Experimental Neurology 255 (2014) 38–48

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

Acute exercise prevents the development of neuropathic pain and the sprouting of non-peptidergic (GDNF- and artemin-responsive) c-fibers after spinal cord injury

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ARTICLE INFO

Article history:
Received 23 September 2013
Revised 29 January 2014
Accepted 14 February 2014
Available online 19 February 2014

Keywords:
Mechanical allodynia
Thermal hyperalgesia
Central pain
Spinal cord injury
Artemin
GDNF

ABSTRACT

Spinal cord injury (SCI) impaired sensory fiber transmission leads to chronic, debilitating neuropathic pain. Sensory afferents are responsive to neurotrophic factors, molecules that are known to promote survival and maintenance of neurons, and regulate sensory neuron transduction of peripheral stimuli. A subset of primary afferent fibers responds only to the glial cell-line derived neurotrophic factor (GDNF) family of ligands (GFLs) and is non-peptidergic. In peripheral nerve injury models, restoration of GDNF or artemin (another GFL) to pre-injury levels within the spinal cord attenuates neuropathic pain. One non-invasive approach to increase the levels of GFLs in the spinal cord is through exercise (Ex), and to date exercise training is the only ameliorative, non-pharmacological treatment for SCI-induced neuropathic pain. The purpose of this study was 3-fold: 1) to determine whether exercise affects the onset of SCI-induced neuropathic pain; 2) to examine the temporal profile of GDNF and artemin in the dorsal root ganglia and spinal cord dorsal horn regions associated with forepaw dermatomes after SCI and Ex; and 3) to characterize GFL-responsive sensory fiber plasticity after SCI and Ex. Adult, female, Sprague–Dawley rats received a moderate, unilateral spinal cord contusion at C5. A subset of rats was exercised (SCI + Ex) on automated running wheels for 20 min, 5 days/week starting at 5 days post-injury (dpi), continuing until 9 or 37 dpi. Hargreaves' and von Frey testing was performed preoperatively and weekly post-SCI. Forty-two percent of rats in the unexercised group exhibited tactile allodynia of the forepaws while the other 58% retained normal sensation. The development of SCI-induced neuropathic pain correlated with a marked decrease in the levels of GDNF and artemin in the spinal cord and DRGs. Additionally, a dramatic increase in the density and the distribution throughout the dorsal horn of GFL-responsive afferents was observed in rats with SCI-induced allodynia. Importantly, in SCI rats that received Ex, the incidence of tactile allodynia decreased to 7% (1/17) and there was maintenance of GDNF and artemin at normal levels, with a normal distribution of GFL-responsive fibers. These data suggest that GFLs and/or their downstream effectors may be important modulators of pain fiber plasticity, representing effective targets for anti-allodynic therapeutics. Furthermore, we highlight the potent beneficial effects of acute exercise after SCI.

Introduction

Damage to the cervical spinal cord that results in chronic debilitating neuropathic pain occurs in more than 60% of human spinal cord traumas (Siddall and Loeser, 2001; Widerstrom-Noga et al., 2008). Clinical hallmarks of central neuropathic pain are the development of allodynia, a condition where normally innocuous stimuli elicit a painful response, and hyperalgesia, a condition where noxious stimuli elicit an amplified pain response (Christensen et al., 1996). These types of neuropathic pain are further delineated based on the location of the pain relative to the SCI epicenter as above-level pain occurring in dermatomes rostral to the lesion site; as at-level pain occurring within 2 segments of the injury epicenter; or as below-level pain occurring in dermatomes caudal to the lesion site (Siddall and Loeser, 2001).

Following cervical spinal cord injury, deficits in sensation and the development of chronic neuropathic pain have been attributed to direct damage to the second order sensory neurons within the gray matter of the dorsal horn and/or direct interruption of their axons that ascend in the anterolateral and spinoreticular tracts. Additionally, peptidergic pain afferent fibers immunolabeled for calcitonin gene-regulated peptide (CGRP) or substance P (SP) sprout and exhibit robust arborization into the deep dorsal horn (laminas III–V) above, at and below the lesion epicenter in response to clinical (Calancie et al., 2005; Kakulas, 2004) and experimental SCI Hagg (Hagg, 2006; Krenz and Weaver, 1998;

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http://dx.doi.org/10.1016/j.expneurol.2014.02.013
0014-4886/

Murray and Goldberger, 1974; Ondarza et al., 2003; Weaver et al., 2001, 2002; Zinck et al., 2007).

Another possible contributor to this change in pain afferent distribution and the concomitant development of neuropathic pain are the glial cell-line derived neurotrophic factor (GDNF) family of ligands (GFLs) within the spinal cord dorsal horn (Boucher and McMahon, 2001). In models of peripheral nerve injury, a decrease in GFLs such as GDNF and artemin correlates to the development of neuropathic pain, and restoration of these GFLs to normal levels is sufficient to attenuate dorsal horn remodeling and the development of neuropathic pain (Boucher et al., 2000; Gardell et al., 2003; Hao et al., 2003; Harvey et al., 2010; Pezet et al., 2006; Wang et al., 2003, 2008). Recent data by Harvey et al. (2010) showed that restoration of artemin levels correlates with the appropriate laminar distribution of regenerating afferents after dorsal root crush injury. It is unlikely that artemin acts directly on peptidergic afferents, but rather on a separate and distinct class of pain afferents that are non-peptidergic and GFL-responsive (Malin et al., 2006; Orozco et al., 2001).

The levels of GDNF and its receptor are reduced in the spinal cord after SCI. One non-invasive, clinically useful approach to enhance the levels of neurotrophic factors in the spinal cord after injury is through exercise (Côté et al., 2011; Gomez-Pinilla et al., 2002), and exercise is the only non-pharmacological treatment that has been shown to reduce SCI-induced neuropathic pain (Hutchinson et al., 2004). In the present study, we evaluated the effect of daily exercise therapy on the development of at-level neuropathic pain, the levels of GFLs in the dorsal horn as well as on the distribution of pain afferents within the dorsal horn after SCI. The results indicate a strong correlation between changes in GFL levels, reduction in primary afferent fiber sprouting and decreased incidence of allodynia in SCI rats that were exercised.

Methods

Subjects and surgeries

Eight-one adult, female Sprague–Dawley rats (225–250 g; Charles River Laboratories) were housed 2–3 per cage in a controlled environment (12 h light–dark cycles) with food and water ad libitum. All experimental procedures were approved by the Drexel University Institutional Animal Care and Use Committee.

We utilized a clinically-relevant and accepted model of SCI pain as described previously (Detloff et al., 2012b). Briefly, rats were anesthetized with ketamine (60 mg/kg), xylazine (6 mg/kg) and acepromazine (6 mg/kg) and given antibiotics (ampicillin, s.c.,100 mg/kg, daily for 7 days). After partial laminectomy at C5, the spinal column was stabilized in the Infinite Horizon Impact Device (Precision Systems and Instrumentation, Lexington, KY, Scheff et al., 2003). A custom impactor probe (0.6 mm in diameter) was lowered to within 2 mm of the mid portion of the right C5 spinal cord, and the spinal cord and surgical field were flooded with sterile saline. The spinal cord was then rapidly contused with a force of 200 kdyn (no dwell time), resulting in tissue displacement of 1600–1800 μm. The incision was closed in layers and 5 mL cc^3 of lactated Ringer's solution was administered subcutaneously to prevent dehydration.

Prior to SCI, rats were randomly assigned to exercise (n = 27), SCI alone (n = 44), or control (no SCI; n = 10) groups. As we have previously shown, SCI alone rats can be partitioned into 2 separate groups based on their paw withdrawal threshold in response to mechanical stimuli during von Frey testing (Detloff et al., 2012b). For a rat to be considered in the SCI Allodynia group, the animal must exhibit a 50% reduction in paw withdrawal threshold in both forepaws that is maintained from 14 dpi to the duration of the experiment. Additionally, each behavioral group was subdivided into short (9 day) or long (37 day) survival, and then separated at time of sacrifice for ELISA or immunohistochemical analysis (See Table 1).

Forced exercise paradigm

Rats in the SCI + Exercise group were acclimated to the forced exercise wheel walking system (Lafayette Instruments, Lafayette, IN). Rungs of the wheels were covered with vinyl material to create a continuous surface in order to minimize the potential of additional injury to SCI rats from limbs falling through the rungs due to a loss of grip and grasping function of the ipsilesional forepaw. Rats were placed in the automated wheels for 5 min prior to the start of exercise, with a subsequent running period of 20 min per day for 5 days per week. Rats began the exercise 5 dpi with an initial speed of 5 m/min with speed increasing at 1 minute intervals according to the forelimb capabilities of the rats or until a maximum speed of 14.0 m/min was achieved. The time spent at each speed, rather than the number of wheel revolutions was recorded. All rats reached the maximum speed by 21 dpi.

Behavioral measures

Rats were acclimated to the individual testing environments for at least 7 days (20 min/day) prior to preoperative testing. Behavioral testing was conducted preoperatively to establish baseline responses and then weekly after SCI by blinded experimenters. Due to the unilateral nature of our injury model, we evaluated the ipsilesional (right) and contralesional (left) forepaws separately.

Tactile allodynia

The up-down method for von Frey hair monofilaments (VFH, Stoelting Co., Wood Dale, Il) was used to measure the degree of tactile sensory changes in the forepaws after SCI (Detloff et al., 2010, 2012a,b). Post-injury assessment of nocifensive behavior was initiated only when there was evidence of weight support during locomotor testing for a given limb (Forelimb Locomotor Scale score ≥ 7; Sandrow et al., 2008). This ensured that the rat had suitable motor control to remove its paw from an unpleasant stimulus. Rats were enclosed in a metal chamber measuring 20 cm × 9 cm by 10 cm, with a wire mesh top and bottom (0.635 cm grid size) allowing access to the plantar surface of the forepaws. A total of ten VFH stimulus applications were collected for each paw for each day of testing, beginning with the 5.18 g VFH. Any supraspinally driven attention given to the tactile stimulus including vocalizing, licking or guarding of the stimulated paw was recorded to ensure that the rat had some supraspinal awareness of stimulus application. The paw withdrawal threshold was determined as the lowest

Table 1
Number of rats at each timepoint used for behavior, ELISA or immunocytochemistry (ICC).

Group	Total n	Days post-spinal cord injury								
		0 dpi			9 dpi			37 dpi		
		Behavior	ELISA	ICC	Behavior	ELISA	ICC	Behavior	ELISA	ICC
Naïve	10	10	5	5						
SCI No Allodynia	24				10	5	5	14	5	8
SCI Allodynia	20				10	5	5	10	5	5
SCI + Exercise	27				10	5	5	17	5	12