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## Effects of interleukin-1 beta, interleukin-6, and tumor necrosis factor on sensitivity of dorsal root ganglion and peripheral receptive fields in rats

Received: 11 June 2005  
Revised: 17 August 2005  
Accepted: 13 September 2005  
Published online: 11 February 2006  
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**Abstract** This study was designed to characterize the effects of low doses (0.5–5 ng) of pro-inflammatory cytokines, interleukin-1 beta (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor (TNF), on the neural activity of dorsal root ganglion (DRG) in rats. The purpose of this study was to examine the effects of cytokines (IL-1 $\beta$ , IL-6, and TNF) on the somatosensory neural response of DRG. The release of inflammatory cytokines by an injured 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 IL-1 $\beta$ , IL-6, TNF, and other algescic chemicals. Their effects on nerve endings in disc and adjacent tissue may lead to low back pain and their effects on dorsal root axons and ganglia may lead to sciatica. Exposed lumbar DRGs were investigated by electrophysiologic techniques. Sham (mineral oil), control (carrier solution), or IL-1 $\beta$ , IL-6, or TNF at doses of 0.5, 1, and 5 ng were applied over the DRG.

Baseline discharge rates as well as mechanosensitivity of the DRG and peripheral receptive fields were evaluated over 30 min. Applications of IL-1 $\beta$  at 1 ng resulted in an increase in the discharge rate, 5 ng resulted in an increased mechanosensitivity of the DRG in group II units. Similarly, after 1 ng TNF applications, group II units also showed an increase in mechanosensitivity of DRG and peripheral receptive fields. At low doses IL-1 $\beta$  and TNF sensitization of receptive fields were observed. The responses observed in the group II units indicate that a sub-population of afferent units might have long-term effects modifying the sensory input to the central nervous system. This study provides added evidence to the role of cytokines in modulating afferent activity following inflammation.

**Keywords** Interleukin-1 beta · Interleukin-6 · Tumor necrosis factor · Dorsal root ganglion · Mechanosensitivity

### Introduction

Several clinical studies indicate that inflamed or irritated spinal roots can be a source of sciatica [21, 37] and that inflammation after disc herniation [12, 34] could be one of the major causes of low back pain. Nucleus pulposus (NP) has been shown to be inflammatory in nature and

was shown to lead to axonal changes [26] through the release of cytokines [4, 17, 25] and other mediators [6, 32, 39].

The presence of interleukin-1 beta (IL-1 $\beta$ ) [11, 39], IL-6 [17, 18, 39], and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [39] in herniated NP was previously reported. Takahashi et al. [39] demonstrated the presence of IL-1 $\beta$ , IL-6, and

TNF- $\alpha$  in the herniated lumbar disc tissue. The levels of IL-1 $\beta$  were 27.5, 38.0, and 74.8 fmol/ml in cultured disc cells obtained from protrusion, extrusion, and sequestration types of disc herniation, respectively. The levels of IL-6 were 137.7, 60.0, and 29.3 pg/ml, respectively. The levels of TNF- $\alpha$  were 58.6, 71.8, and 9.1 pg/ml in the three types of disc herniation tissue, respectively [39]. Similarly, very high levels of IL-6 (>200,000 pg/ml) were detected in the harvested cells of cervical disc specimens [17].

Evidence for the role of these cytokines in painful conditions also comes from several animal studies. The effects of various doses of IL-1 $\beta$ , IL-6, and TNF (0.001–1000 ng) on the expression of inflammatory substances, neural activity, and hyperalgesia were shown to be both time and dose dependent [6, 8–10, 33]. *IL-1 $\beta$* : In a study by Fukuoka et al. [9], various doses of IL-1 $\beta$ , 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-1 $\beta$  produced hypoalgesia in the same study [9]. *IL-6*: IL-6 (6 pg–300 ng) had hyperalgesic effect in the hot plate test when given intracerebroventricularly [24]. *TNF*: TNF- $\alpha$  applied topically at low concentrations (0.001–0.01 ng/ml) along a restricted portion of sciatic nerve elicited a dose-dependent rapid onset (1–3 min) increase in discharging C fibers and to a lesser extent in A-delta fibers; higher concentrations led to reduced firing rates [38]. TNF- $\alpha$  injected subcutaneously sensitized C nociceptors in rats. The optimal dose of 5 ng lowered thresholds in two-thirds of the single fibers tested [15]. In a study with pigs, epidural application of 1.66  $\mu$ g of TNF- $\alpha$  showed a reduction in nerve conduction velocity (CV) recorded from tail muscles similar to the application of nucleus pulposus [2].

Furthermore the reduced conduction velocity in NP-exposed porcine cauda equina [19, 25, 29] was shown to be prevented by selective inhibition of TNF- $\alpha$  [27]. Similarly, NP release following a disc incision was also shown to decrease CV in dogs [20]. Neurophysiologically, cytokine application on dorsal nerve roots in rats was shown to cause a statistically significant decrease in neural activity with increased sensitivity of the tail receptive field [31]. Dorsal root ganglion (DRG) exposed to NP was also shown to develop edema as evidenced by the presence of enlarged space between cell bodies and axons [28].

However, studies aimed at understanding the neurophysiological effects of cytokine application on DRG are limited. Mechanosensitivity of DRG was shown to increase after the exposure of the L<sub>5</sub> nerve root to autologous NP [40]. This may be related to the excitatory effects of NP on DRG [40]. Significant levels of glutamate, the excitatory neurotransmitter, were also shown in herniated disc material, and a modulation of glutamate receptors has been suggested [13].

Considering the effects of various NP-derived chemical mediators on spinal nerve root, this study attempts to investigate the acute effects of low doses of cytokines IL-1 $\beta$ , IL-6, and TNF on DRG. It has been hypothesized that sensitization of DRG may be an important factor in the etiology of low back pain and sciatica.

## Materials and methods

### Preparation of the animals

All procedures were approved by the institutional animal investigation committee. Nineteen 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 L<sub>3</sub>/S<sub>2</sub> spinous processes. An L<sub>4</sub>/S<sub>1</sub> laminectomy was performed exposing the dura and L<sub>5</sub> DRG. The rat spine was secured at the second lumbar and third sacral spinous processes by a spine-holding device. After opening the dura, the left L<sub>5</sub> dorsal root innervating the left hind paw was exposed. A pool was formed from skin flaps, and the spinal cord was covered in warm (37°C) mineral oil (Fig. 1).

### Neurophysiological recording

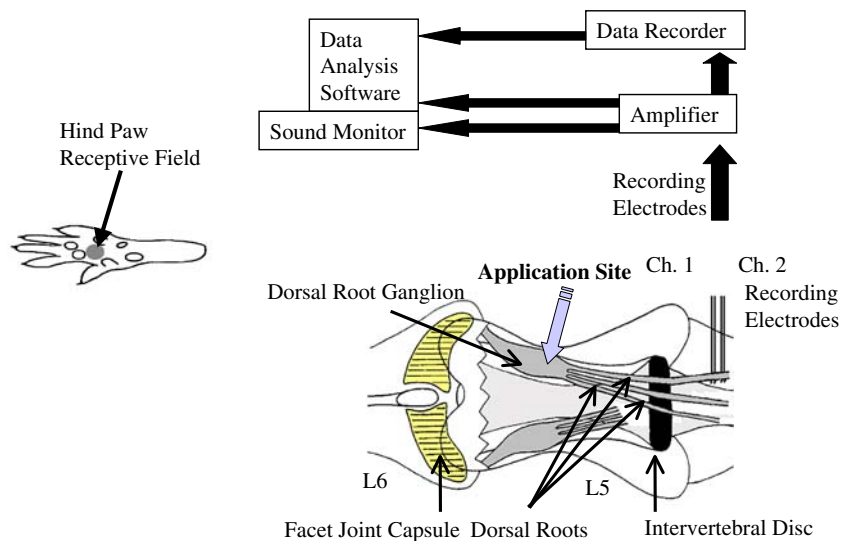
The L<sub>5</sub> dorsal roots were cut at their proximal ends and split longitudinally into fine rootlets. One of the split rootlets was draped over a dual-bipolar platinum-recording electrode to record the afferent impulses (units) from nerve fibers innervating the hind paw (receptive field).

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 were part of the Enhanced Graphics Acquisition and Analysis system (EGAA, R.C. Electronics, Goleta, CA, USA). The data were also simultaneously recorded on an analog tape recorder (MR-30, TEAC, Montebello, CA, USA).

### Identification of single units

In each experiment, the recorded multi-unit activity was analyzed by the EGAA software to distinguish the single units. A single unit, extant in both channels, was identified by its amplitude and conduction velocity (CV). To calculate the CV, the distance between the recording

**Fig. 1** Diagram of rat lumbar spine showing electrophysiologic experimental set-up



electrodes (millimeters) was divided by the onset latency (in milliseconds) of the evoked response. They were further classified based on their CVs into: group IV fibers (0.5–2  $\mu\text{m}$  diameter unmyelinated C fibers; CV < 2.5 m/s), group III fibers (1–5  $\mu\text{m}$  diameter finely myelinated A-delta fibers; CV 2.5–20 m/s), and group II fibers (5–12  $\mu\text{m}$  diameter myelinated A-beta fibers; CV 20–70 m/s) [36].

#### *Mechanosensitivity measurements*

In each experiment, the hind paw receptive field and the corresponding L<sub>5</sub> DRG were identified. This was accomplished by observing the neural activity responses obtained from the corresponding dorsal roots while mechanically stimulating the hind paw receptive field. The mechanical threshold was characterized using successively stronger nylon filaments (compression magnitude ranging from 0.17 to 24.4 g; Aesthesiometer; Stoelting, Wood Dale, IL, USA) until an increase in discharge rate could be elicited. Mechanosensitivity was determined 15 min before (baseline) and 30 min after each application of a particular cytokine, saline solution (control), or mineral oil (sham).

#### *Application of IL-1 $\beta$ , IL-6, and TNF*

In all experiments ( $n=19$ ), after characterizing the receptive field, 10  $\mu\text{l}$  of carrier solution as controls was applied on the surface of the left L<sub>5</sub> DRG distal to the recording electrodes using a micropipette. This was followed by successive applications of 0.5, 1, and 5 ng of the cytokine of interest: IL-1 $\beta$  ( $n=4$ ), IL-6 ( $n=5$ ), TNF ( $n=5$ ) (Pepro Tech, Rocky Hill, NJ, USA). Each cytokine application was prepared in 10  $\mu\text{l}$  of carrier saline solution (0.9% buffered at pH 7) and the mineral oil

pool kept the solution at the site of application on DRG. In a subgroup of experiments ( $n=5$ ) mineral oil (sham) was also applied over the DRG prior to the cytokine studies.

#### *Discharge rate changes*

Multiple applications were performed in each experiment. The discharge rate of the units was followed for over 45 min in each application as described below. First, 15 min prior to the application, the spontaneous baseline neural activity (SDR) was recorded. Then the hind paw receptive field and the DRG were tested for their mechanosensitivities. The SDR was then recorded continuously during the application period beginning 1 min before the application until 5 min post-application. The SDR was further recorded every 5 min with 1 min duration for a total period of 30 min after the application. After this, the hind paw and the DRG receptive fields were tested again to compare with the pre-application threshold values. Additionally, any sudden changes in activity observed on the oscilloscope, audio-monitor, or EGAA histogram were also recorded until they were stabilized for at least 2 min. Between each application, the spinal canal was rinsed by replacing the mineral oil, and waited for 30 min till the next application.

#### *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 or window discrimination system software was used to discriminate and count spikes. The SDR was calculated as impulses per second (imp/s). The change in discharge

rate for each experiment was expressed as a percentage change from the baseline SDR. Mechanosensitivity thresholds 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 CVs. For statistical analysis, time effects were analyzed by repeated measures analysis of variance (ANOVA) within groups as well as between subjects. Pair-wise 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 mean  $\pm$  standard error of the mean (SEM). Statistical results were categorized by the following changes in discharge rate: (a) entire trend over time, (b) at individual time points compared to previous activity, (c) similarities of each unit within its own group, and (d) comparison of the response patterns of different groups.

## Results

### Comparison of the response patterns

The multi-unit discharge rate in all the applications was followed over a 30 min period and was grouped accordingly (control, sham, IL-1 $\beta$ , IL-6, and TNF). When each group was compared to the others, there were no statistical differences observed with their baseline and the response patterns over time.

### IL-1 $\beta$ applications

*Multi-unit discharge rate (n=4 each application)* The only significant transient increase observed was at the time of application of 0.5 ng IL-1 $\beta$  (data not shown, *P*<0.04). The discharge rate stayed very stable for the remainder of the 30 min. In applications of both 1 and 5 ng of IL-1 $\beta$ , there were no changes over time in the multi-unit discharge rates.

*Single units* Eight group II units (39.75  $\pm$  6.14 m/s) and four group I units (>70 m/s) were characterized. One minute after the application of 1 ng IL-1 $\beta$  application, the SDR of group II units displayed a significant decrease. The SDR then showed a significant increase over 30 min (Fig. 2a.1, *P*<0.0001). There was no other significant change in the SDR over time in all other IL-1 $\beta$  applications.

*Mechanosensitivity* Significant decrease in DRG mechanical threshold (increased sensitivity) was observed in group II units from 0.36  $\pm$  0.09 to 0.19  $\pm$  0.05 g 30 min after 5 ng IL-1 $\beta$  applications (Fig. 2b.2, *P*<0.005).

### IL-6 applications

*Multi-unit discharge rate (n=5 each application)* There was no change in multi-unit discharge rate over time after the 0.5, 1, and 5 ng IL-6 applications.

*Single units* Six group III (9.83  $\pm$  2.07 m/s), four group II (35.00  $\pm$  8.29 m/s), and six group I units (>70 m/s) were identified. All showed similar responses within their groups (*P*<0.009) but none showed significant changes over time. There were only four group II units and they did not display any change over time. After the applications of 1 and 5 ng of IL-6, their SDRs were not similar to each other within their own group. This may indicate that a sub-population of these group II units may respond to 1 and 5 ng of IL-6 differently.

*Mechanosensitivity* There was no change in the mechanosensitivity of the receptive fields. They all showed a similar pattern in their responses (*P*<0.0001).

### TNF applications

*Multi-unit discharge rate [0.5 ng (n=5), 1 ng (n=4), and 5 ng (n=4)]* After each application, the discharge rates showed similar response patterns (*P*<0.009) but no change was observed over 30 min after the 0.5, 1, and 5 ng TNF applications.

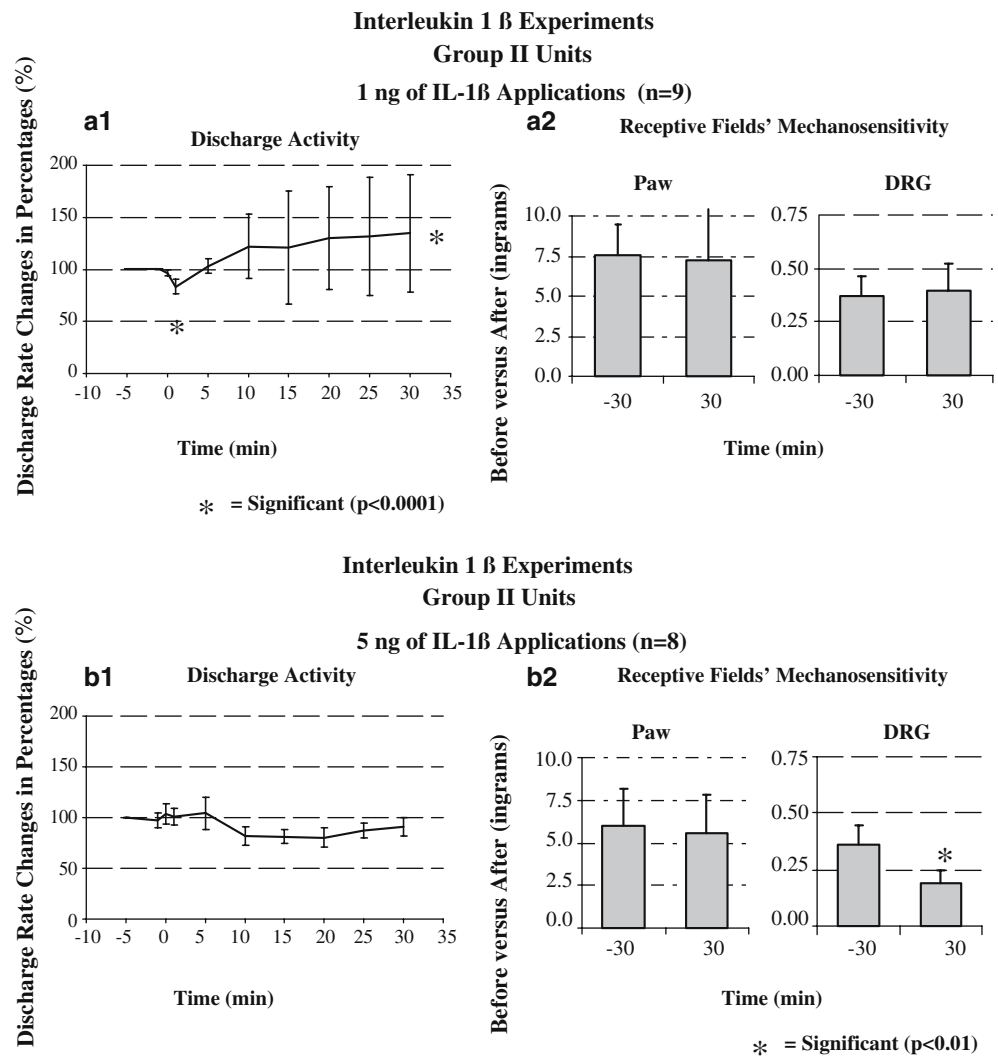
*Single units* A total of four group III (7.25  $\pm$  2.28 m/s), ten group II (41.70  $\pm$  2.91 m/s), and four group I units (>70 m/s) were analyzed. They all displayed similar patterns in their groups (*P*<0.003) but none showed any significant change over time in their discharge rate. Following 1 ng TNF application, two group III and two of the ten group II units were undetectable. Due to the limited number of units in 5 ng of TNF applications only group II units (*n*=7) were analyzed statistically. They showed no significant changes over time.

*Mechanosensitivity* After the 1 ng TNF applications, only group II units (*n*=8) showed decreased thresholds from 27.70  $\pm$  11.70 to 13.68  $\pm$  4.07 g in hind paw; from 0.65  $\pm$  0.20 to 0.46  $\pm$  0.18 g in DRG (Fig. 3a, b, respectively, *P*<0.02). No other statistically significant changes were observed.

### Control solution applications

*Multi-unit discharge rate (n=19)* A statistically insignificant increase in discharge rate was observed during the saline solution application. This immediately returned to baseline after the application without further changes over 30 min. They all showed a similar pattern throughout the experiments (*P*<0.0001).

**Fig. 2 a1** Group II units' response to the applications of 1 ng of IL-1 $\beta$  have displayed a transient decrease in discharge rate immediately after the application ( $P < 0.018$ ), then showed increasing trend over time ( $P < 0.0001$ ). **a2** However, no change in mechanosensitivities of the receptive fields was observed in these IL-1 $\beta$  applications. **b** Group II units' response to the applications of 5 ng IL-1 $\beta$ . There was no change in the response pattern of the group II units in 5 ng of IL-1 $\beta$  applications. On the other hand only 30 min after the 5 ng IL-1 $\beta$  applications, DRGs have displayed increased sensitivity when compared prior to the application ( $P < 0.005$ )



**Single units** A total of 45 individually characterized units were followed over 30 min after each saline solution application. Six group III ( $16.50 \pm 2.09$  m/s), 23 group II ( $48.22 \pm 2.89$  m/s), and 16 group I units ( $> 70$  m/s) were identified. Each group displayed a similar pattern within their groups ( $P < 0.007$ ) but no changes in their discharge rate over time were observed.

**Mechanosensitivity** After the saline solution application, only group II units ( $n = 22$ ) showed decreased thresholds from  $0.70 \pm 0.08$  to  $0.50 \pm 0.06$  g in DRG (Fig. 4b,  $P = 0.042$ ). No other changes in mechanosensitivity were observed in other receptive fields and groups.

**Sham (mineral oil) studies**

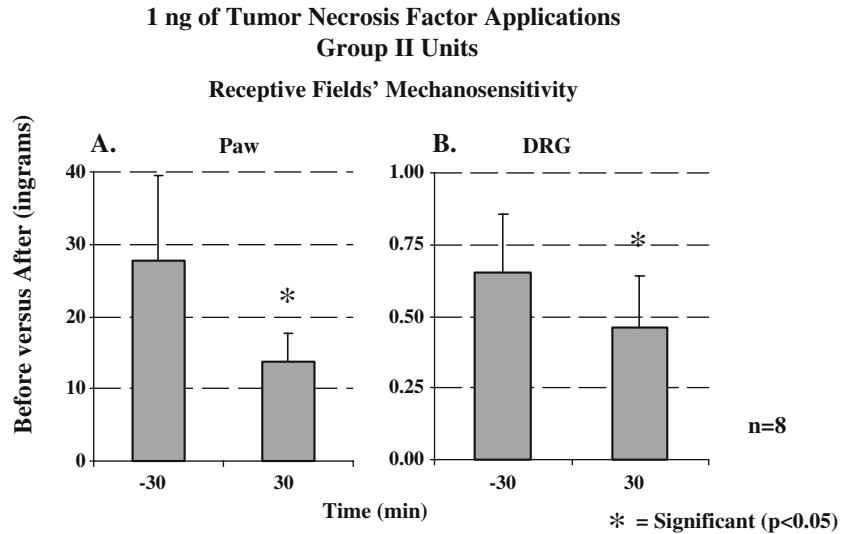
Although the discharge rates displayed a similar pattern, no change was observed after the mineral oil

applications ( $n = 6$ ) in both mechanosensitivities of the receptive fields and the discharge rate of units. We identified five group III units ( $11.00 \pm 2.29$  m/s), six group II ( $46.00 \pm 8.42$  m/s), and eight group I units. None showed any change in discharge rate and mechanosensitivity of the DRG and paw receptive fields. Group III and group I units also displayed a similar pattern ( $P < 0.0001$ ) in their groups but not the group II units. This may indicate that a sub-population of these group II units may respond differently.

## Discussion

The present study demonstrated that applications of low doses of IL-1 $\beta$  and TNF resulted in the sensitization of receptive fields. Applications of 1 ng IL-1 $\beta$  resulted in an increased neural activity (discharge rate), 5 ng resulted in an increased mechanosensitivity (more sensitive) of

**Fig. 3** Changes in group II units' ( $n=8$ ) mechanosensitivity of both the receptive fields, **a** paw and **b** DRG, after the 1 ng TNF application fields exhibited increased sensitivity ( $P < 0.02$ )



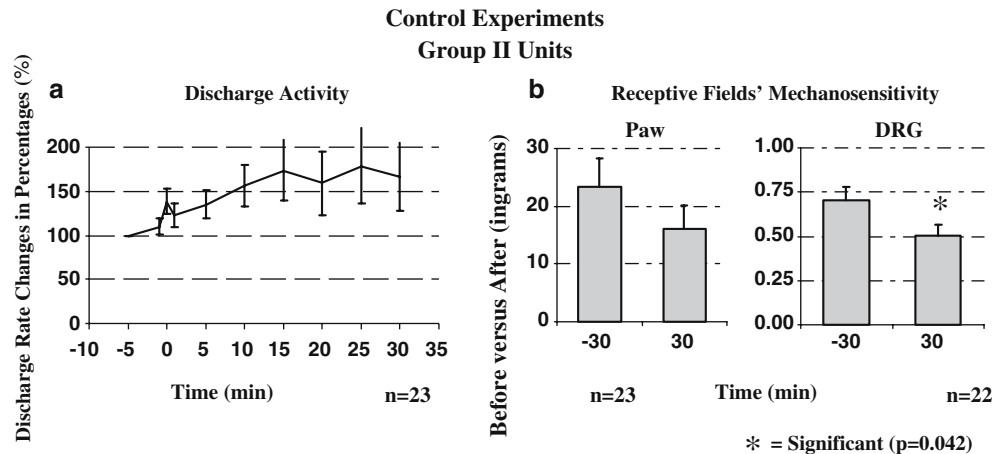
the DRG in group II units. Similarly, after 1 ng TNF applications, group II units also showed an increase in mechanosensitivity of DRG and peripheral receptive fields.

In our previous study with the same cytokine applications on the dorsal roots, TNF applications (100 ng) resulted in a significant decrease in multi-unit discharge activity that correlated with increased mechanosensitivity after 75 min. An increase in group III units for 15 min and in peripheral mechanosensitivity over 3 h were also observed. Both group III and group II units later displayed a decrease in activity after 1 h. The peripheral mechanosensitivity of group II units was also increased at the half hour and 2 h later [31]. The increased mechanosensitivity following TNF application in the current study provides added credence to the role of TNF and other cytokines in the initiation and maintenance of peripheral hypersensitivity [31]. Homma

et al. [14] have shown that infusion of TNF to normal L<sub>5</sub> DRG resulted in ipsilateral mechanical allodynia lasting for 2 weeks while infusion to an injured L<sub>5</sub> DRG resulted in the enhancement of ipsilateral allodynia after the first postoperative week. Schafer et al. [35] have shown that TNF (0.1–10 pg/ml) injected into L<sub>5</sub> DRG of rats results in long-lasting allodynia in naïve rats and faster onset with spontaneous behavior in rats with spinal nerve ligation (SNL).

Although there was no change in the paw mechanical threshold following IL-1 $\beta$  application, the DRG receptive fields (group II units) were more sensitive as to indicate a role for spinal IL-1 $\beta$  in the mechanisms underlying hyperalgesic states. Bianchi et al. [3] reported that intrathecal administration of IL-1 $\beta$  (500–5 pg) induced significant hyperalgesic effect in the hot plate test and enhanced nociceptive thresholds to mechanical stimuli. In another study, electrophysiological record-

**Fig. 4** Group II units' responses to the applications of control solutions. **a** Their discharge activity over time did not make any significant change. **b** Only the DRG receptive fields ( $n=22$ ) were more sensitive to the mechanical stimuli 30 min after the applications ( $P=0.042$ ) but not the paw receptive fields ( $n=23$ )



ings have shown that small-diameter cutaneous nerves are activated by subcutaneous IL-1 $\beta$  resulting in behavioral hyperalgesia [9]. In our previous study with IL-1 $\beta$  (100 ng) application on dorsal roots, an immediate but transient increase in multi-unit discharge activity and increased peripheral mechanosensitivity after 90 min were observed. Furthermore, the group III units' discharge rate also increased initially and decreased after 10 min. This was also correlated with their peripheral mechanosensitivities as it increased over 3 h [31]. In addition, Anzai et al. [1] showed that exposure of nerve roots to NP resulted in remarkably enhanced responses of wide dynamic range neurons to noxious stimuli and suggested that pathogenic factors in NP may have a crucial role in the induction of hyperalgesia.

**DRG mechanosensitivity** Our results have also shown altered mechanical sensitivity of DRG group II units following both IL-1 $\beta$  and TNF applications. Mechanical threshold of DRG decreased (i.e., *increased sensitivity*) after application of IL-1 $\beta$  (5 ng from 0.36 to 0.19 g) and TNF (1 ng from 0.65 to 0.46 g), but not with IL-6 application. Notably, all the three doses of TNF resulted in reduced DRG threshold although the statistically significant decrease was observed only at 1 ng applications. Increased sensitivity of group II units in our study could be similar to the results of Liu et al. [22] who have shown that acute topical application of TNF to the DRG for 15 min evokes C and A-beta fiber responses in both normal and chronically compressed DRG with short response latency and high peak discharge rate in chronically constricted DRG fibers. Schafers et al. [35] have shown that perfusion of TNF (100–1000 pg/ml) to naïve DRG resulted in short-lasting neural discharges. In injured DRG, TNF at concentrations that were sub-threshold in naïve resulted in earlier onset, markedly higher and longer lasting discharges. They concluded that injured and adjacent uninjured DRG neurons are sensitized to TNF after SNL. Sensitization to endogenous TNF and even IL-1 $\beta$  may be essential for the development and maintenance of neuropathic pain or painful states. Takebayashi et al. [40] have shown that the application of autologous NP on L<sub>5</sub> nerve root just proximal to DRG resulted in increased spontaneous neural activity with increased mechanosensitivity of DRG after 6 h. Similarly, the application of NP was also shown to result in reduced CV [19, 25, 29]. Nevertheless, the application of TNF resulted in more pronounced CV reduction than NP. In a similar study, the application of IL-1 $\beta$  was also shown to result in reduced CV when compared to fat [2].

In this study, there were no marked effects by the application of IL-6. This may be similar to studies by Oprea and Kress [30] who have shown that IL-6 alone

may not be sufficient to induce heat sensitization but was effective only in the presence of a soluble IL-6 receptor unlike IL-1 $\beta$  and TNF. This may support the need for the presence of constitutively expressed IL-6. The application of IL-6 (100 ng) on dorsal roots showed a decrease in multi-unit discharge rate after 105 min with no changes in mechanosensitivity. An initial increase in discharge rates of group III units with a decrease after 135 min was also observed [31]. Above all, the doses that were tested in this study, 0.1, 1, and 5 ng, were considerably lower than we have reported in the earlier study (100 ng to dorsal roots) [31].

The reduced mechanical threshold in control applications is merely significant ( $P=0.042$ ). The subtle response in control applications could be attributed to an experimental artifact, as it was never experienced in any of our previous control studies. Above all, the reduced threshold was indeed a result of chemical sensitization is well supported by IL-1 $\beta$  and TNF applications, particularly with TNF showing a dose-dependent response.

The observation of a changed peripheral sensitivity in a distant receptive field such as paw following IL-1 $\beta$  and TNF application may also be similar to the development of altered paw sensitivity following experimental neuritis in rats [7]. Local application of IL-1 $\beta$  and TNF around nerve root may be similar to the release of inflammatory mediators following a disc rupture. This has wide ranging implications such as an innocent bystander effect as described by Eliav et al. [7] in which a disease process near a nerve or root may secondarily involve it in an inflammatory milieu. In the etiology of radicular and low back pain this may be particularly significant, as there is a leakage of NP, which is known to be inflammatory in nature. It is also important to note the increased activity of group II units and their changed mechanical threshold may be a neurophysiological equivalent of peripheral hypersensitization leading to the onset of allodynia. The presence of cytokines in NP [11, 17, 19] and their ability to induce various inflammatory [23], neurophysiological [5, 27, 29], and altered peripheral sensitivity changes [16, 25, 26] have been previously reported.

Overall, this study shows that low doses of IL- $\beta$  and TNF are capable of mediating sensitivity changes at the periphery and on DRG. The ability of TNF to trigger the changes at both locations in the light of its ability to induce other changes in axons and DRG supports the role of inflammatory mediators in low back pain and sciatica.

**Acknowledgements** The authors wish to thank Dr. Elaine Hockman, Wayne State University, Research Support Laboratory, for valuable contributions regarding statistical analysis of the data. Supported by the NIH/NIAMS-AR41739 (JMC), AR44757 (JAD), the Orthopedic Research and Education Foundation (JAD) and Bristol-Myers Squibb/Zimmer Foundation (JAD and JNW).

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