PAIN® xxx (2014) xxx–xxx

PAIN®

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Peripheral noxious stimulation reduces withdrawal threshold to mechanical stimuli after spinal cord injury: Role of tumor necrosis factor alpha and apoptosis

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 12 August 2014
Accepted 26 August 2014
Available online xxxx

Keywords:
Apoptosis
Mechanical allodynia
Nociceptive stimulation
Pain
Spinal cord injury
TNFα

ABSTRACT

We previously showed that peripheral noxious input after spinal cord injury (SCI) inhibits beneficial spinal plasticity and impairs recovery of locomotor and bladder functions. These observations suggest that noxious input may similarly affect the development and maintenance of chronic neuropathic pain, an important consequence of SCI. In adult rats with a moderate contusion SCI, we investigated the effect of noxious tail stimulation, administered 1 day after SCI on mechanical withdrawal responses to von Frey stimuli from 1 to 28 days after treatment. In addition, because the proinflammatory cytokine tumor necrosis factor alpha (TNFα) is implicated in numerous injury-induced processes including pain hypersensitivity, we assessed the temporal and spatial expression of TNFα, TNF receptors, and several downstream signaling targets after stimulation. Our results showed that unlike sham surgery or SCI only, nociceptive stimulation after SCI induced mechanical sensitivity by 24 h. These behavioral changes were accompanied by increased expression of TNFα. Cellular assessments of downstream targets of TNFα revealed that nociceptive stimulation increased the expression of caspase 8 and the active subunit (12 kDa) of caspase 3, indicative of active apoptosis at a time point consistent with the onset of mechanical allodynia. In addition, immunohistochemical analysis revealed distinct morphological signs of apoptosis in neurons and microglia at 24 h after stimulation. Interestingly, expression of the inflammatory mediator NFκB was unaltered by nociceptive stimulation. These results suggest that noxious input caudal to the level of SCI can increase the onset and expression of behavioral responses indicative of pain, potentially involving TNFα signaling.

1. Introduction

Chronic neuropathic pain is a frequent consequence of spinal cord injury (SCI) [28,78]. Despite its prevalence, limited progress is made in the development of effective treatments, a reflection of how little is known about the underlying mechanisms. This lack of knowledge may result from the complex interactions between the primary insult and various secondary processes caused by the injury [4,71]. Additionally, noxious input arising from concomitant peripheral injuries may significantly contribute to maladaptive plasticity and chronic pain after SCI.

We previously investigated the effect of peripheral nociceptive stimulation on spinal plasticity and functional recovery after SCI [31,38]. In adult rats with a complete spinal transection, noxious input derived from electrical stimulation or peripheral inflammation, inhibited beneficial plasticity producing a spinal learning deficit that resembles learned helplessness [18,45]. Stimulation also increased withdrawal sensitivity to an innocuous stimulus [2,30], a behavioral response analogous to mechanical allodynia. As demonstrated by Baumbauer et al. [2], these negative effects are

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http://dx.doi.org/10.1016/j.pain.2014.08.034
0304-3959/

produced only when stimulation intensity reliably engages C fibers, indicating that these behavioral effects reflect alterations in plasticity within pain pathways. When extended to a contusion SCI model, nociceptive stimulation decreased signaling in brain-derived neurotrophic factor pathways in the lumbar spinal cord [36], and undermined long-term locomotor recovery [39].

SCI produces several cellular and morphological changes, including proliferation and activation of microglia and astrocytes [22,42], excitotoxicity, and cell death resulting from necrotic and apoptotic processes [17]. One key mediator implicated in each of these processes is the cytokine tumor necrosis factor alpha (TNFα) [3,29,92]. TNFα has been shown to influence neural survival, exerting both neuroprotective and neurodegenerative actions [32,62,66]. The diverse actions of TNFα reflect the many signaling pathways it engages, among which are the proinflammatory, nuclear factor kappa B (NFκB) and the proapoptotic caspase 8 pathways. TNFα also activates JNK and ERK1/2 pathways.

Although a large body of literature has detailed the expression and downstream effects of TNFα after SCI alone, little is known about the role peripheral input plays in altering the effects of TNFα in the injured spinal cord. Recent work has suggested that peripheral injury can exacerbate nociceptive plasticity by overdriving TNFα expression [13]. Similarly, we have shown that the spinal learning deficit induced by peripheral nociceptive stimulation after complete SCI is mediated by TNFα [47]. Although apoptosis contributes to the loss of tissue after SCI [17,59], it is not known whether peripheral noxious input exacerbates apoptosis after SCI. In this study, we proposed that the development and maintenance of chronic neuropathic pain after SCI is modulated by peripheral nociceptive input accompanying the injury and by increased TNFα expression. Using adult rats with a moderate thoracic contusion SCI, we examined the effect of nociceptive stimulation on hind limb withdrawal threshold that may reflect mechanical allodynia. In addition, we investigated the temporal, spatial, and cell-specific expression of TNFα and its downstream targets. We show that peripheral nociceptive input after SCI induces a sustained mechanical sensitivity that is paralleled by increased TNFα expression and the engagement of the apoptotic caspase-signaling pathway in spinal neurons and glia.

2. Methods

Male Sprague Dawley rats obtained from Harlan (Houston, TX) served as subjects. Rats were approximately 90 to 110 days old and weighed between 350 and 400 g. They were housed individually and maintained on a 12 h light/dark cycle, with all behavioral testing performed during the light cycle. Food and water were available ad libitum. All experiments were carried out in accordance with National Institutes of Health standards for the care and use of laboratory animals (NIH publication 80-23) and were approved by the University Laboratory Animal Care Committee at Texas A&M University. Every effort was made to minimize suffering and limit the number of animals used.

2.1. Surgery and spinal contusion injury

Subjects were anesthetized with isoflurane (5%, gas). Once a stable level of anesthesia was achieved, the concentration of isoflurane was lowered to 2% to 3%. An area extending approximately 4.5 cm above and below the injury site was shaved and disinfected with iodine, and a 7.0 cm incision was made over the spinal cord. Next, 2 incisions were made on either side of the vertebral column, extending about 3 cm rostral and caudal to the T12 segment. The dorsal spinous processes at T12 were removed and the spinal tissue exposed. The dura remained intact. For the contusion injury, administered to the lower thoracic spinal cord, the vertebral column was fixed within the MASCIS device [41], and a moderate injury was produced by allowing the 10 g impactor (outfitted with a 2.5 mm tip) to drop 12.5 mm unto the dorsal surface of the spinal cord, with zero dwell time. The wound was then closed with Michel clips.

To help prevent infection, subjects were treated with 10^5 units/kg Pfizerpen (penicillin G potassium) immediately after surgery and again 2 days later. For the first 24 h after surgery, rats were placed in a recovery room maintained at 26.6 °C. To compensate for fluid loss, subjects were given a 2.5 mL intraperitoneal injection of 0.9% saline after surgery. Bladders were expressed twice daily (morning and evening) until the animals had empty bladders for 3 consecutive days at the times of expression.

Rats used as sham controls underwent the surgical procedure but did not receive a contusion SCI.

2.2. Experimental design

These experiments were designed to investigate the effect of peripheral nociceptive stimulation after SCI (SCI + STIM) on the temporal and spatial expression of the proinflammatory cytokine, TNFα, its receptors, and downstream signaling targets and on hind limb mechanical withdrawal responses. The study consisted of 4 experiments, each involving a separate cohort of subjects. For all experiments, procedures for noxious tail shock or no shock (as described below) were conducted 24 h after surgery. Each experiment consisted of 3 groups of subjects: contusion SCI alone (SCI), subjects administered noxious tail shock after injury (SCI + STIM), and sham-operated controls, which did not receive any stimulation. The first experiment sought to establish the acute effect of contusion injury and the impact of nociceptive stimulation treatment after SCI on the expression of TNFα signaling genes. In this experiment, SCI + STIM subjects were sacrificed 1 h after noxious stimulation (1 h group). The subsequent experiments were conducted to extend the observations arising from experiment 1 and to determine the lasting behavioral and cellular effects of stimulation. In these experiments, SCI + STIM subjects were sacrificed at 24 h (24 h group), 7 days (7 day group), and 28 days (28 day group) after noxious stimulation. In all experiments, SCI and sham control subjects were sacrificed at the time equivalent to the SCI + STIM subjects within the individual group. In addition, to determine whether noxious stimulation had a differential effect across the dorsal/ventral regions of the spinal cord, the tissue was further divided to collect dorsal and ventral halves, which were subsequently processed for mRNA and protein individually. This manipulation was introduced for subjects in the 24 h, 7 day, and 28 day groups (after the acute experiments were undertaken) because concurrent work had revealed that controllable stimulation (delivered when the leg is in an extended position) affected TrkB signaling in the dorsal, but not the ventral, spinal cord [48]. Furthermore, previous work showed that intermittent noxious stimulation after SCI differentially altered the expression of BDNF-TrkB signaling genes in the dorsal and ventral spinal cord [36]. A total of 70 subjects were used as follows: 22 sham controls [6 used for behavior and 16 ($n = 4$, each time point) for cellular assays], 24 SCI ($n = 6$, each time point), and 24 SCI + STIM ($n = 6$, each time point).

2.3. Behavioral locomotor assessment

Twenty-four hours after surgery, before shock/no shock treatment, locomotor behavior was assessed using the Basso, Beattie, and Bresnahan (BBB) scale [1] in an open enclosure for all subjects to ensure the effectiveness of the contusion injury [39]. After baseline scores were obtained, the subjects were assigned to a treatment group (shock or no shock) in a manner that ensured that

Please cite this article in press as: Garraway SM et al. Peripheral noxious stimulation reduces withdrawal threshold to mechanical stimuli after spinal cord injury: Role of tumor necrosis factor alpha and apoptosis. PAIN® (2014), http://dx.doi.org/10.1016/j.pain.2014.08.034