Eur Spine J (2002) 11 : 467–475
DOI 10.1007/s00586-002-0430-x

ORIGINAL ARTICLE

A. Cüneyt Özaktay
John M. Cavanaugh
Ibrahim Asik
Joyce A. DeLeo
James N. Weinstein

Dorsal root sensitivity to interleukin-1 beta, interleukin-6 and tumor necrosis factor in rats

Received: 31 March 2001
Revised: 17 January 2002
Accepted: 12 April 2002
Published online: 4 June 2002

A.C. Özaktay (✉) · J.M. Cavanaugh
Wayne State University,
Bioengineering Center, 818W Hancock,
Detroit, MI 48201, USA
e-mail: ozaktay@wayne.edu,
Tel.: +1-313-5778883,
Fax: +1-603-9490275

I. Asik
University of Ankara,
School of Medicine, Ibn-I Sina Hospital,
Department of Anesthesiology
and Intensive Care, Sihhiye,
Ankara, Turkey

J.A. DeLeo · J.N. Weinstein
Dartmouth-Hitchcock Medical Center,
Department of Anesthesiology, Lebanon,
New Hampshire, USA

Abstract The release of inflammatory cytokines caused by a disrupted disc may play a critical role in pain production at nerve endings, axons, and nerve cell bodies. Herniated disc tissue has been shown to release inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF), and other algesic chemicals. This study was designed to characterize the effects of these proinflammatory cytokines on the somatosensory neural response at the dorsal root level in rats. It is hypothesized that their effects on nerve endings in disc and adjacent tissue contribute to low-back pain, and the effects on dorsal root axons and ganglia contribute to radiculopathy and sciatica. Surgically isolated sacral dorsal roots were investigated by electrophysiologic techniques. IL-1β, IL-6, or TNF (100 ng, each) were applied onto the dorsal roots. Neural responses and mechanosensitivity of the receptive fields were evaluated over time. The results showed that 3 h after each cytokine application, the neural activity was statistically decreased. The mechanical sensitivity of the receptive fields increased at 90 min following IL-1β or TNF application, and returned to normal more than 3 h after IL-1β application. IL-1β, IL-6, and TNF may be neurotoxic to dorsal root axons. Furthermore IL-1β and TNF may sensitize the peripheral receptive fields. This study suggests that dorsal roots may be impaired by these proinflammatory cytokines.

Keywords Interleukin-1 beta · Interleukin-6 · Tumor necrosis factor · Radiculopathy · Low-back pain

Introduction

Low-back pain and sciatica are significant clinical problems in modern society. However, despite extensive research there are still insufficient data to explain fully the pathophysiology of low-back pain and sciatica, and associated paresthesia. Sciatica, the radiating leg pain that often accompanies low-back pain, may be due to irritation of nerve roots caused by mechanical compression, venous congestion or exposure to inflammatory chemicals. Results of clinical studies indicate that inflamed or irritated spinal roots could be the source of sciatica [21, 35]. Other studies suggest that injury-induced inflammation in disc herniation could be one of the possible reasons for low-back pain [17, 32]. It has also been shown that autologous nucleus pulposus itself is able to produce inflammatory and degenerative changes when injected into the epidural space in dogs [24] and in pigs [27]. Inflammation in these cases may potentially be induced by direct chemical irritation from the nucleus pulposus material [23, 25, 27] and/or it may be an autoimmune response to the nucleus pulposus from the surrounding tissues [17].

Several algesic chemicals have been shown to be expressed in human herniated disc specimens, including interleukin-1 beta (IL-1β) [37], interleukin-6 (IL-6) [20, 37] and tumor necrosis factor (TNF) [37]. There is an extensive body of research that supports the critical roles of

these cytokines in the etiology of pain. In arthritic patients, IL-1β is elevated in both their plasma and synovial fluid level as compared to levels obtained from normal populations [10]. IL-1β shows a potent proinflammatory and hyperalgesic action in animal models [6, 11, 13] by stimulating the synthesis and release of other mediators of inflammation and hyperalgesia including nitric oxide, prostaglandins, and substance P [9]. Furthermore, in association with IL-1β expression, there appears to be an increased expression of nerve growth factor [22] and bradykinin B1 receptors [6] along with alterations in sympathetic fiber activity [2, 34].

In addition to IL-1β, there are significant findings that implicate the role of IL-6 in the inflammatory response of the nervous system. IL-6 has been shown to be produced both locally, at the site of peripheral nerve injury, and centrally, in response to nerve damage [7]. Kang et al. [20] demonstrated that herniated human lumbar intervertebral disc tissue spontaneously produces high levels of IL-6. Elevated human serum IL-6 levels have been detected in patients with rheumatoid arthritis, ankylosing spondylitis or non-inflammatory back pain [15]. Xu et al. [41] reported that IL-6 deficient mice had a significantly lower response threshold to both mechanical and thermal stimulation in comparison to normal controls after localized carrageenan injection. DeLeo et al. [7] showed that IL-6 produced allodynia in normal rats and thermal hyperalgesia in rats with previously lesioned sciatic cryoneurolysis. Hori et al. [18] demonstrated that IL-6 and TNF induced hyperalgesia in a prostanoid-dependent way in rats. These reports suggest that IL-6 may be involved in the cascade of events leading to the development and maintenance of neuropathic pain.

Another important cytokine related to inflammatory responses is TNF. Intra-plantar injection of exogenous TNF induces mechanical [5] and thermal [31] hyperalgesia, presumably by activation and/or sensitization of nerve terminals. In addition, TNF induces ectopic activity in nociceptive primary afferent fibers [36]. TNF and IL-1β were reported to enhance pain response following administration of illness-inducing substances such as lithium chloride and endotoxin [26].

Given the suggested important roles of IL-1β, IL-6, and TNF in the literature, the current study sought to determine the response of the application of these cytokines on dorsal roots in an established electrophysiological model in the rat. In the present study, changes in the electrophysiological parameters were followed over time. These parameters were multi-unit discharge activity, identified single-unit discharge activity, and mechanosensitivity of the receptive fields. Elucidation of the physiological effects of these proinflammatory cytokines on nerve roots will provide insight into understanding the etiology of low-back pain and sciatica.

Materials and methods

Preparation of the animals

All procedures were approved by the Animal Investigation Committee at Wayne State University. Twenty-three adult male Sprague-Dawley rats weighing 350–400 g were used. They were sedated and anesthetized by intramuscular injection of ketamine hydrochloride (43 mg/kg), xylazine (7 mg/kg) and torbutrol (0.1 mg/kg) as needed throughout the experiment.

A midline dorsal longitudinal incision was made over the lumbar spine, and the multifidus muscles were removed along the

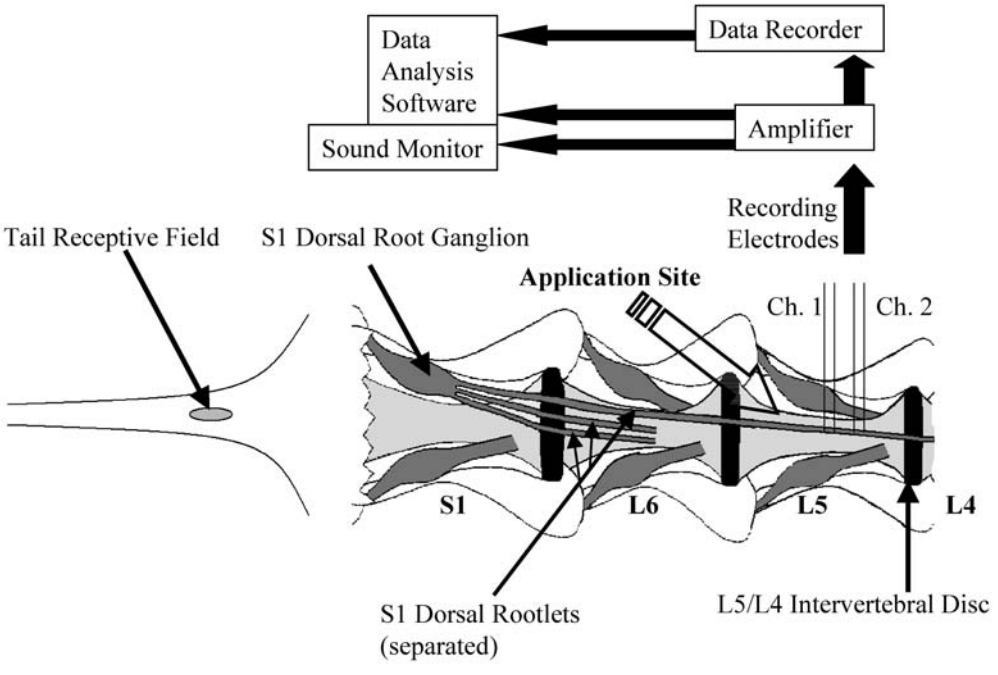

Fig. 1 Diagram of rat lumbar spine showing the electrophysiologic experimental set-up. The *hollow arrow* indicates the site of application. The S1 dorsal root was separated into the three rootlets. All dorsal roots and rootlets were intact to the spinal cord (shown cut for illustration purposes)

L3/S2 spinous processes. An L4/S1 laminectomy was performed with fine bone rongeurs, and the dura was exposed. The rat spine was secured at the third lumbar and second sacral spinous processes by means of a spine-holding device. A pool was formed from skin flaps, and the spinal cord was covered in warm (37°C) mineral oil (Fig. 1).

Extracellular recording

Identification of multiple units

After opening the dura, the left S1 dorsal root was carefully separated into two to three rootlets and draped over a dual-bipolar platinum-recording electrode at the L5 disc level. The S1 dorsal rootlets contained afferent impulses (units) from nerve fibers innervating the base of the tail (receptive field) with higher mechanical thresholds. This rootlet was identified and studied. Multi-unit recordings were made from intact sacral dorsal roots.

The impulses on two channels were amplified, monitored on an oscilloscope and an audio-monitor, digitized and analyzed using PC-based spike discrimination and frequency analysis software. All software was part of the Enhanced Graphics Acquisition and Analysis system (EGAA; R.C. Electronics, Goleta, Calif.). For detailed analysis later, the data were also simultaneously recorded on an analog tape recorder (MR-30; TEAC, Montebello, Calif.).

Identification of single units

In each experiment, recorded multi-unit activity was later analyzed via the EGAA software system for differentiating the single units. A single unit present in both channels was identified by its amplitude and its conduction velocity. They were discriminated and analyzed by the EGAA system software in the same way as the multi-unit recordings were analyzed. The advantage of characterizing conduction velocities (CVs) of single units is that implications can be drawn about the effect of cytokines on neurons with different axonal diameters that serve different functions. Group IV, C fibers (0.5–2 μm diameter, unmyelinated) primarily carry pain, itch, temperature, and crude touch; group III, A-delta fibers (1–5 μm diameter, finely myelinated) [33] transmit pricking pain, temperature, and crude touch sensations to the central nervous system; and group II, A-beta fibers (5–12 μm diameter, myelinated) predominantly innervate muscle spindles.

To calculate the conduction velocities (CVs), the distance between the recording electrodes was measured (in millimeters), and this was divided by the onset latency (in milliseconds) of the evoked response. The units that had a conduction velocity of less than 2.5 m/s were classified as group IV, and those with a velocity of between 2.5 and 20 m/s as group III units. Group II units were in the range of 20–70 m/s. Units with CVs more than 70 m/s were classified as group I units (12–20 μm diameter, large myelinated; A-alpha fibers).

Mechanosensitivity measurements

In each experiment, mechanosensitivity of the receptive field was probed. The receptive field (responsive area) was identified at the base of the tail with a fine-tipped glass probe. It was then stimulated with calibrated nylon filaments, with the compression magnitude ranging from 0.4 to 24.4 g (Aesthesiometer; Stoelting, Wood Dale, Ill.). Mechanical threshold was characterized by using successively stronger nylon filaments until the nerve began to fire (increase in discharge rate). Mechanosensitivity was determined 30 min before cytokine application (baseline) and every 30 min subsequent to the application of cytokine.

Application of IL-1β, IL-6, and TNF

IL-1β, IL-6, or TNF (Pepro Tech, Rocky Hill, N.J.) were diluted to 5 μg/ml in 0.9% buffered saline, pH 7.4. After characterizing the receptive field, 20 μl (100 ng) of IL-1β (n=6), IL-6 (n=5), TNF (n=6) or vehicle alone (n=6) was applied onto the dorsal root of interest, distal to the recording electrodes. Since the vehicle was water based, the mineral oil pool kept the solution in place where it was applied onto the dorsal root.

Discharge rate changes

The discharge rate of the units was followed for over 3.5 h for each experiment. First, spontaneous baseline neural activity (discharge rate) was recorded. Once the stable baseline activity was established, successive recordings were made throughout the experiment; 15 and 5 min prior to application, continuous recording 1 min before until 5 min after the application, 10 and 15 min after the application, and every 15 min thereafter. In addition, any sudden change of activity, observed on the oscilloscope, audiomonitor, or simultaneous EGAA histogram, was recorded until the activity stabilized for at least 2 min.

Data acquisition and statistical tests

The data, stored on an analog cassette tape, were later digitized and analyzed using spike discrimination and frequency analysis software. EGAA template matching, pattern recognition or window discrimination system software was used to discriminate and count spikes. The spontaneous background activity from each experiment was studied as spontaneous discharge rate and calculated as impulses per second (imp/s). The change in discharge rate for each experiment was also studied and expressed as a percentage change from the baseline spontaneous discharge rate. Data of the threshold for mechanosensitivities were also examined in detail for each analysis via EGAA system software. The data were grouped with respect to the type of applications and the conduction velocities. For statistical analysis, time effects were analyzed by repeated-measures analysis of variance (ANOVA) within groups as well as between-subjects within the groups. Pairwise comparison [Post Hoc (Scheffe)] tests were used to analyze the differences between the groups at corresponding time points. P-values less than 0.05 were considered significant. The averages are given as the mean ± standard error of the mean (SEM). The statistical results were categorized as (i) changes in the entire trend over time, (ii) changes at individual time points compared to previous activity, (iii) for each unit, whether that unit represents its group in the application under analysis, and (iv) comparison of the response patterns of different groups.

Results

Multi-unit discharge rate

Comparison of the response patterns

When the average discharge rate of each cytokine and vehicle application group was analyzed over time, a different response pattern was generated for each (Fig. 2A, Fig. 3A, Fig. 4A; P<0.003).

- *IL-1β applications* (Fig. 2A, n=6): For IL-1β, the only significant increase observed was at the time of appli-

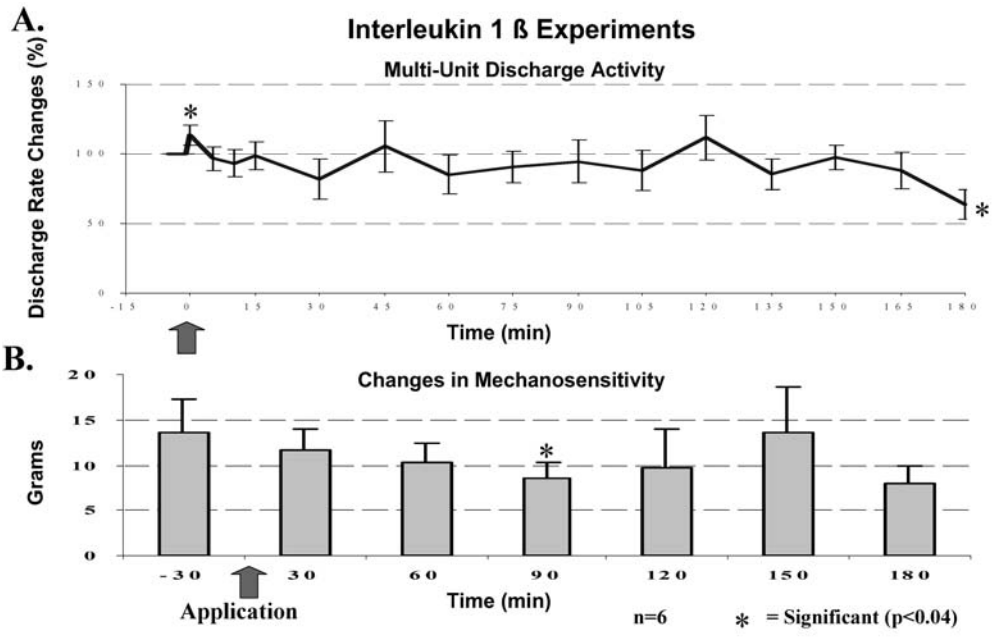

Fig. 2A,B Application of 100 ng of interleukin-1 β (IL-1β; *n*=6) in 20 µl buffered saline solution (pH 7.4). **A** The ratio of the average multi-unit discharge rate after the IL-1β application to the rate before the application over time. In all IL-1β experiments the response patterns showed similarity ($P<0.00001$). There was an increase in discharge rate during the application ($P<0.04$) following a decrease in trend over 3 h ($P<0.02$). **B** Changes in mechanosensitivity of the receptive fields after the IL-1β applications (*n*=6). At 90 min after the application the receptive fields were activated by lower mechanical stimuli than before the application (indicating hypersensitivity)

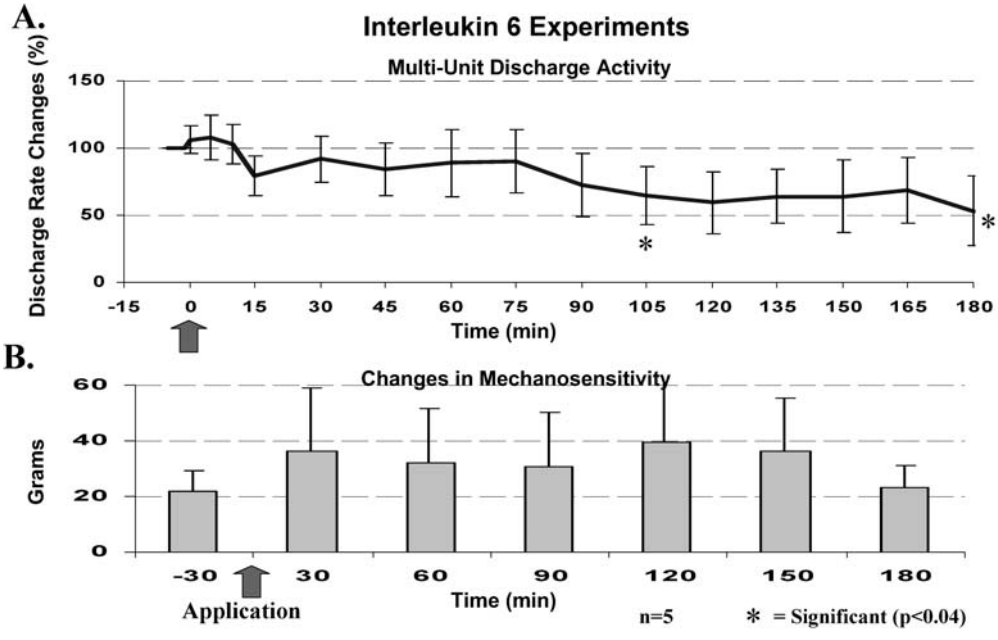

Fig. 3A,B Application of 100 ng of interleukin 6 (IL-6, *n*=5). **A** At 105 min there was a significant decrease ($P<0.037$). Overall, in all IL-6 experiments, the response patterns showed similarity, and all showed statistically significant decreases over time ($P<0.007$). **B** No changes in mechanosensitivity of the receptive fields were observed over time

cation ($P<0.04$). The discharge rate remained very stable for over 2 h 45 min, and significantly decreased after 3 h ($P<0.02$).

- *IL-6 applications* (Fig. 3A, *n*=5): IL-6 demonstrated a trend of decreased rate of multi-unit activity over 3 h ($P<0.007$). The turning point of this significant decrease was at 1 h 45 min after the application ($P<0.037$).
- *TNF applications* (Fig. 4A, *n*=6): Among the four applications, the multi-unit discharge rate was the most significantly decreased over time with TNF ($P<0.0001$). The first significant decrease was observed at 75 min after the application, and remained low for another hour ($P<0.047$). After a brief return to baseline at 135 min, the discharge rate decreased again at 150 min ($P<0.003$).
- *Vehicle applications* (*n*=6): After vehicle applications no statistically significant changes in discharge rate were observed.

Mechanosensitivity of the receptive fields

- *IL-1β applications* (Fig. 2B, *n*=6): The threshold for the mechanical sensitivity of receptive fields showed a sig-

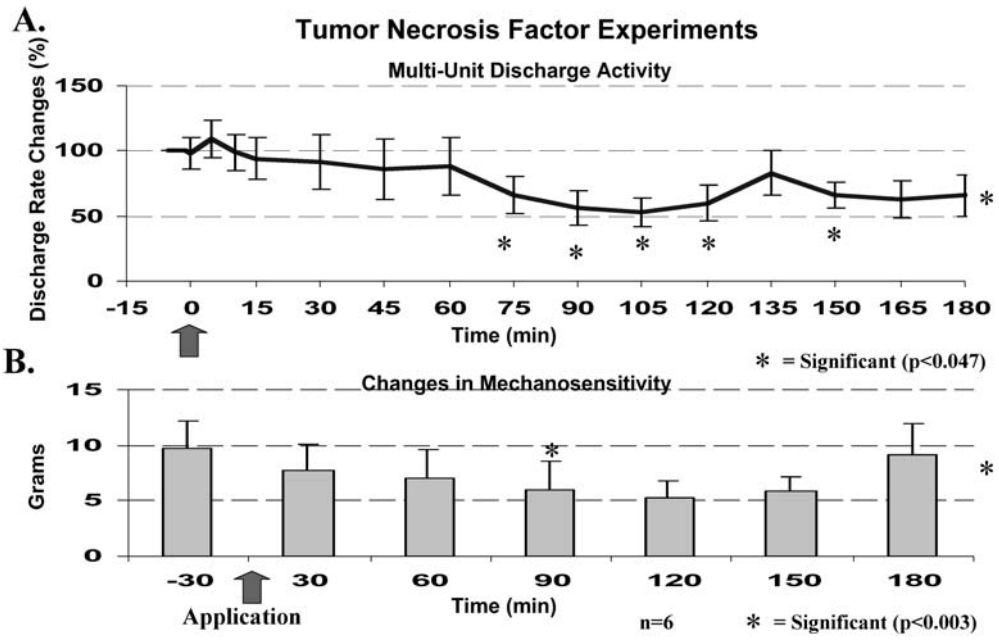

Fig. 4A,B Application of 100 ng of tumor necrosis factor (TNF, n=6). **A** The discharge rates in all six TNF experiments showed similarity in the response patterns and all decreased over time (P<0.05). **B** The mechanosensitivity of the receptive fields displayed similar response patterns and all decreased at 90 min for 1 h (P<0.04)

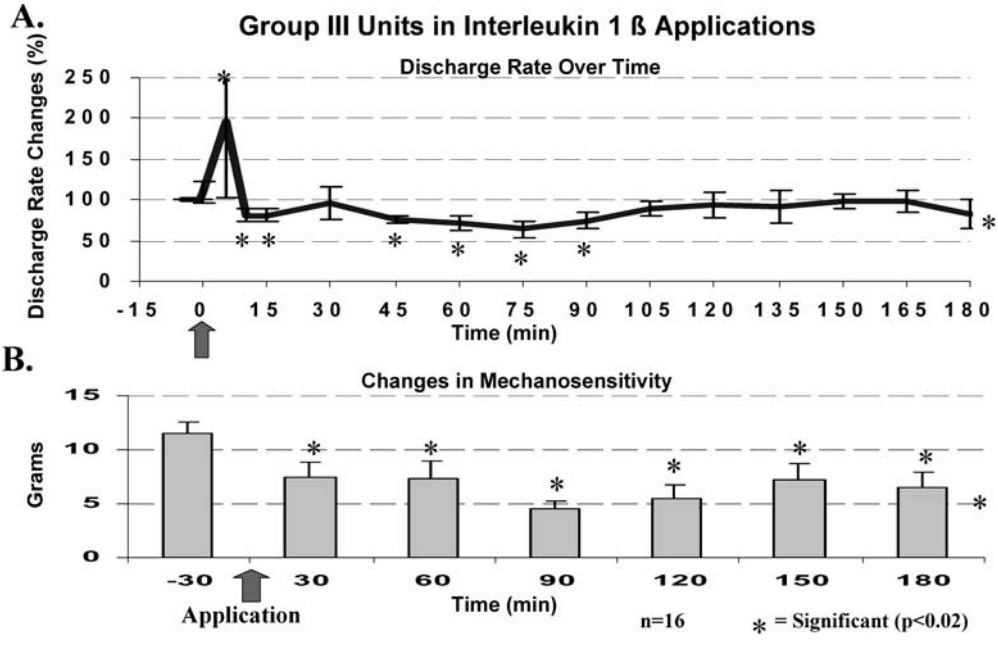

Fig. 5A,B The group III unit (8.81±1.44 m/s; n=16) after the 100 ng IL-1β applications. **A** The discharge rate showed a significant decrease over time (P<0.0004) after a transient increase at 5 min (P<0.006). **B** The receptive field mechanosensitivity of the group III units were first decreased from 11.5±1.0 g to 7.5±1.3 g at 30 min after the applications (P<0.0005) and remained significantly lower over 3 h (P<0.0005)

nificant decrease (increased sensitivity) only at 1.5 h after the applications (P<0.04).

- *IL-6 applications* (Fig. 3B, n=5): No change in mechanosensitivity was observed at any point.
- *TNF applications* (Fig. 4B, n=6): Overall, the trend of changes in mechanosensitivity throughout the experiments was statistically significant (P<0.035). A significant decrease in mechanical threshold (increased mechanosensitivity) was observed only at 1.5 h and 2 h after the TNF application (P<0.046 in comparison to the previous threshold levels).
- *Vehicle applications* (n=6): No change in mechanosensitivity was observed at any point.

Conduction velocities (CVs) and classification of single units

A total of 70 units were individually identified. Forty-eight of the 70 units in this study were in the range of group III (2.5–20 m/s). Twenty-two units with CVs of 20–70 m/s were classified as group II.

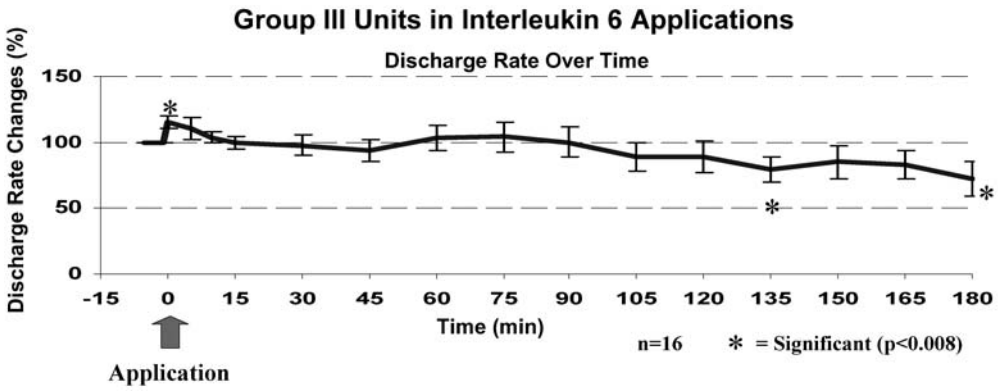

Fig. 6 The discharge rates of the 14 group III units (8.29± 0.85 m/s) after the 100 ng IL-6 applications. The discharge rate displayed a significant decrease over time, except for during the application, when the rate increased (P<0.008)

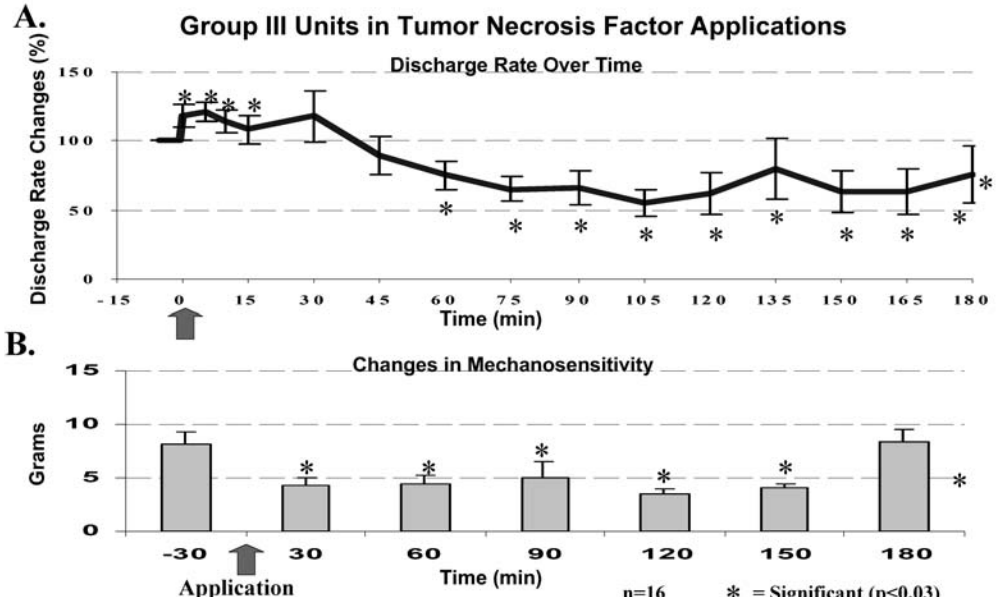

Fig. 7A,B The group III unit (7.44±1.18 m/s, n=16) after the 100 ng TNF applications. **A** The discharge rate of the units was increased significantly for 15 min (P<0.03), then decreased over 1 h (P< 0.03) and remained decreased over time (P<0.0001). **B** The mechanosensitivity of the receptive fields of the group III units displayed a significant response pattern over time (P< 0.0005). Thirty minutes after the application, the mechanical thresholds decreased significantly, and remained low (P< 0.0008) until 3 h, when they returned to baseline values

IL-1β applications

In the six animals, 16 group III units (8.81±1.44 m/s) were identified. They showed a significant decrease in discharge rate at 10 and 15 min after the IL-1β applications. After a brief return to the baseline at 30 min, the discharge rate was significantly decreased again at 45 min and remained at a decreased level until 105 min (Fig. 5A, P<0.02). In the remainder of the experiment the discharge rate returned to the baseline. The receptive fields became more sensitive to the mechanical stimuli beginning at 30 min after the IL-1β applications. The threshold decreased from 11.5±1.0 to 7.5±1.3 g (P<0.0005) and remained significantly lower over 3 h (Fig. 5B, P<0.0005). The largest decrease was observed at 90 min after the application (P<0.0001).

Group II (36.75±6.33 m/s, n=10) units, however, did not show any significant change in discharge rate over time. Their mechanosensitivity on the other hand displayed a decrease at 2 h after the IL-1β application (P<0.02, data not shown).

IL-6 applications

In the five animals subjected to IL-6 applications, group III units (8.29±0.85 m/s, n=14) exhibited an initial increase during the application (P<0.0002), then the discharge rate gradually decreased over 3 h (Fig. 6, P<0.008). On the other hand, five group II units (57.40±7.48 m/s) examined did not display any changes over time (data not shown). There was also no significant change in mechanosensitivity in either group II and III units over time (data not shown).

TNF applications

In six applications of TNF, group III units (7.44±1.18 m/s, n=16) demonstrated a significant increase in discharge rate at the time of application up to 15 min (P<0.03), then decreased over 1 h (P<0.03), and remained decreased over time (Fig. 7A, P<0.0001). The mechanosensitivity of the

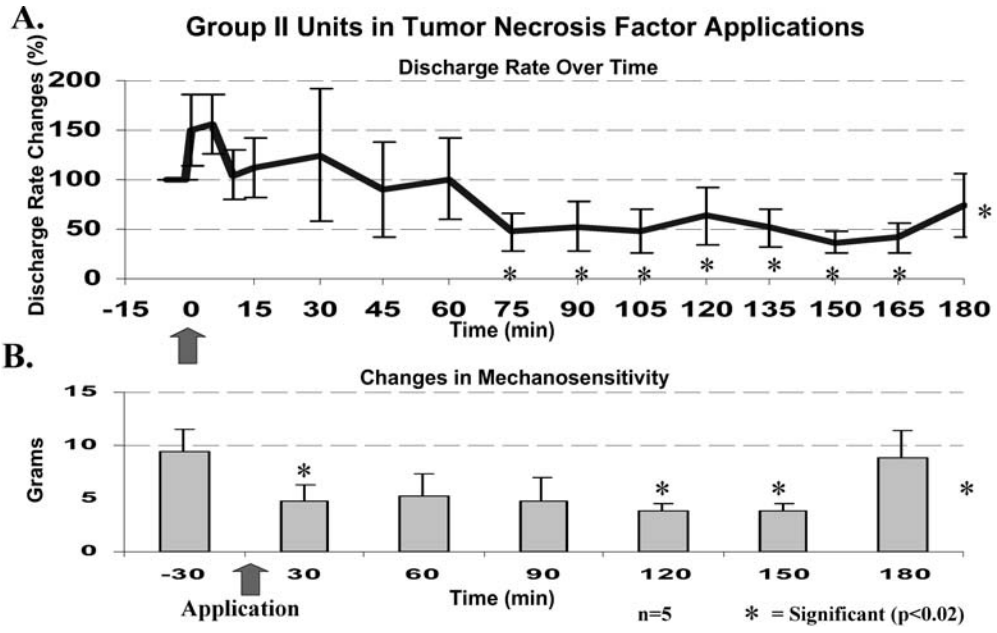

Fig. 8A,B The group II units (36.20±4.57 m/s, n=5) after the 100 ng TNF applications. **A** The discharge rate of the units showed a significant decrease over time (P<0.02), beginning at 75 min after the application (P<0.04). **B** The mechanosensitivity of these group II units was decreased only at 30, 120 and 150 min after the 100 ng TNF applications (P<0.02)

receptive fields displayed a significant response pattern over time (Fig. 7B, P<0.0005). Thirty minutes after the application, the mechanical thresholds decreased significantly and remained low (P<0.0008) until 3 h, with a return to the baseline values. Group II units (36.20±4.57 m/s, n=5) also showed a significant decrease in their discharge rates over time (Fig. 8A, P<0.02). The first significant decrease was noted at 75 min after the application (P<0.04). The threshold for mechanical stimuli was also decreased, at 30, 120 and 150 min after the applications (Fig. 8B, P<0.02).

Vehicle applications

Only two group II and two group III units were identified in six control experiments. Their responses were not analyzed statistically, due to the small sample size.

Discussion

Our results demonstrated that both the discharge rate of sensory units and mechanosensitivity of the tail receptive fields in rats are altered upon direct application of the proinflammatory cytokines IL-1β, IL-6, and TNF on dorsal roots. The most striking finding in our results was the decrease in neural activity after all three cytokine applications.

We analyzed mechanosensitivities and discharge rates of both multiple and single units. By analyzing single units, assumptions can be made regarding the effects these cytokines have on the known physiological roles of nerve axons of different diameters. Our results have shown that group III units (A-delta, small myelinated nerves) displayed decreased discharge rate with each cytokine application. Group III units are known to transmit sensory information relating to pricking pain, temperature, and crude touch to the central nervous system [16]. Group II units only showed a decrease in their discharge rate with TNF application. Group II units (A-beta fibers) carry afferent sensory information from specialized nerve endings, which sense touch, pressure, and stretch and velocity of stretch (namely Meissner's, Pacinian corpuscles, and muscle spindles, respectively) [19].

The decreased discharge activity of the sensory nerves may indicate cytokine-specific responses of the nerve fibers and/or neurotoxic effects at the dose used in this study. Several researchers have shown the effects of various doses of IL-1β, IL-6, and TNF (0.001–1000 ng) on the expression of inflammatory substances, neural activity, and hyperalgesia to be both time and dose dependent [4, 11, 12, 13, 14, 18, 31, 36]. In a study by Fukuoka et al. [13], various doses of IL-1β injected into the rat hind paw led to an increased discharge rate, lowered thresholds and higher discharge to stimulus compared to controls at low doses of 0.1–100 pg. On the other hand 100 ng IL-1β produced hypoalgesia in the same study [13]. In a study with pigs, epidural application of 1.66 μg of TNF-α showed a reduction in nerve conduction velocity recorded from tail muscles similar to the application of nucleus pulposus [1]. The dose used in our study (100 ng) was moderate. Further work is warranted to study time- and dose-dependent responses, to fully understand the effects of IL-1β, IL-6, and TNF on neuronal functions. The preliminary data obtained from our current work indicate that at doses of 0.5, 1, and 5 ng, the same cytokines had a little or no effect on neural functions using a similar setup (unpublished data).

While the neural activity decreased in these studies, the overall mechanosensitivity of the peripheral receptive fields increased in response to IL-1β and TNF dorsal root applications (but not with IL-6 or vehicle). In particular, the increased mechanosensitivity of group III units to IL-1β and TNF may suggest a possible role in nociception. Action potentials travel both antegrade and retrograde. Retrograde signals are transmitted through the dorsal root ganglion down to the periphery [19, 40]. This may possibly cause the release of neurogenic inflammatory mediators such as substance P to decrease the threshold. Unfortunately, we were not able to characterize the response of group IV units, which transmit primarily pain and temperature. Small-amplitude units, which may include group IV units, were present in the multi-unit activity, but these are difficult to evaluate in multi-unit data.

The neurophysiology model we used in this study was modified from earlier studies [3, 28, 29, 30, 38, 42]. Our previous studies revealed unique characteristics of neural response to inflammatory chemicals thought to play a role in low-back pain and sciatica. In those studies, we monitored neural activity in response to direct application of substance P (a neurogenic inflammatory agent) [42], carrageenan (a producer of tissue inflammation) [28], phospholipase A_2 (a principle component of the arachidonic acid cascade) [3, 29, 30], and nucleus pulposus (an agent that may cause all these previously mentioned effects) [38]. Substance P appeared to have a direct excitatory effect on receptor sites at or near nerve endings [42]. Carrageenan had a more prolonged effect, which likely involved the release of several inflammatory agents over several hours [28]. Phospholipase A_2 appeared to be neurotoxic in acute studies in which it is applied on receptive fields [29], and on the dorsal root ganglion [30], and in chronic studies in which it produced demyelination of the dorsal roots [3]. When applied to dorsal roots, nucleus pulposus produced excitation as well as increased mechanosensitivity of the dorsal root ganglion [38].

At the cellular level, hyperalgesic inflammatory substances (i.e., carrageenan [39], prostaglandin E_2, serotonin and adenosine) decrease the activation threshold, increase the rates of activation and inactivation, and increase the magnitude of tetrodotoxin-resistant Na^+ current [25]. Selective expression of tetrodotoxin-resistant Na^+ channels has been found to be largely restricted to a subpopulation of neurons with nociceptor characteristics [25]. An increase in the Na^+ channel concentration, also reported by Chen et al. [2], may play a role in the generation of ectopic discharges in dorsal roots after the exposure to nucleus pulposus.

Conclusion

Our results showed that application of IL-1β, IL-6, and TNF on the lumbar dorsal roots produced a decreased sensory neural activity, i.e., decreased activity of group III units (A-delta fibers). Possible neurotoxicity of these substances and diminished input to the dorsal spinal horn neurons may lead to sensory changes. In the presence of a spinal hyperexcitability state such as "central sensitization," A-delta and A-beta touch afferents may give rise to the sensation of pain [8].

In our studies, application of IL-1β and TNF on the lumbar dorsal roots also produced increased mechanosensitivity of the peripheral receptive fields. This phenomenon might suggest that dorsal roots may be impaired by these pro-inflammatory cytokines.

Overall, these findings warrant future studies to investigate the effects of dose-response relationships of proinflammatory cytokines and application of multiple cytokines on nerve root and dorsal root ganglion in both acute and chronic studies in order to fully elucidate their potential pathophysiological role in radiculopathy/sciatica.

Acknowledgements The authors wish to thank Srinivasu Kallakuri, MS, and Tsuneo Takebayashi, MD, for their input into this study and Dr. Elaine Hockman, Wayne State University, Research Support Laboratory, for valuable contributions regarding statistical analysis of the data. This study was supported by the NIH/NIAMS-AR44757 (J.A.D.), AR41739 (J.M.C.), the Orthopedic Research and Education Foundation (J.A.D.) and the Bristol-Myers Squibb/Zimmer Foundation (J.A.D. and J.N.W.).

References

1. Aoki Y, Rydevik B, Larsson K, Olmarker K (2000) Local application of disk-related cytokines on spinal nerve roots. Proceedings of the Annual Meeting of the International Society for the Study of the Lumbar Spine, Abstract 92, Adelaide
2. Chen CY, Cavanaugh JM, Ozaktay AC, Kallakuri S, King AI (1997) Effects of phospholipase A_2 on lumbar nerve root structure and function. Spine 22:1057–1064
3. Chen C, Cavanaugh JM, Song Z, Wooley PH, Kallakuri S, Özaktay AC (1999) The effect of nucleus pulposus on lumbar nerve root structure and function. Presented at the 45th Annual Meeting of the Orthopedic Research Society, Anaheim, California
4. Cunha FQ, Lorenzetti BB, Poole S, Ferreira SH (1991) Interleukin-8 as a mediator of sympathetic pain. Br J Pharmacol 104:765–767
5. Cunha FQ, Poole S, Lorenzetti B, Ferreria SH (1992) The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. Br J Pharmacol 107:660–664

6. Davis AJ, Perkins MN (1994) The involvement of bradykinin B1 and B2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat. Br J Pharmacol 113:63–68
7. DeLeo JA, Raymond WC, Michelle N, Malhotra A (1996) Interleukin-6 mediated hyperalgesia/allodynia and increased spinal interleukin-6 expression in a rat mononeuropathy model. J Interferon Cytokine Res 16:695–700
8. Devor M (1994) The pathophysiology of damaged peripheral nerves. In: Wall PD, Melzack R (eds) Textbook of pain. Churchill Livingstone, New York, pp 293–303
9. Dray A, Urban L (1996) New pharmacological strategies for pain relief. Ann Rev Pharmacol Toxicol 36:253–280
10. Eastgate JA, Symons JA, Wood NC, Grinlinton FM, Di Giovine FS, Duff GW (1988) Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet 2:706–709
11. Ferreria SH, Lorenzetti BB, Bristow AF, Poole S (1988) Interleukin 1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. Nature 334:698–700
12. Follenfalt RL, Nakamura-Craig M, Henderson B, Higgs GA (1989) Inhibition by neuropeptides of interleukin-1 β induced, prostaglandin-independent hyperalgesia. Br J Pharmacol 98:41–43
13. Fukuoka H, Hawatami M, Hisamitsi T, Takeshige C (1994) Cutaneous hyperalgesia induced by peripheral injection of interleukin-1 β in the rat. Brain Res 657:133–140
14. Garabedian BS, Poole S, Allchorne A, Winter J, Woolf CJ (1995) Contribution of interleukin 1beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. Br J Pharmacol 115:1265–1275
15. Gratacos J, Collado A, Filella X, Sanmarti R, Cante J, Llena J, Molina R, Ballesta A, Munoz-Gomez J (1994) Serum cytokines (IL-6, TNF-α, IL-1β, and IFN-gamma) in ankylosing spondylitis: a close correlation between serum IL-6 and disease activity and severity. Br J Rheum 33:927–931
16. Guyton AC (1981) Sensory receptors and their mechanisms of action. In: Guyton AC (ed) Textbook of medical physiology. WB Saunders, Philadelphia, pp 588–596
17. Habtemariam A, Gronblad M, Virri J, Seitsalo S, Karaharju E (1998) A comparative immunohistochemical study of inflammatory cells in acute-stage and chronic-stage disc herniations. Spine 23:2159–2166
18. Hori T, Oka T, Hosoi M, Aou S (1998) Pain modulatory actions of cytokines and prostaglandin E2 in the brain. Ann New York Acad Sci 840:269–281
19. Kandel ER, Schwartz JH, Jessell TM (1999) The bodily senses. In: Kandel ER, Schwartz JH, Jessel TM (eds) Principles of neural sciences, 4th edn. V. Perception. McGraw-Hill, New York, pp 430–450
20. Kang JD, Georgescu HI, Larkin LM, Racic MJ, Donaldson III WF, Evans CH (1996) Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6 and prostaglandin E2. Spine 21:271–277
21. Kuslich SD, Ulstrom CL, Michael CJ (1991) The tissue origin of low-back pain and sciatica: a report of pain response to tissue stimulation during operation on the lumbar spine using local anesthesia. Orthop Clin North Am 22: 181–187
22. Lindholm D, Heumann R, Hengerer B, Thoenen H (1988) Interleukin 1 increases stability and transcription of mRNA encoding nerve growth factor in cultured rat fibroblasts. J Biol Chem 263:16348–16351
23. Marshall LL, Trethewie ER, Curtain CC (1977) Chemical radiculitis: a clinical, physiological and immunological study. Clin Orthop 129:61–67
24. McCarron RF, Wimpee MW, Hudkins PG, Laros GS (1987) The inflammatory effects of nucleus pulposus: a possible element in the pathogenesis of low back pain. Spine 12:760–764
25. McCleskey EW, Gold S (1999) Ion channels of nociception. Annu Rev Physiol 61:835–856
26. Oka T, Aou S, Hori T (1993) Intracerebroventricular injection of interleukin-1-beta induces hyperalgesia in rats. Brain Res 624:21–68
27. Olmarker K, Blomquist J, Stromberg J, Nannmark U, Thomsen P, Rydevik B (1995) Inflammatogenic properties of nucleus pulposus. Spine 20:665–669
28. Özaktay AC, Cavanaugh JM, Blagoev DC, Getchell TV, King AI (1994) Effects of a carrageenan-induced inflammation in rabbit lumbar facet joint capsule and adjacent tissues. Neurosci Res 20:355–364
29. Özaktay AC, Cavanaugh JM, Blagoev DC, King AI (1995) Phospholipase A_2 induced electrophysiological and histological changes in rabbit dorsal lumbar spine tissues. Spine 20:2659–2668
30. Özaktay AC, Kallakuri S, Cavanaugh JM (1998) Phospholipase A_2 sensitivity of the dorsal root and dorsal root ganglion. Spine 23:1297–1306
31. Perkins MN, Kelly D (1994) Interleukin-1β induced desArg9bradykinin-mediated thermal hyperalgesia in the rat. Neuropharmacology 33:657–660
32. Rothoerl RD, Woertgen C, Holzschuh M, Rueschoff J, Brawanski A (1998) Is there a clinical correlate to the histologic evidence of inflammation in herniated lumbar disc tissue? Spine 23: 1197–1201
33. Schaible H-G, Schmidt RF (1983) Activation of groups III and IV sensory units in medial articular nerve by local mechanical stimulation of knee joint. J Neurophysiol 49:35–44
34. Seabrook GR, Bowey BJ, Hill RG (1995) Bradykinin receptors in mouse and rat isolated superior cervical ganglia. Br J Pharmacol 115:368–372
35. Smyth MJ, Wright V (1958) Sciatica and intervertebral disc. J Bone Joint Surg Am 40:1401–1418
36. Sorkin LS, Xiao WH, Wagner R, Myers RR (1997) Tumour necrosis factor-alpha induces ectopic activity in nociceptive primary afferent fibers. Neuroscience 81:255–262
37. Takahashi H, Suguro T, Okajima Y, Motegi M, Okada Y, Kauchi M (1996) Inflammatory cytokines in the herniated disc of the lumbar spine. Spine 21:218–224
38. Takebayashi T, Cavanaugh JM, Özaktay AC, Kallakuri S, Chen CY (2001) Effect of nucleus pulposus on the neural activity of dorsal root ganglion. Spine 26:940–945
39. Tanaka M, Cummins TR, Ishikawa K, Dib-Hajj SD, Black JA, Waxman SG (1998) SNS Na^+ channel expression increases in dorsal root ganglion neurons in the carrageenan inflammatory pain model. Neuroreport 9:967–972
40. Wall PD, Devor M (1983) Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve injured rats. Pain 17:321–339
41. Xu XJ, Hao JX, Andell-Jonsson S, Poli V, Bartfai T, Wiesenfeld-Hallin Z (1997) Nociceptive responses in interleukin-6-deficient mice to peripheral inflammation and peripheral nerve section. Cytokine 9:1028–1033
42. Yamashita T, Cavanaugh JM, Özaktay AC, Avramov AI, Getchell TV, King AI (1993) Effect of substance P on mechanosensitive units of tissues around and in the lumbar facet joint. J Orthop Res 11:205–214