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Research Report

Spinal cord injury triggers sensitization of wide dynamic range dorsal horn neurons in segments rostral to the injury

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Abstract

A spinal cord injury (SCI) was produced in adult rats by complete spinal cord transection at L6–S1. Neuropathic pain behaviors similar to the chronic central pain (CCP) syndrome in human, such as thermal hyperalgesia, mechanical allodynia and autotomy, were present in these rats after spinal cord injury. Meanwhile, wide dynamic range (WDR) neurons recorded in the spinal dorsal horn rostral to the lesion responded as high frequency of spontaneous activities, long duration of after-discharges to noxious electrical stimuli and an augmented wind-up to 0.5 Hz stimuli. By using bupivacaine powder, a sodium channel blocker, at the locus of transection immediate after nerve injury, the chronic pain behaviors were prevented; the hyperexcitability of WDR neurons was also substantially reduced. It is suggested that spinal cord transection induces the CCP syndromes, which may be evoked and maintained by the hyperexcitability in WDR neurons rostrally. Reducing the neuronal activity at the site of lesion following injury may prevent the development of CCP after SCI.

Theme: Sensory system
Topic: Pain modulation: anatomy and physiology

Keywords: Spinal cord injury; Chronic central pain; Wide dynamic range neuron

1. Introduction

Spinal cord injury (SCI) is a devastating event that may result in the development of chronic central pain (CCP) syndrome within weeks or months following the injury [4–6,11]. According to the body part perceiving pain, three types of chronic neuropathic pain resulted from SCI: above-level, at-level and below-level pain [37]. The management of post-SCI pain is difficult due to the poor understanding of the mechanisms of the SCI pain. Many studies have been done on animal models of partial spinal injury such as contusion model [12], photochemical model [22], excito-toxic model [42] and hemisection model [8]. Bare data from animal models with completive spinal injury are available. Recently, significant changes in the frequency and pattern of the background discharges have been observed in the non-specific spinal dorsal horn neurons just rostral to the spinal transection lesion [27,33]. Since several lines of evidence suggest that wide dynamic range (WDR) neurons may be more responsible for the generation of chronic pain than other types of spinal dorsal horn neurons including nociceptive specific (NS) neurons [10,28], we presently intended to investigate the characteristics of WDR neurons rostral to the site of spinal injury after SCI and their possible involvements in the development of CCP.

Neuronal trauma usually causes tremendous and acute increases in abnormal neuronal activities and inputs at the time of injury. It has been suggested that a robust increase in abnormal neuronal inputs in a short period may be enough to produce central sensitization and initiate neuropathic pain after peripheral nerve injury. In the present study, we

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proposed that the neuronal activity at the very beginning of SCI is very important for the initiation of CCP and central sensitization.

2. Materials and methods

Sixteen adult female Sprague–Dawley rats (180–250 g) were used in this study. The experimental procedures were approved by the animal use and care advisory committee of Chinese Academy of Medical Sciences.

2.1. Surgery

The rats were randomly divided into three groups: spinal-transected (ST, $n = 6$), bupivacaine-treated (BUP, $n = 6$) and sham-operated (SHAM, $n = 4$). For the spinal transection surgery, rats were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). The L5–L6 intervertebral space was identified by palpation; and a midline incision was made between vertebrae L1 and L4. The paraspinous muscles were freed from the spinous processes on both sides. A partial laminectomy was performed at two vertebrae, L2–L3, and approximately 0.5 cm length spinal cord was exposed. The dura was torn to open and the L6–S1 dorsal roots were identified under the dissecting microscope. The spinal cord was completely transected at the L6 dorsal root entry zone with a micro-knife without damage to any other spinal dorsal or ventral roots. To ensure complete transection, a 2-mm segment of the spinal cord caudal to the lesion was removed. After careful hemostasis, the musculature and fascia were sutured and the skin was closed. In BUP rats, to produce prolonged and stable nerve blockade, we applied 50 mg insoluble bupivacaine OH powder (made from its hydrochloride (Sigma, St Louis, MI) by titrating sodium hydroxide) directly to the lesion site and filled the gap between the spinal cord stumps. According to the observation on the recovering from motor defect, a single application of 50 mg bupivacaine powder can keep blocking neuronal activity for 5–7 days in this model. More detailed description about this bupivacaine powder and its effect in neuronal blockade was discussed by Xie W. et al. (Pain, in press). Sham-operated animals have undergone all surgical procedures performed in ST rats except spinal cord injury. Prophylactic antibiotic penicillin-G (20,000 units, i.p.) was given twice daily for postoperative treatments until bladder control returned. Feces and urine were manually expressed twice daily until automatic anus and bladder control recovered, usually by the postoperative day 7.

2.2. Behavior testing

Behaviors representing mechanical and thermal sensitivity were tested on all animals at 7 days before and 28–35 days following surgery. The rational that we chose these time points is that both mechanical allodynia and thermal hyperalgesia have been well developed in rats by 4 weeks after spinal hemisection [8,9]. The preoperative testing was used to establish both individual and group baseline of behavior. Because the L4–L6 ventral and dorsal roots were intact, the afferent and efferent pathways of both hind paws were not impaired. To avoid including motor reflexives caused by increased neuronal activity in the lesion site of spinal cord as withdrawal responses, we carefully observed if each withdrawal response was also accompanied with supraspinal behaviors such as licking, stimulus attack or vocalization.

2.2.1. Hot beam stimuli

Using the previously described methods, the hind paw withdrawal latency (PWL) to a noxious radiant heat stimulus was measured [2,26]. The rat was placed under a transparent plastic cylinder on an elevated glass floor and allowed to acclimate for 30 min. Then a radiant heat source was focused onto the plantar (heel) of hind paw. The latency of the withdrawal reflex was recorded as the thermal pain threshold. A cut-off latency of 20 s was used to avoid tissue damage. The hind paws were tested alternately with 5 min intervals between consecutive tests. Three latencies were taken for each hind paw in each test session, and were averaged. The development of thermal hyperalgesia was defined as a statistically significant decrease in PWL compared to baseline ($P < 0.05$).

2.2.2. Mechanical stimuli

Paw withdrawal frequency (PWF) in response to repeated mechanical stimuli to the glabrous surface of the hind paws was used to quantify mechanical sensitivity. In this test, the frequency of paw withdrawals in response to a non-noxious mechanical stimulus, which usually does not cause pain sensation in normal rats, was measured as described elsewhere [7,8]. The rats were placed in individual Plexiglas enclosures on a mesh floor and allowed to acclimate for 30 min. The hairless plantar surface of the hind paw was probed by a von Frey filament of 46.55 mN for 1–2 s and five times on each hind paw at a 30 s intervals. A significant increase in the frequency of brisk foot withdrawals in response to these innocuous mechanical stimuli compared to baseline was interpreted as mechanical allodynia ($P < 0.05$).

2.3. Electrophysiological experiments

After behavioral tests, rats were used as electrophysiological recording. Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.), and supplemented (5 mg/kg per h) through a jugular vein catheter as required. The left sciatic nerve was exposed and bathed in warmed mineral oil for electrical stimulation. A pair of bipolar stimulation electrodes was put on the site of nerve 1–2 mm proximal to the point of trifurcation. The spinal column was rigidly secured