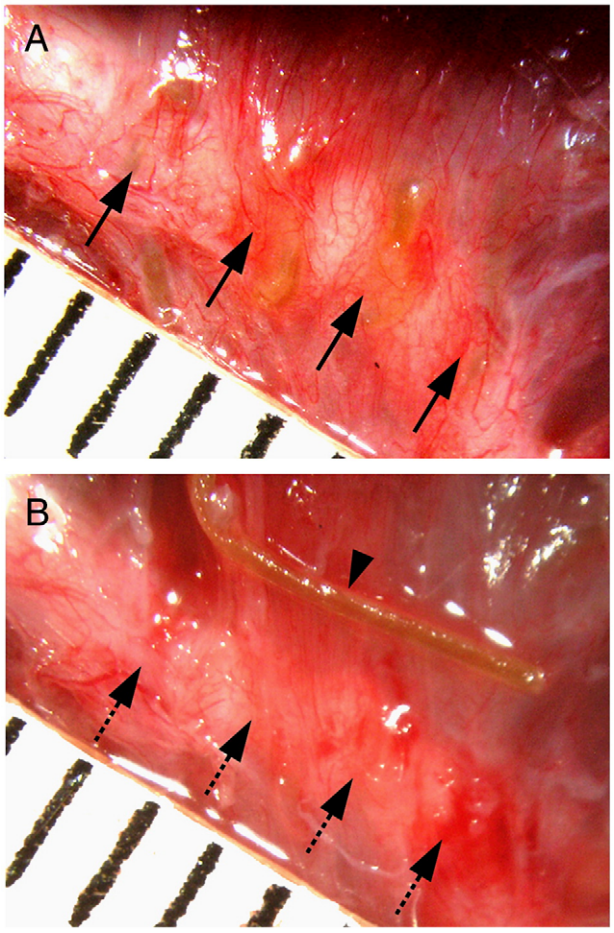

Fig. 1. Ligatures in chronic constriction injury. (A) At postoperative week (POW) 4, ligatures (yellow in color, arrows) can still be recognized beneath the fibrous tissue surrounding the sciatic nerve. (B) After careful untying of the ligatures under a dissecting microscope, the sciatic nerve remains intact with indentations (dot-tailed arrows) indicating the previous sites of ligations. A removed ligature (arrowhead) is placed near the sciatic nerve.

with limited reinnervation (Tseng et al., 2007). These findings raise the possibility on whether the changes in ERK activation of the dorsal horn may reflect the neuropathic pain behaviors. Specifically, we asked whether ERK activation was correlated with neuropathic pain behaviors in long-term CCI and whether decompression can reverse the above changes.

Materials and methods

Animals and surgery

Adult male Sprague–Dawley rats, weighing 250–300 g, were used in this study. 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), and the protocol was approved by the Animal Committee of National Taiwan University College of Medicine, Taipei, Taiwan.

CCI was performed in animals following established surgical procedures (Bennett and Xie, 1988; Lin et al., 2001). Briefly, under intraperitoneal chloral hydrate anesthesia (400 mg/kg, Sigma, St. Louis, MO), the right sciatic nerve was exposed at the mid-thigh level and four ligatures (of 4/0 chromic gut) were loosely tied around the sciatic nerve at 1-mm intervals proximal to the sciatic nerve's trifurcation. This side was defined as the operated side, with the contralateral side being used for comparison to normalize individual variations of different animals.

To examine the effect of decompression, animals were randomly assigned to two groups. In the decompression group, all four ligatures were carefully removed without destroying the surrounding vessels at postoperative week (POW) 4; the other group was designated the CCI group, with ligatures remaining throughout the entire experimental period. We chose this time point for two considerations: (1) both neuropathic pain behaviors and skin denervation were well established from previous studies (Lin et al., 2001; Tseng et al., 2007) and (2) the effect of decompression on the dorsal horn plasticity could be compared with that on the skin denervation at same time points. At POW 4, ligatures could still be visible although reactive fibrosis became prominent (Fig. 1A); under dissecting microscope, ligatures could be untied without destroying the surrounding tissues (Fig. 1B). Examiners were blinded to the grouping information, and this information was only decoded during the final analyses.

Thermal hyperalgesia

Thermal thresholds defined as the paw withdrawal latencies upon heat stimulation were measured with a Hargreaves-type analgesiometer (Ugo Basile, Comerio-Varese, Italy). Animals were individually placed in one of three separate Plexiglas containers (22 × 17 × 14 cm) and allowed 30 min to habituate to the apparatus. A radiant heat source was placed directly beneath the plantar surface of the hindpaw, and the withdrawal latency was automatically measured as the time elapsed from the onset of radiant heat stimulation to the withdrawal of the hindpaw. Each hindpaw was alternatively tested seven times with a minimal interval of 5 min between measurements, and each reading was recorded to the nearest 0.1 s with the mean of the last five consecutive measurements used for the analysis. Based on a preliminary analysis, a difference in the mean withdrawal latencies (the operated side minus the contralateral side) of less than 2 s was defined as thermal hyperalgesia (Chiang et al., 2005).

Fig. 2. Activation of extracellular signal-regulated kinase (ERK) in the dorsal horn after chronic constriction injury. Spinal cords at lumbar segments 4 and 5 were immunostained with the phosphorylated form of ERK (pERK) from postoperative week (POW) 1. The graphs show representative changes in the dorsal horn on the contralateral side (A, C) and operated side (B, D) at POW 1 (A, B), POW 12 (C, D). The above changes were quantitatively analyzed with pERK(+) cell density (E). (A, C, A′, C′) On the contralateral side, there are constitutive pERK(+) cells in the dorsal horn. (B, B′) pERK(+) cells are increased in the medial part of the dorsal horn (arrows) corresponding to the receptive field of the sciatic nerve. (D, D′) The number of pERK(+) cells on the medial side of the dorsal horn (arrow) on the operated side is similar to that on the contralateral side at POW 12, and is significantly reduced compared to that of the operated side at POW 1. (E) Data are expressed as pERK(+) cell densities of the operated side and contralateral side. *$p<0.05$ indicates a significant difference between the operated side and contralateral side (scale bar: A–D = 100 μm, A′–D′ = 50 μm).