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BASIC SCIENCE

Increased Interleukin-1α and Prostaglandin E_2 Expression in the Spinal Cord at 1 Day After Painful Facet Joint Injury

Evidence of Early Spinal Inflammation

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Study Design. This study used immunohistochemistry and an enzyme immunoassay to quantify interleukin-1α (IL-1α) and prostaglandin E_2 (PGE_2) levels in the spinal cord of rats at 1 day after painful cervical facet joint injury.

Objective. The objective of this study was to determine to what extent spinal inflammation is initiated early after a painful loading-induced injury of the C6–C7 facet joint in a rat model.

Summary of Background Data. A common source of neck pain, the cervical facet joint is susceptible to loading-induced injury, which can lead to persistent pain. IL-1α and PGE_2 are associated with joint inflammation and pain, both locally in the joint and centrally in the spinal cord. Joint inflammation has been shown to contribute to pain after facet joint injury. Although spinal neuronal hyperactivity is evident within 1 day of painful facet injury, it is unknown if inflammatory mediators, such as IL-1α and PGE_2, are also induced early after painful injury.

Methods. Rats underwent either a painful C6–C7 facet joint distraction or sham procedure. Mechanical sensitivity was assessed, and immunohistochemical and enzyme immunoassay techniques were used to quantify IL-1α and PGE_2 expression in the spinal cord at day 1.

Results. Both IL-1α and PGE_2 were significantly elevated ($P \leq 0.04$) at day 1 after painful injury. Moreover, although both spinal IL-1α and PGE_2 levels were correlated with the withdrawal threshold in response to mechanical stimulation of the forepaw, this correlation was only significant ($P = 0.01$) for PGE_2.

Conclusion. The increased expression of 2 inflammatory markers in the spinal cord at 1 day after painful joint injury suggests that spinal inflammation may contribute to the initiation of pain after cervical facet joint injury. Further studies will help identify functional roles of both spinal IL-1α and PGE_2 in loading-induced joint pain.

Key words: IL-1, spinal cord, PGE_2, facet, joint, pain, inflammation.

Level of Evidence: N/A

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Chronic neck pain is a widespread problem that carries a tremendous economic burden.[1,2] The facet joint has been identified as one of the most common sources of neck pain and remains a likely candidate for injury due to its mechanical vulnerability and its innervation by nerve fibers, and nociceptors in particular, in its capsular ligament.[3–5] Painful facet joint injury and inflammation have been reported to upregulate inflammatory cytokines and the prostaglandin E_2 (PGE_2) receptor, EP2, in primary afferent neurons,[6–8] implicating inflammation as a key component of pain from the facet joint. Moreover, loading-induced joint pain can be alleviated through the intra-articular injection of ketorolac, a nonsteroidal anti-inflammatory drug,[9] supporting the assertion that inflammation contributes to facet joint pain. Furthermore, inflammatory cytokines are upregulated in the spinal cord within 1 day after mechanical loading of nerve tissue in combination with an inflammatory stimulus that produces pain.[10–12] Numerous studies have demonstrated the role of cytokines and glial activation in the development and maintenance of chronic pain.[13–17] Those studies demonstrate that peripheral neural tissue loading induces an early inflammatory response in the spinal cord, yet despite mounting evidence of an important role for inflammatory cascades in loading-induced joint pain,[7,9] it remains unknown if painful mechanical facet joint injury induces a similar early inflammatory response in the spinal cord as is reported for neuropathic pain conditions.

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Prostaglandins and cytokines, including PGE_2, interleukin-1 (IL-1), and tumor necrosis factor α, are implicated in both joint inflammation and pain.[8,18–23] Both IL-1α and PGE_2 have been reported to increase in the membranes and synovial fluid of painful joints, with increasing PGE_2 levels correlating to increased severity of pathology in the joint,[23,24] suggesting a contribution of these molecules to joint pain. Although the role of spinal IL-1α has not been identified for joint pain, increased spinal IL-1α is associated with painful mechanical loading of the dorsal nerve root,[11] so it may be possible that painful joint loading will also alter its expression in the spinal cord. Furthermore, the attenuation of neuropathic and inflammatory pain by blocking IL-1 signaling demonstrates its potential contribution to pain.[11,25,26] Separately, joint inflammation increases the release of PGE_2 in the spinal cord, and intrathecal injection of PGE_2 by itself induces mechanical hypersensitivity in the paw.[18,27] Together with the likely role of joint inflammation in loading-induced facet pain, studies imply that spinal inflammatory mediators may contribute to pain after mechanical facet joint injury, but this relationship has not been investigated.

We have previously demonstrated that painful cervical facet joint distraction in the rat induces changes in proinflammatory cytokine (IL-1β and tumor necrosis factor α) mRNA in the spinal cord at day 7 after joint injury.[7] Although changes in gene expression of inflammatory cytokines have been quantified at this late time point after injury when pain persists,[7] joint inflammation is known to induce molecular responses in the spinal cord within 1 day.[18] Because pain after facet joint distraction is associated with joint inflammation and develops within 1 day of the injury,[7,9,28] it is likely that painful joint loading may induce similar changes in inflammatory mediators in the spinal cord as early as 1 day after injury. This study tests the hypothesis that painful facet joint injury induces an early upregulation of a spinal proinflammatory cytokine and prostaglandin and that both inflammatory mediators are positively correlated with the degree of pain after injury. As such, inflammatory responses were quantified in the spinal cord at day 1 after a painful mechanical facet joint injury in the rat, using IL-1α and PGE_2 as markers of inflammation, and expression levels of both IL-1α and PGE_2 were separately correlated with the mechanical withdrawal thresholds for those rats at day 1 after the painful injury.

MATERIALS AND METHODS

Experiments were performed using male Holtzman rats weighing 405 ± 25 g (Harlan Laboratories; Indianapois, IN). Procedures were approved by the Institutional Animal Care and Use Committee and carried out according to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[29] Studies were performed to define the early spinal inflammatory responses associated with a painful facet joint distraction. Protein levels of IL-1α and PGE_2 were quantified in the spinal cord at day 1 after a painful facet joint injury. The surgical procedures and behavioral hypersensitivity testing methods were performed as described previously.[28,30,31] Behavioral hypersensitivity was measured in the bilateral forepaws by quantifying the mechanical withdrawal threshold before any surgical procedure and at day 1 after injury or sham, corresponding to the time point when spinal cord tissue was harvested for assessment.

Rats underwent either a C6–C7 facet joint injury distraction to produce behavioral hypersensitivity (n = 12) or a sham procedure (n = 8) as surgical controls, using previously described methods.[30,31] Under inhalation isoflurane anesthesia, a midline incision was made along the back of the neck, and the C6–C7 facet joints and their capsules were exposed. After transecting the interspinous ligaments and ligamentum flavum from C5 to T1, the C6 and C7 laminae were attached to a customized loading device *via* microforceps. For the injury group, the bilateral C6–C7 facet joints were distracted by displacing the C6 vertebra rostrally while holding the C7 vertebra fixed.[28,30,31] A camera mounted to a surgical dissecting scope tracked a grid of markers on the C6–C7 facet joint capsular ligament during injury to quantify the joint distraction.[30] An identical sham procedure without any joint distraction served as a control. The mechanical withdrawal threshold was evaluated in the forepaws prior to surgery and at postoperative day 1 to confirm the onset or absence of behavioral hypersensitivity in each group. Average withdrawal thresholds were compared between groups using a 2-way analysis of variance with the Tukey HSD test, with time point and group as factors.

At day 1 after surgery, spinal cord tissue at the injury levels (C6–C7) was harvested to evaluate spinal IL-1α and PGE_2 using immunohistochemistry and enzyme immunoassay, respectively. The spinal cord was assayed (injury, n = 5; sham, n = 4) for IL-1α expression using immunofluorescent labeling. Tissue from a naive unoperated rat was included as a normal control. Rats were deeply anesthetized followed by transcardiac perfusion with phosphate-buffered saline and 4% paraformaldehyde. Samples were postfixed in paraformaldehyde overnight and transferred to 30% sucrose for 3 to 5 days at 4°C. Thin cryosections (16 μm, 6 sections per rat) were mounted onto APES-coated slides and blocked with 5% normal donkey serum (Invitrogen; Carlsbad, CA). Sections were then treated with goat anti-IL-1α (1:100; Santa Cruz Biotechnology; Santa Cruz, CA), followed by a secondary incubation with donkey anti-goat Alexa 488–conjugated antibody (1:250; Invitrogen). Sections were imaged at 10× magnification using a Carl Zeiss LSM 510 (Carl Zeiss Microscopy; Thornwood, NY) microscope and cropped to include the superficial dorsal horn (650 × 200 pixels). Total IL-1α immunoreactivity was measured as the percentage of positive pixels above a threshold that was defined on the basis of staining of naive unoperated tissue. Values were averaged for each group.

In a separate group of rats (injury, n = 7; sham, n = 4), spinal cord tissue was harvested with sodium pentobarbital followed by transcardiac perfusion with 300 mL of phosphate-buffered saline. Tissue from 3 naive unoperated rats was included for normalization controls. Tissue was rapidly frozen on dry ice and homogenized in lysis buffer containing

0.1 M phosphate (pH 7.4), 1 mM EDTA, and 10 μM indomethacin (Cayman Chemicals; Ann Arbor, MI). Total protein concentration was determined using a BCA assay (Pierce; Rockford, IL), and the PGE_2 concentration was measured using an enzyme immunoassay kit (Cayman Chemicals). Samples were run in duplicate and expressed as the average amount of PGE_2 in total protein relative to normal (pg/mg).

Separate *t* tests compared responses between the injury group and sham group at day 1 for IL-1α expression and PGE_2 levels, with significance at $P < 0.05$. Separate linear regressions were used to evaluate whether or not the mechanical withdrawal threshold at day 1 was correlated with the amount of either IL-1α or PGE_2 in the spinal cord on day 1, corresponding to the time of tissue harvest. Both the injury group and sham rats were included for the correlations. Separate analyses of variance tested the significance of the correlations for both regressions. All statistical analyses were performed using JMP, version 8 (SAS Institute; Cary, NC).

RESULTS

All rats that underwent a C6–C7 facet joint distraction received the same magnitude of injury regardless of whether they were used for the IL-1α or PGE_2 assays. In the group of rats used to analyze IL-1α expression, the average capsular ligament distraction was 0.35 ± 0.06 mm, which was not different from the distraction applied to the rats for PGE_2 analysis (0.39 ± 0.06 mm). Moreover, the mechanical withdrawal threshold for the forepaw at day 1 was significantly lower after injury than sham for the rats in both the IL-1α study ($P = 0.01$) and those in the PGE_2 study ($P < 0.01$). There was no difference in the withdrawal threshold of the injury rats used in either the IL-1α or PGE_2 study, nor was there any difference in withdrawal threshold between the sham groups used for those 2 assays.

Painful facet joint distraction significantly increased the levels of IL-1α expression in the superficial dorsal horn of the spinal cord (Figure 1). Specifically, spinal IL-1α expression after injury exhibited a higher intensity of staining in the spinal dorsal horn than sham (Figure 1A,B), relative to normal (Figure 1C). Quantification using densitometry showed a significant, nearly 2-fold increase ($P = 0.03$) after injury relative to sham (Figure 1D). Similarly, spinal PGE_2 at day 1 was also significantly increased ($P = 0.04$) after an injury to more than twice the levels in the spinal cord after a sham procedure (Figure 2).

Although IL-1α expression was increased in the spinal cord after a painful injury and exhibited a slight trend with increased behavioral sensitivity, the correlation between paw withdrawal threshold and IL-1α expression was weak and not significant ($R^2 = 0.13$, $P = 0.35$) (Figure 3A). However, behavioral sensitivity was found to be significantly correlated ($R^2 = 0.54$, $P = 0.01$) with spinal PGE_2 at day 1 (Figure 3B), with a correlation coefficient of 0.74.

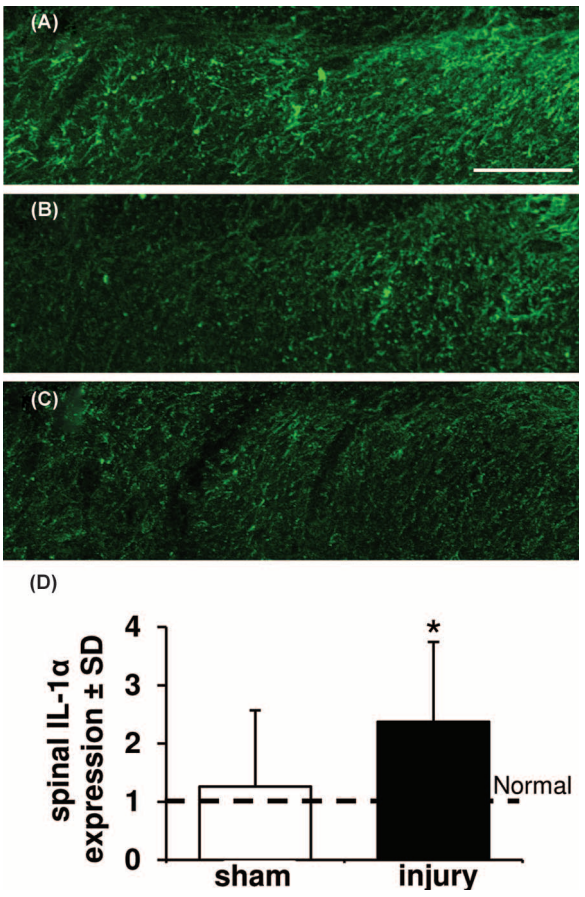

Figure 1. IL-1α expression and quantification in the spinal cord at day 1 after injury and sham relative to normal expression. IL-1α immunoreactivity increases after injury (**A**) compared with sham (**B**) when normalized to normal tissue (**C**). Scale bar (100 μm) applies to panels **A–C**. **(D)**, Quantification of IL-1α in the superficial dorsal horn shows a significant increase (*$P = 0.03$) in injury levels over sham levels. IL-1α indicates interleukin-1α; SD, standard deviation.

DISCUSSION

The proinflammatory cytokine, IL-1α, and prostaglandin E_2 are both significantly increased in the spinal cord at day 1 after a painful facet joint injury (Figures 1, 2). Furthermore, spinal PGE_2 levels are significantly correlated with the degree of behavioral sensitivity at day 1 (Figure 3B), suggesting spinal PGE_2 to have a potentially important role in regulating the early pain response after mechanical facet injury. Yet, IL-1α expression does not correlate with behavioral sensitivity despite its increase after injury (Figures 1, 3A). Prostaglandins and cytokines

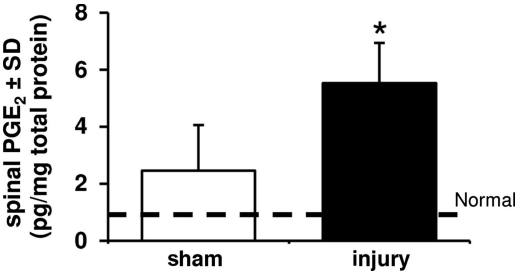

Figure 2. Quantification of spinal PGE_2 levels at day 1 after sham and injury. Spinal PGE_2 expression in the injury group is significantly greater (*$P = 0.04$) than that of sham. SD indicates standard deviation; PGE_2, prostaglandin E_2.

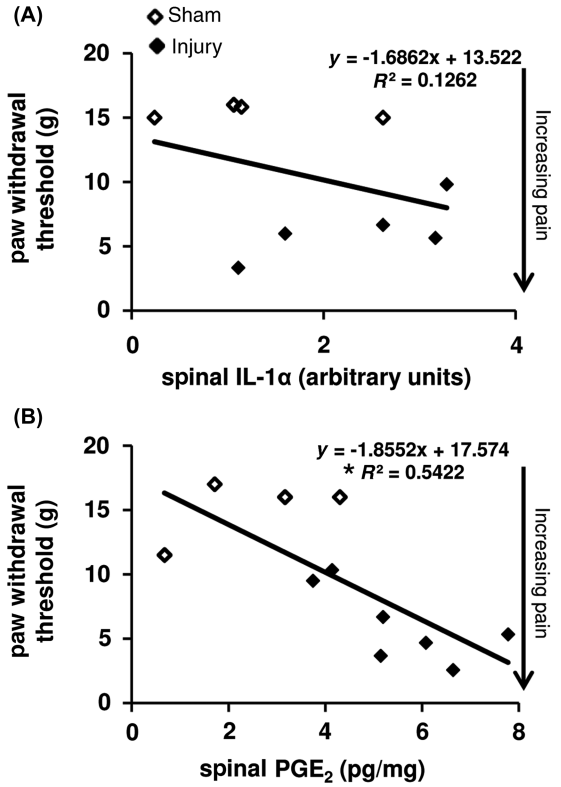

Figure 3. Correlations of spinal IL-1α and PGE_2 with paw withdrawal threshold at day 1. (**A**) The correlation between paw withdrawal threshold and the IL-1α expression in the dorsal horn of the spinal cord at day 1 is not significant ($P = 0.35$). (**B**) The paw withdrawal threshold is significantly ($P = 0.01$) correlated with the spinal PGE_2 levels at day 1 after facet joint injury, with a decrease in the paw withdrawal threshold (more sensitivity) related to greater expression of PGE_2. IL-1α indicates interleukin-1α; PGE_2, prostaglandin E_2.

increase within arthritic and inflamed joints,[23,24,32] but this is the first study to document increased expression of both IL-1α and PGE_2 in the spinal cord at a time when pain symptoms are first evident after a mechanical joint injury. Although the role of spinal PGE_2 in joint inflammation is well known[18,33,34] and IL-1α has been shown to be a regulator of nociceptive cascades in other pain states,[11,17,25] the possible contribution of IL-1α to the onset of joint pain has not been well characterized. Our study demonstrates that painful joint injury is associated with an immediate increase in spinal IL-1α and PGE_2 expression (Figures 1, 2), demonstrating a potential role for spinal inflammation in mechanical joint pain. Although these increases in IL-1α and PGE_2 were significant ($P \leq 0.04$) (Figures 1, 2), the degree of variability in the IL-1α and PGE_2 responses after a sham procedure (Figure 3), together with our limitation in using only a single naive rat for analysis of IL-1α, suggests fully defining the normal level of these and other inflammatory mediators in naive tissue would certainly provide improved context for the current findings.

Although both IL-1α and IL-1β share comparable biological activities, their spinal effects can be distinctly different.[11,35,36] Mika *et al*[25] reported that IL-1α, but not IL-1β, attenuated neuropathic pain in a dose-dependent manner.[25] In contrast, systemic administration of IL-1β is approximately 3,000 times more potent than IL-1α in eliciting hyperalgesia in naive rats.[19] Although a significant increase in spinal IL-1α was observed 1 day after painful injury (Figure 1), the increased expression was not correlated with behavioral sensitivity (Figure 3A). IL-1β was not evaluated at that time point; its contribution could be equally important in the pathogenicity of facet pain.[16,19] Both IL-1α and IL-1β separately increase expression of the pain-associated neuropeptide substance P in primary afferents *in vitro*, but when applied in combination, substance P expression remains at control levels, possibly a result of competitive inhibition.[37] Furthermore, a single intraperitoneal dose of IL-1α increases substance P release in the central nervous system within 2 hours.[38] Despite our finding of increased spinal IL-1α expression immediately after a painful joint distraction, a previous study using this same model found that substance P actually *decreased* in the spinal cord at day 1.[39] Taken together, the finding of increased IL-1α at a time point after joint injury when substance P expression is decreased suggests that additional factors may inhibit the ability of IL-1α to upregulate substance P. Additional studies evaluating the timing and extent of both IL-1α and IL-1β expression in the spinal cord are needed to fully understand their roles in facet pain.

IL-1α expression was only quantified in the superficial dorsal horn in our study, and was not evaluated in the ventral horn of the spinal cord. Although IL-1β expression has been reported to be increased after a painful sciatic nerve chronic constriction injury in *both* the dorsal and ventral horns, IL-1α expression was not investigated in either spinal location in that study.[40] However, Mika *et al* found no change in IL-1α mRNA and was unable to detect "any" IL-1α protein in both the dorsal and ventral horns after the same injury,[25] suggesting that IL-1α and IL-1β may be differentially regulated in the spinal cord. However, the techniques (reverse transcription-polymerase chain reaction and Western blot) used in that study prohibit the cellular localization of IL-1α expression, and immunolabeling techniques that preserve the cytoarchitecture of the spinal cord may be able to detect regional and/or cell-specific differences in IL-1α expression in the ventral horn. In fact, DeLeo *et al*[40] identified an increase in the number of neurons labeled for IL-1β in the ventral horn after a chronic constriction injury. It may be possible that a similar cell-specific increase in IL-1α expression may occur in the ventral horn after a painful joint injury. Additional studies are needed to evaluate whether or not IL-1α expression in the ventral horn is modulated by painful facet joint injury to more fully characterize the spinal inflammatory responses to joint injury.

Spinal PGE_2 expression increased after painful joint injury and was significantly correlated with increased behavioral sensitivity (Figures 2, 3B). Increased PGE_2 in the spinal cord at day 1 after injury agrees with previous work with this pain model demonstrating that both behavioral sensitivity and spinal neuronal hyperexcitability are induced by day 1, but

not sooner.[41] Intrathecal administration of PGE_2 is sufficient to induce behavioral sensitivity to mechanical stimuli applied to the paw.[27] In a separate study, spinal PGE_2 administration was reported to increase the firing rate of spinal neurons during mechanical stimulation of the knee, ankle, or paw.[34] As such, the increased spinal PGE_2 observed here likely contributes to both the neuronal hyperexcitability and behavioral sensitivity observed at this same time point.[41] Indirect inhibition of prostaglandin synthesis *via* intra-articular injection of the nonselective cyclooxygenase inhibitor ketorolac alleviates facet joint-mediated pain.[9] Furthermore, PGE_2 contributes to increased brain-derived neurotrophic factor expression 1 to 2 weeks after a nerve injury.[42] Although PGE_2 levels were only quantified at day 1 in this study, painful facet joint distraction also increases spinal brain-derived neurotrophic factor by day 7,[28] suggesting that spinal PGE_2 may contribute to that increase in brain-derived neurotrophic factor expression after painful joint injury. However, characterization of the temporal expression and identification of the functional role of PGE_2 after joint injury is still needed. PGE_2 was not assessed after a nonpainful joint distraction, so it is not known if PGE_2 is modulated by joint loading or specifically by *painful* joint loading. Interestingly, spinal substance P is greater after a nonpainful joint distraction than after a painful distraction,[39] suggesting that even nonpainful joint loading can induce spinal responses. However, the significant correlation between withdrawal threshold and spinal PGE_2 expression supports that a nonpainful joint distraction would likely have no effect on PGE_2 expression (Figure 3B) since the withdrawal threshold is not different from sham after a nonpainful distraction.[39,43]

Ferreira and Lorenzetti[27] showed that spinal PGE_2 induces behavioral sensitivity by sensitizing primary afferent neurons. Prostaglandin receptors are present on both the presynaptic and postsynaptic neurons in the spinal cord.[33,44] The PGE_2 receptors, EP1, EP2, and EP4, have all been shown to contribute to PGE_2-induced hyperalgesia.[45,46] We previously reported an increase in expression of the PGE_2 receptor EP2 in the dorsal root ganglia after this same joint injury.[6] As such, it is likely that increased EP2 expression in the dorsal root ganglia corresponds to increased EP2 in the presynaptic terminals of the spinal cord; however, spinal expression of any of the PGE_2 receptors was not quantified in our study but would help to identify those receptors through which PGE_2 acts and would more completely define its mechanism of action related to joint pain.

After facet joint distraction, the forepaw withdrawal threshold significantly decreased compared with the sham groups. Because hypersensitivity to mechanical stimulation is a common symptom of whiplash-associated disorders in humans,[47] our finding of increased spinal PGE_2 correlating with behavioral sensitivity (Figure 3B) suggests that a similar such inflammatory response may also contribute to, or be associated with, injury-induced sensitivity in humans. However, care must be taken when translating results from animal studies to the human condition because a number of confounding factors, including the use of a single sex or measuring evoked pain rather than the more clinically relevant spontaneous pain, may prevent responses documented in animal studies from predicting those observed in humans. For example, this was the case of NK_1 receptor antagonists failing to relieve pain in humans despite their effectiveness in animal models.[48,49] This study only included male rats, yet in humans with whiplash-associated disorders, the pressure pain threshold is significantly lower in females than in males,[47] suggesting that inclusion of female rats in addition to male rats may improve the translatability of our results to human patients with neck pain. Nevertheless, studies determining the functional role of spinal PGE_2 in pain after joint injury and the efficacy of targeted interventions in the rat do provide an initial framework for the development of treatment options for human neck pain.

CONCLUSION

Overall, this study provides the first evidence of an early spinal inflammatory response after painful facet joint distraction. Because PGE_2 and IL-1α responses parallel each other at day 1 after painful injury, it is possible that they may be modulated by the same initial injury stimulus. Furthermore, because spinal PGE_2 is significantly correlated with behavioral sensitivity (Figure 3), PGE_2 is suggested as having a strong relationship to pain in this model, and blocking spinal prostaglandin signaling may provide a potential treatment approach. Although additional work is needed to determine the functional roles of these inflammatory mediators, and others, in loading-induced joint pain, this study identifies spinal prostaglandins and cytokines as potential early contributors to the complex cellular response in this pain syndrome.

➢ Key Points

- ❑ IL-1α and PGE_2 expression increase in the spinal cord at day 1 after painful cervical facet joint injury in the rat.
- ❑ Spinal PGE_2, but not IL-1α, levels are correlated with the degree of behavioral sensitivity after injury or sham procedures.
- ❑ These findings support an early role for PGE_2 in facet joint-mediated pain, but additional work is needed to define the contributions of both IL-1α and PGE_2 to facet pain.

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