Experimental Neurology 264 (2015) 111–120

Contents lists available at ScienceDirect

Experimental Neurology

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

Regular Article

Thrombospondin-4 and excitatory synaptogenesis promote spinal sensitization after painful mechanical joint injury

Nathan D. Crosby [a], Frank Zaucke [b], Jeffrey V. Kras [a], Ling Dong [a], Z. David Luo [c,d], Beth A. Winkelstein [a,e,*]

[a] Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, United States
[b] Center for Biochemistry, Medical Faculty, University of Cologne, D-50931 Cologne, Germany
[c] Department of Anesthesiology and Perioperative Care, University of California Irvine Medical Center, Irvine, CA 92868, United States
[d] Department of Pharmacology, University of California Irvine Medical Center, Irvine, CA 92868, United States
[e] Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104, United States

ARTICLE INFO

Article history:
Received 26 September 2014
Revised 24 November 2014
Accepted 26 November 2014
Available online 5 December 2014

ABSTRACT

Facet joint injury induces persistent pain that may be maintained by structural plasticity in the spinal cord. Astrocyte-derived thrombospondins, especially thrombospondin-4 (TSP4), have been implicated in synaptogenesis and spinal sensitization in neuropathic pain, but the TSP4 response and its relationship to synaptic changes in the spinal cord have not been investigated for painful joint injury. This study investigates the role of TSP4 in the development and maintenance of persistent pain following injurious facet joint distraction in rats and tests the hypothesis that excitatory synaptogenesis contributes to such pain. Painful facet joint loading induces dorsal horn excitatory synaptogenesis along with decreased TSP4 in the DRG and increased astrocytic release of TSP4 in the spinal cord, all of which parallel the time course of sustained tactile allodynia. Blocking injury-induced spinal TSP4 expression with antisense oligonucleotides or reducing TSP4 activity at its neuronal receptor in the spinal cord with gabapentin treatment both attenuate the allodynia and dorsal horn synaptogenesis that develop after painful facet joint loading. Increased spinal TSP4 also facilitates the development of allodynia and spinal hyperexcitability, even after non-painful physiological loading of the facet joint. These results suggest that spinal TSP4 plays an important role in the development and maintenance of persistent joint-mediated pain by inducing excitatory synaptogenesis and facilitating the transduction of mechanical loading of the facet joint that leads to spinal hyperexcitability.

Introduction

Chronic spine pain is a prevalent and costly medical problem. The cervical facet joints are common sources of chronic pain, and are susceptible to injury during neck trauma and degeneration (Hogg-Johnson et al., 2008). Abnormal motions of the cervical facets can load the joint tissues that are innervated by mechanoreceptive and nociceptive fibers, including the facet capsular ligament (Pearson et al., 2004; Siegmund et al., 2009). Facet joint loading that produces pain initiates a host of changes in the spinal cord that are characteristic of central sensitization, including hyperexcitability and increased spontaneous activity in dorsal horn neurons (Crosby et al., 2013; Lee et al., 2008; Quinn et al., 2010). Effective clinical treatment requires a better understanding of the spinal mechanisms contributing to central sensitization from joint pain.

Structural plasticity is one mechanism by which pain is maintained through the potentiation of both nociceptive and non-nociceptive pathways (Jaken et al., 2010; Latremoliere and Woolf, 2009; Woolf et al., 1992). Although increases in synapse number are observed in the dorsal horn in neuropathic pain states (Jaken et al., 2010; Lin et al., 2011; Peng et al., 2010), the spinal signals initiating synaptogenesis remain unclear. One family of extracellular matrix proteins, the thrombospondins (TSPs), promotes synaptogenesis (Christopherson et al., 2005; Eroglu et al., 2009; Lo et al., 2011). The thrombospondins are glycoproteins that are produced by numerous cell types and have widely varying roles in cell signaling (Adams, 2001; Adams and Lawler, 2004). For example, platelets, fibroblasts, skeletal muscle, endothelial cells, and astrocytes and neurons in the CNS each express one or more TSP isoforms (Adams, 2001; Arber and Caroni, 1995; Christopherson et al., 2005; Stenina et al., 2003). Expression of TSPs can be radically altered in pathophysiological conditions (Adams and Lawler, 2004; Chen et al., 2000). In particular, thrombospondin-4 (TSP4) is upregulated in spinal astrocytes following nerve ligation and enhances excitatory synaptic transmission in the dorsal horn, but spinal TSP1 and TSP2 are unchanged after that injury (Kim et al., 2012). Despite its potential involvement

* Corresponding author at: Department of Bioengineering, University of Pennsylvania, 240 Skirkanich Hall, 210 S. 33rd St, Philadelphia, PA 19104-6321, United States. Fax: +1 215 573 2071.
E-mail address: winkelst@seas.upenn.edu (B.A. Winkelstein).

http://dx.doi.org/10.1016/j.expneurol.2014.11.015
0014-4886/

in the development of pain from neural trauma, the role of TSP4 in joint-mediated pain and synaptogenesis is unknown.

Given the involvement of thrombospondin-4 in spinal sensitization and neuropathic pain (Kim et al., 2012), and its potential to induce synaptogenesis (Christopherson et al., 2005), we hypothesized that TSP4 plays a role in joint-mediated pain by promoting dorsal horn excitatory synaptogenesis following painful facet joint loading. The temporal regulation of TSP4 is unknown after injurious facet joint distraction, so TSP4 expression was evaluated at day 1 and day 7. However, synaptogenesis has been reported to occur by 3–5 days after a peripheral nerve injury (Lo et al., 2011). As such, excitatory synaptogenesis was evaluated at day 7 following injurious joint loading, which corresponds to a time after the reported 3–5 day development period for new synapses and when behavioral sensitivity and spinal neuronal hyperexcitability are observed after injurious facet joint loading (Crosby et al., 2014; Quinn et al., 2010). Spinal TSP4 levels were then modulated to directly assess the role of TSP4 in synaptogenesis and joint-mediated pain after different severities of facet joint distraction that simulate injurious or physiological loading of tissues in the joint. Findings indicate that TSP4 promotes excitatory synaptogenesis and neuronal hyperexcitability in the spinal cord, and is required for the development of persistent pain after facet joint loading that is injurious.

Materials and methods

Facet joint distraction procedures

All surgical procedures were performed using adult male Holtzman rats (362–464 g) under inhalation isoflurane anesthesia (4% induction, 2–3% maintenance). Facet joint loading was performed using a distraction of the bilateral C6/C7 facet joints, which has been described previously to produce physiological or injurious tissue loading under different magnitudes of distraction (Dong and Winkelstein, 2010; Lee et al., 2004; Lee and Winkelstein, 2009). Briefly, the overlying paraspinal musculature was separated to expose the vertebrae from C4-T1. The C6 and C7 vertebrae were attached to a custom loading device using microforceps and the C6 vertebra was distracted rostrally to stretch the facet capsule across the C6/C7 joints (Fig. 1). Distraction of 0.2 mm was applied for physiological loading and 0.7 mm imposed for injurious loading, in separate rats. Bead markers were placed on the C6 and C7 vertebrae, as well as in a grid on the right C6/C7 facet capsule, and were tracked with a Phantom v4.3 CCD camera (Fig. 1) (Vision Research, Wayne, IN) during distraction of the joint. Using the marker locations before joint loading and at the peak of loading, both the vertebral and facet capsule displacements were quantified, and the maximum principal capsular strain was calculated during each joint distraction (Fig. 1). Sham surgery included all of the same procedures with no joint distraction applied and controlled for the effect of the surgical procedures. Following surgery, the incision was closed with 3–0 polyester suture and surgical staples, and rats recovered under supervision in room air.

Assessment of behavioral sensitivity

Tactile allodynia was measured in the forepaws of each rat by quantifying the paw withdrawal threshold (PWT) during application of a series of von Frey filaments, with increasing strength (1.4, 2, 4, 6, 8, 10, 15, and 26 g), to the plantar surface of the forepaw (Lee and Winkelstein, 2009). Each filament weight was applied five times, and a positive response was recorded if a rat responded to von Frey stimulation by licking, shaking, or withdrawing the forepaw. If a rat displayed a positive response to two consecutive filaments, the lower weight filament was taken as the paw withdrawal threshold. Rats not responding to any of the filaments were assigned the maximum threshold of 26 g. Testing was repeated in three rounds on each day, separated by at least 10 min, and the average threshold from the three rounds was calculated for each rat.

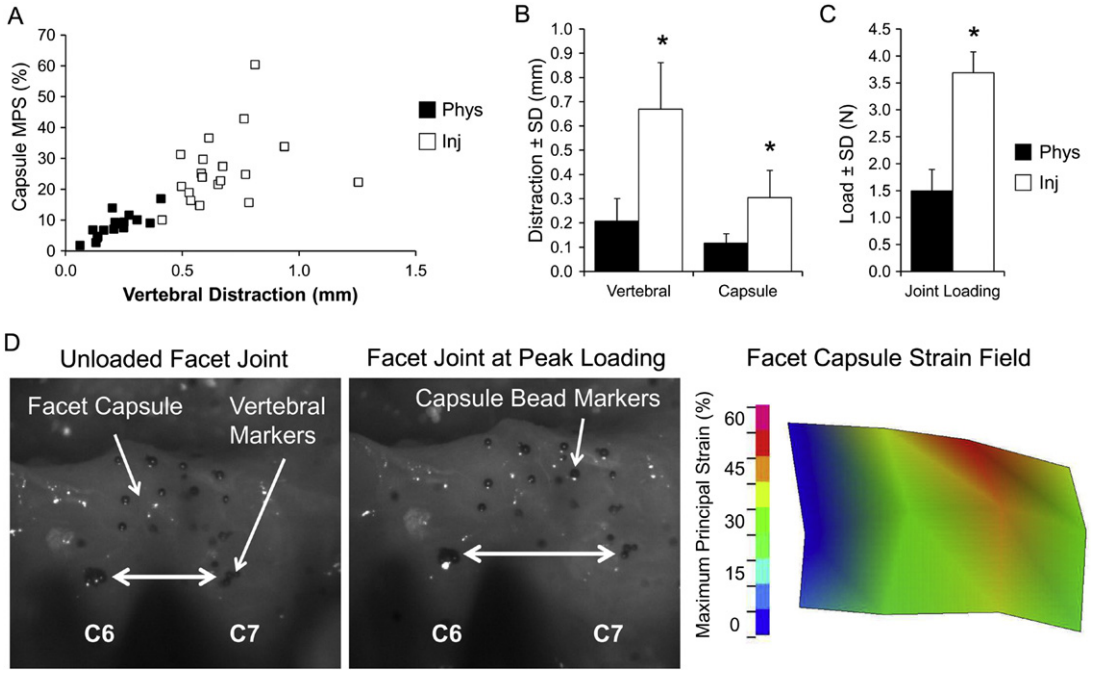

Fig. 1. Characterization of the mechanical loading of the C6/C7 facet joint. **(A)** Injurious loading (Inj) imposes greater C6/C7 vertebral distraction and generates higher maximum principal strains (MPS) in the facet capsule than does physiological loading (Phys). **(B, C)** Vertebral distraction, capsule distraction, and load across the facet joint are significantly higher during injurious loading than physiological loading. Data are mean ± SD (n = 18–19 rats per group). *$p < 0.0001$ compared by Student's t-test. **(D)** Representative images of the facet joint are shown before distraction (unloaded), and at the peak of injurious loading of the joint. The maximum principal strain field is shown for the corresponding loaded facet capsule of a representative rat, with injurious joint loading inducing 0.92 mm of vertebral distraction and a mean MPS of 34%.