Neuroscience Letters 604 (2015) 193–198

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Pain from intra-articular NGF or joint injury in the rat requires contributions from peptidergic joint afferents

Jeffrey V. Kras [a], Christine L. Weisshaar [a], Parul S. Pall [a], Beth A. Winkelstein [a,b,*]

[a] Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104, USA

HIGHLIGHTS

- NGF injected in the cervical facet induces mechanical and thermal hypersensitivity.
- Spinal neuronal hyperexcitability develops 1 day after intra-articular NGF.
- Intact peptidergic signaling in the joint is necessary for NGF-induced sensitivity.
- Cervical facet injury induces behavioral sensitivity and increases NGF in the DRG.
- Injury-induced sensitivity and NGF require peptidergic signaling in the joint.

ARTICLE INFO

Article history:
Received 20 June 2015
Received in revised form 28 July 2015
Accepted 30 July 2015
Available online 1 August 2015

Keywords:
NGF
Facet joint
Pain
Trauma
Neuronal hyperexcitability
Afferent

ABSTRACT

Non-physiological stretch of the cervical facet joint's capsular ligament induces persistent behavioral hypersensitivity and spinal neuronal hyperexcitability via an intra-articular NGF-dependent mechanism. Although that ligament is innervated by nociceptors, it is unknown if a subpopulation is exclusively responsible for the behavioral and spinal neuronal responses to intra-articular NGF and/or facet joint injury. This study ablated joint afferents using the neurotoxin saporin targeted to neurons involved in either peptidergic ([Sar^9,Met $(O_2)^{11}$]-substance P-saporin (SSP-Sap)) or non-peptidergic (isolectin B4-saporin (IB4-Sap)) signaling to investigate the contributions of those neuronal populations to facet-mediated pain. SSP-Sap, but not IB4-Sap, injected into the bilateral C6/C7 facet joints 14 days prior to an intra- articular NGF injection prevents NGF-induced mechanical and thermal hypersensitivity in the forepaws. Similarly, only SSP- Sap prevents the increase in mechanical forepaw stimulation- induced firing of spinal neurons after intra-articular NGF. In addition, intra-articular SSP-Sap prevents both behavioral hypersensitivity and upregulation of NGF in the dorsal root ganglion after a facet joint distraction that normally induces pain. These findings collectively suggest that disruption of peptidergic signaling within the joint may be a potential treatment for facet pain, as well as other painful joint conditions associated with elevated NGF, such as osteoarthritis.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Chronic pain affects nearly 1/3 of adults in the US annually, with neck and back pain being the most common [1]. The facet joint is the source of pain in up to 60% of neck trauma cases [2], with non-physiological capsular ligament stretch a common cause of pain [3]. Proprioceptors and nociceptors innervating the capsular ligament respond to its stretch [4–6]. Both peptidergic (expressing neuropeptides like substance P (SP)) and non-peptidergic (binding isolectin B4 (IB4)) primary afferents innervate the facet joint [7–10]. Clinically and experimentally, anesthetic nerve blocks demonstrate that joint afferents contribute to facet-mediated pain [11–13]. Although excessive facet capsular stretch induces persistent pain and spinal and afferent neuronal hyperexcitability [4,14–16], no study has identified whether a subpopulation of joint afferents mediates injury-induced facet pain.

Peptidergic afferents are sensitive to nerve growth factor (NGF), which is increased in the facet joint by day 1 after its injury [17]. Up to 1/3 of NGF-sensitive afferents bind IB4 [18]. NGF sensitizes adult sensory neurons [7]; intra-articular NGF induces pain and spinal neuronal hyperexcitability within 1 day [17]. Administration of anti-NGF antibodies attenuates experimental and clinical

* Corresponding author. Department of Bioengineering, University of Pennsylvania, 210 S. 33rd Street, 240 Skirkanich Hall, Philadelphia, PA 19104-6392, USA. Fax: +1 215 573 2071.
E-mail address: winkelst@seas.upenn.edu (B.A. Winkelstein).

http://dx.doi.org/10.1016/j.neulet.2015.07.043
0304-3940/© 2015 Elsevier Ireland Ltd. All rights reserved.

joint pain [19,20], and blocking intra-articular NGF after facet injury prevents pain and spinal neuronal dysfunction [17]. Although these findings support NGF's role in joint pain, the relative contributions of peptidergic and non-peptidergic afferents to NGF-induced joint pain is unknown.

This study investigated the separate contributions of peptidergic or non-peptidergic facet joint afferents in the development of behavioral hypersensitivity after intra-articular administration of NGF in the rat using targeted neuronal ablation. Because spinal neuronal hyperexcitability is a hallmark of persistent pain [21], extracellular potentials were recorded from the deep laminae (III–VI) of the dorsal horn, which contain mostly wide dynamic range (WDR) neurons [22], to assess neuronal firing evoked by peripheral mechanical stimuli. Based on those findings and the known role of intra-articular NGF in injury-induced joint pain [17], peptidergic signaling via SP within the facet was eliminated prior to imposing a typically painful facet injury in order to define the role of those joint afferents in pain initiation from joint trauma.

2. Materials and methods

Male Holtzman rats (Harlan Sprague–Dawley) weighing 393 ± 30 g were housed under USDA- and AAALAC-compliant conditions with free access to food and water. All procedures were approved by our institutional IACUC and carried out under the guidelines of the Committee for Research and Ethical Issues of the IASP [23]. Rats were doubly-housed with 12-h light/dark cycles and randomly assigned to groups before surgical or behavioral procedures. Quantitative analyses were performed without group identification to eliminate bias.

2.1. Joint injection of saporin followed by NGF

Neurons expressing the NK1 receptor (NK1R) were ablated using a targeted SP conjugate of the neurotoxin saporin, [Sar^9,$Met(O_2)^{11}$]-substance P-saporin (SSP-Sap), via intra- articular injection as described previously [10]: 100 ng of SSP-Sap dissolved in PBS (5 μL) and injected into the bilateral C6/C7 facet joints (n = 20). Non-targeted saporin (100 ng) was injected in separate rats as controls (Blank-Sap n = 16). Additional rats received intra-articular injections of 5 μg of saporin conjugated to isolectin B4 (IB4-Sap n = 14) in 5 μL of PBS to ablate non-peptidergic neurons, with similar control injections of 5 μg of unconjugated saporin (Saporin n = 9) in separate rats.

Fourteen days after saporin injections, rats were given an intra-articular injection of 3 μg of rat β-NGF (R&D Systems) in 5 μL of PBS (SSP-Sap + NGF n = 12; Blank-Sap + NGF n = 12; IB4-Sap + NGF n = 8; Saporin + NGF n = 9) or PBS (5 μL) as vehicle (SSP-Sap + veh n = 8; Blank-Sap + veh n = 4; IB4-Sap + veh n = 6), using the same procedures. That NGF dose induces behavioral hypersensitivity and spinal neuronal hyperexcitability within 1 day [17]. Weight gain was monitored regularly after all procedures until each rat's study endpoint.

2.1.1. Behavioral assessments

All behavioral tests were performed between 8 a.m. and noon. Forepaw mechanical withdrawal thresholds were quantified for all rats using customary methods [10,24]. Rats were acclimated to the testing environment for 20 min prior to application of an ascending series of von Frey filaments to each forepaw; the lower of two consecutive filaments eliciting emphatic lifting of the forepaw was taken as the threshold. Thresholds in the bilateral forepaws were quantified and averaged over three testing rounds prior to NGF or vehicle injection (baseline) and on day 1 following it. Baseline responses were quantified and averaged over two days before injections.

A subset of rats in each group also was evaluated for thermal sensitivity after NGF or vehicle injection (SSP-Sap + NGF n = 8; SSP-Sap + veh n = 8; Blank-Sap + NGF n = 4; Blank-Sap + veh n = 4; IB4-Sap + NGF n = 8; IB4-Sap + veh n = 6; Saporin + NGF n = 9). Thermal hypersensitivity was measured following mechanical assessment using a commercially available device (UC San Diego) and customary methods [25]. After acclimation to the apparatus for 30 min, the withdrawal latency for each rat was averaged across forepaws over three testing rounds on each day; baseline measurements were averaged over two days before injections.

2.1.2. Spinal electrophysiological recordings

After behavioral testing on day 1, spinal neuronal excitability was quantified in a subset of rats (SSP-Sap + NGF n = 4; SSP-Sap + veh n = 4; Blank-Sap + NGF n = 4; IB4-Sap + NGF n = 5; IB4-Sap + veh n = 6; Saporin + NGF n = 4) using customary methods [14,17]. Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and maintained with supplementary doses (5–10 mg/kg, i.p.). The C6–C8 spinal cord was exposed and bathed in 37 °C mineral oil. Rats were mounted onto a stereotaxic frame; core temperature was maintained at 35–37 °C.

Extracellular potentials were recorded using tungsten electrodes lowered into laminae III–VI via a micropositioner [14,17]. Sensory neurons were selected for recording if brushing the forepaw plantar surface induced firing. A stimulus train, consisting of light brush (for 10-s), a series of von Frey filaments (1.4 g, 4 g, 10 g, 26 g) each applied for 5 stimulations of 1-s followed by 1-s of recovery, and a noxious pinch (60 g, for 10- s), was applied to the forepaw. Stimuli were applied at 30-s intervals. This stimulation protocol was repeated for each identified mechanosensitive neuron.

Recordings were spike-sorted using Spike2 (CED). Evoked spikes were summed over the continuous 10-s stimulus period for both the brush and pinch. Neurons were classified as low threshold mechanoreceptive (LTM) or WDR, based on pinch-evoked firing [16]. The number of spikes evoked from the initial application of a von Frey filament until 1- second after its 5th application was summed. For each filament, the baseline spikes 1-second prior to its initial application were subtracted from the total spike count.

2.1.3. Facet joint injury and assessment of NGF in the dorsal root ganglion (DRG)

Separate groups of rats received SSP-Sap (SSP-Sap n = 11) or non-targeted saporin (Blank-Sap n = 7) as described above. Fourteen days later under inhalation isoflurane anesthesia, rats underwent either a facet joint distraction (FJD) (SSP-Sap + FJD n = 7; Blank-Sap + FJD n = 7) or sham (SSP-Sap + sham n = 4), as previously described [10,24]. A loading device distracted the bilateral C6/C7 facet joints by displacing the C6 vertebra rostrally and holding C7 fixed. Sham procedures included mounting onto the device with no applied distraction. Forepaw mechanical withdrawal thresholds were measured at baseline (day 0) and days 1, 3, 5, and 7 after injury or sham procedures.

On day 7, rats were given an overdose of sodium pentobarbital and transcardially perfused with PBS and 4% paraformaldehyde. Bilateral C7 DRGs were harvested, post-fixed, and cryo-protected. Serial axial DRG sections (14 μm thick) were thaw-mounted onto slides and incubated in DeCal Antigen Retrieval (BioGenex) solution for 2 hrs. Slides were washed, blocked with goat serum, and incubated overnight with rabbit- anti-NGF antibody (1:100; Santa Cruz) at 4 °C. Sections were incubated in an Alexa488-conjugated goat-anti-rabbit secondary antibody (Life Technologies) for 2 hrs at room temperature, washed, and coverslipped. Sections from naïve rats and with no primary antibody incubation were included as controls. NGF labeling was quantified using densitometry in four sections per rat [24]. The percentage of neurons expressing NGF

Download English Version:

https://daneshyari.com/en/article/6280467

Download Persian Version:

https://daneshyari.com/article/6280467

Daneshyari.com