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Increased nerve growth factor after rat plantar incision contributes to guarding behavior and heat hyperalgesia

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Abstract

Acutely, nerve growth factor (NGF) exerts profound effects on nociceptive transmission and produces pain and hyperalgesia. In the present study, we sought to determine the tissue levels and role of NGF after a plantar incision. A substantial increase in NGF protein expression occurred in skin 4-h, 1-day and 2-days and 5-days after incision comparing contralateral uninjured skin. Plantar incision did not change NGF levels in the tibial nerve and L4-L6 dorsal root ganglia. The therapeutic effect of a monoclonal antibody against endogenous NGF was evaluated by intraperitoneal administration of a single preoperative dose of anti-NGF. Of three different doses of anti-NGF used, the highest dose 2.8 mg/kg anti-NGF attenuated or almost abolished guarding pain scores at 4-h, 1-day (>80% decrease) and 2-days after incision. This effect is dose dependent in that lower doses (1, 0.1 mg/kg) were effective only at 1-day after incision. Anti-NGF also attenuated heat hyperalgesia at 1-day and 2-days after incision when the highest dose was used. However, treatment by anti-NGF did not affect C-fibers sensitized 1-day after incision in a glabrous skin-tibial nerve in vitro preparation. In conclusion, increased NGF was present in skin after plantar incision. NGF contributes to some incision-induced pain behaviors, guarding and heat hyperalgesia. Anti-NGF did not affect the extent of sensitization of C-fibers observed in vitro.

1. Introduction

After tissue damage a number of substances are released within the local tissue environment, which increase the excitability of nociceptors. One of these substances is nerve growth factor (NGF), a prototypic neurotrophin that was initially characterized for its role in survival and development of sensory and autonomic neurons. NGF has far broader biological activities including modulation of nociceptive sensory neuron properties (for review, see Levi-Montalcini 1995). NGF is produced from non-neuronal cells and binds to its receptors located on nociceptors, and this complex is retrogradely transported to the sensory nerve cell body (Richardson and Riopelle, 1984). NGF produces localized pain and tenderness when injected intradermally in humans (Dyck et al., 1997). The parenteral administration of NGF in rodents results in profound heat and mechanical hyperalgesia (Lewin et al., 1993). NGF induces these exaggerated responses through several putative mechanisms. It has been shown to upregulate neuropeptides (Donnerer et al., 1992), tetrodoxin-resistant, voltage-dependent sodium channels (Gould et al., 2000), acid sensing ion channels (Mamet et al., 2003), and the capsaicin receptor VR1 (Ji et al., 2002). Neutralization of endogenous NGF decreases inflammatory hyperalgesia (Koltzenburg et al., 1999; McMahon et al., 1995; Woolf et al., 1994).

In this study, we systematically carried out experiments in a rat model of incisional pain (Brennan et al., 1996) to determine the role of NGF in pain behaviors and C-fiber sensitization caused by plantar incision. The plantar incision model has spontaneous pain-like behaviors and mechanical and heat hyperalgesia which are very similar to human postoperative pain and the responses of humans to a small incision (Kawamata et al., 2002). During the last few years this animal model has improved our understanding of

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postoperative pain; thus far, data indicate that incisional pain mechanisms are distinct from other experimentally induced inflammation models. For example, the response to anti-inflammatory agents (Whiteside et al., 2004; Zahn et al., 2004), spinal *N*-Methyle-D-aspartate (NMDA) receptor blockade (Zahn and Brennan, 1998), spinal non-NMDA receptor blockade (Zahn et al., 1998) and parenteral $P2X_{2/3}$ receptor antagonists (Jarvis et al., 2002) are different in incisional pain compared to other models.

In the present study we examined the level of NGF at different times after incision in skin, tibial nerve and dorsal root ganglia (DRG). We also injected a single preoperative dose of anti-NGF antibody to inhibit incision induced pain-related behaviors. To determine the role of NGF on primary afferent sensitization, we examined the effect of anti-NGF treatment on afferent fibers in the incised glabrous skin. The preliminary account of our study was reported (Banik et al., 2003).

2. Materials and methods

The studies followed the proposals of the Committee for Research and Ethical Issues of the IASP (Zimmermann, 1983) and were approved by the institutional Animal Care and Use committee, The University of Iowa.

2.1. Animals and surgery

Adult male, 225–275 g, Sprague-Dawley (Harlan, Indianapolis, IN) rats were used for all the experiments. They were housed postoperatively in groups of two to three each in clear plastic cages. Food and water were available ad libitum.

A previously described plantar incision animal model (Brennan et al., 1996) was used. Rats were anesthetized with 1.5–2% halothane and the surgical field was prepared in a sterile manner. A 1-cm longitudinal incision was made in the plantar aspect of hindpaw beginning 0.5 cm from the end of heel; skin, fascia and muscle were incised and the skin was closed with 5-0 nylon suture. Topical antibiotics were administered. At the second postoperative day, sutures were removed under a brief anesthesia.

2.2. Quantitative NGF estimation by enzyme-linked immunosorbent assay

The hindpaw skin, tibial nerve, and lumbar (L4-L6) dorsal root ganglia were taken from rats killed with CO_2. Tissue samples were taken from control rats and rats 4-h, 1, 2, 5 and 10 days following plantar incision. The number of animals was five in each group. All tissue samples were rapidly frozen on dry ice and homogenized in ice-cold lysis buffer, containing 137 mmol/L NaCl, 20 mmol/L Tris–HCl buffered to pH 8, 1% NP40, 10% glycerol, 1 mmol/L phenomethanesulfonyl fluoride, 10 μg/mL aprotinine, 1 μg/mL leupeptin and 0.5 mmol/L sodium vanadate. The homogenates were centrifuged twice at 15,000 rpm for 20 min at 4 °C and the supernatant was taken. The protein concentration was determined using modified Lowry protein assay (Pierce, Rockford, IL). The NGF concentration was measured using a commercially available assay following the manufacturer's instructions (NGF Emax Immunoassay, Promega, Madison, WI). The assay quantitates a minimum of 7.8 pg/ml NGF and has very low cross reactivity with structurally related growth factors.

2.3. Immunological neutralization of endogenous NGF

2.3.1. Anti-NGF or IgG (vehicle) administration

Each group of 6–8 rats received a single dose (0.1, 1 or 2.8 mg/kg, i.p.) of anti-NGF (generously provided by David Shelton, Rinat Neuroscience, Palo Alto, CA) and the other group received 5 mg/kg of human Immunoglobulin G (IgG) (Michigan Department of Public Safety, Lansing, MI) 1-day before plantar incision. The anti-NGF antibody was raised in mouse and found to neutralize NGF protein. It has 1:10,000 affinity for NGF over brain derived growth factor and neurotrophin-3. In small number of rats, a commonly used commercially available rat anti-NGF (Sigma) or IgG 0.3 mg/kg were injected intravenously 30 min before incision. The persons performing behavioral experiments were blinded to the drug administered.

2.3.2. Assessment of pain behaviors

Sham operated rats, without incision, were used as controls. Pain behaviors were measured before incision, 4-h, 1-day, 2-days, 3-days and 5-days after incision.

2.3.3. Guarding behaviors

In a separate groups of rats, a pain score was determined to assess guarding pain behaviors as described previously (Zahn et al., 1997). Unrestrained rats were placed on a small plastic mesh floor (grid 8×8 mm). Using an angled magnifying mirror, the incised and non-incised paw was viewed. Both paws of each animal were closely observed during one-minute period repeated for every 5-min for 1-h. Depending on the position in which each paw was found during the majority of 1-min scoring period a 0, 1 or 2 was given. A score of 0 was given for full weight bearing with the area of the wound blanched or distorted by the mesh, 1 for the wound area just touching the mesh without blanching or distortion and 2 for the wound area completely off of the mesh. The sum of the 12 scores (0–24) obtained during 1-h session for each paw was obtained.

2.3.4. Heat sensitivity

Rats were placed individually on glass floor covered with clear plastic cage and allowed to acclimate. Withdrawal latencies to radiant heat were assessed by applying a focused radiant heat source underneath a glass floor on the middle of incision. The latency time to evoke a withdrawal was determined with a cut-off value of 30 s. The intensity of the heat was adjusted to produce withdrawal latency in normal rats of 25–30 s. Each rat was tested at least three times, at an interval of 10 min. The average of at least three trials was used to obtain paw withdrawal latency.

2.3.5. Mechanosensitivity

Rats were placed individually on plastic mesh floor covered with clear plastic cage and allowed to acclimate. Withdrawal response to punctate mechanical stimulation was determined using calibrated von Frey hairs applied underneath the cage to an area adjacent to the incision. Each filament was applied once starting with 10 mN and continuing until a withdrawal response occurred

or 250 mN was reached. If a rat did not respond to the 250 mN filaments, 522 mN, the next filament was recorded. This was repeated a total of three times with at least a 5–10 min test free period between withdrawal responses. The lowest force from the three tests producing a response was considered the withdrawal threshold.

2.3.6. Neurophysiological studies: glabrous skin-nerve in vitro preparation

Activities of C-fibers recorded in an in vitro glabrous skin-nerve preparation from incised rats receiving anti-NGF (2.8 mg/kg) 1-day before incision.

2.3.6.1. General. The rat glabrous skin-nerve in vitro preparation, modeled as saphenous nerve-skin preparation (Reeh, 1986), has been described elsewhere (Banik and Brennan, 2004). In brief, rats were killed with CO_2; the medial and lateral plantar nerves and their innervated territory on the glabrous hindpaw skin were subcutaneously dissected until the nerve and skin could be removed. The skin was placed 'epidermal side down' in the in vitro perfusion chamber, and it was superfused with a modified Krebs-Hensleit solution, which was saturated with a gas mixture of 95% O_2 and 5% CO_2. The temperature of bath solution was maintained at 32 ± 0.5 °C. The plantar nerves were drawn through a small hole into the recording chamber where aqueous solution was overlaid by a layer of paraffin oil. The nerve was placed on a mirror, and small filaments of nerve were repeatedly split with sharp forceps and thin needles until single-unit activity could be recorded. Neural activity was amplified and filtered using standard techniques. Amplified signals were led to a digital oscilloscope and an audio monitor and fed into PC computer via a data acquisition system (spike2/CED1401 program).

2.3.6.2. Recording and stimulation procedures. In this study we focused on the mechanosensitive C-fiber nociceptors. Receptive fields of the units were identified by probing with a blunt glass rod in the corium side of skin. Conduction velocity of a fiber was determined by monopolar electrical stimulation into the receptive field. Then the distance between receptive field and the recording electrode (conduction distance) was divided by the latency of the action potential.

2.3.6.3. Heat stimulation. A standard heat ramp was delivered by a feedback-controlled heat stimulator. The receptive field of unit was isolated with a small metal ring, which could seal by its own weight. The bath solution within the ring was manually removed with a syringe. A thermocouple gently placed to measure intracutaneous temperature. A radiant lamp was placed in the translucent area underneath of the organ bath and the light beam was focused onto the epidermal side of the skin. A computer-controlled standard heat ramp was applied starting from 33 °C and rising to 48 °C in 15-s.

2.3.6.4. Data analysis. Action potentials collected on a computer were analyzed off-line with a template-matching function of Spike 2 software (Cambridge Electronic Design Ltd, Cambridge, UK). If a unit had discharges at a rate of 0.1 imp/s or more without any intentional stimuli, it was categorized as spontaneously active. The threshold of a unit was considered if the temperature that elicited the second action potential when background activity was absent or greater than 50% increase of any spontaneous activity. The quantitative analysis was carried out by averaging responses in 1-s bins and by counting total evoked action potentials in a response. For counting total impulses generated in a unit that had spontaneous activity, total spikes in 30-s control period were subtracted from that in 30-s after the stimulation.

2.4. Statistics

At each time point, NGF levels in the incised tissues were compared with that in unincised contralateral tissues by an unpaired *t*-test. Among behavioral data, Mann-Whitney test was used for comparing paw withdrawal latencies and Dunnett's test was used for comparing guarding pain scores. A chi-square test was used to compare the percentage of spontaneously discharging C-fibers and percentage of heat sensitive fibers between groups. The heat response thresholds were compared using Dunnett's test. The total evoked action potentials during each heat stimulation were compared by a nonparametric Dunn's multiple comparison test. $P < 0.05$ was considered statistically significant. Data are presented as mean $\pm$ SD in the text and mean $\pm$ SEM in the figures.

3. Results

3.1. NGF levels in skin, tibial nerve and DRG tissues

The basal NGF expression in the glabrous part of the right and left hindpaw skin respectively was 0.20 ± 0.05 and 0.16 ± 0.04 ng/mg of total protein. As shown in Fig. 1(A), the levels of incised skin NGF gradually increased over time with a peak at 2 days after incision. The increases from the contralateral sides were 2.6-($P < 0.001$), 5.6-($P < 0.001$) and 15.5-fold ($P > 0.005$), respectively, at 4 h, 1 day and 2-days after incision. The increase in incised skin NGF levels had dropped to 9.4-fold ($P < 0.05$) and 5.5-fold by 5 and 10 days after incision. The NGF expression in the tibial nerve and L4-L6 DRG were not influenced by plantar incision (Fig. 1(B, C))

3.2. Effects of anti-NGF treatment on incision induced pain related behaviors

3.2.1. Mechanical hyperalgesia

Before incision, the median withdrawal threshold to von Frey hair stimulation was 522 mN and this decreased to 10 mN in the incised paw at 4-h and 1-day after incision. The paw withdrawal thresholds in the incised rats were not changed after anti-NGF 2.8 mg/kg treatment when compared with incised rats with IgG treatment (Fig. 2(A)).

3.2.2. Guarding pain scores

Incision caused guarding pain scores (see Section 2) to increase at 4 h, 1-day and 2-days after incision. As seen in Fig. 2(B), anti-NGF treatment reduced the pain score in a dose dependent manner. The pain scores were significantly less in rats treated with anti-NGF 2.8 mg/kg 4 h after

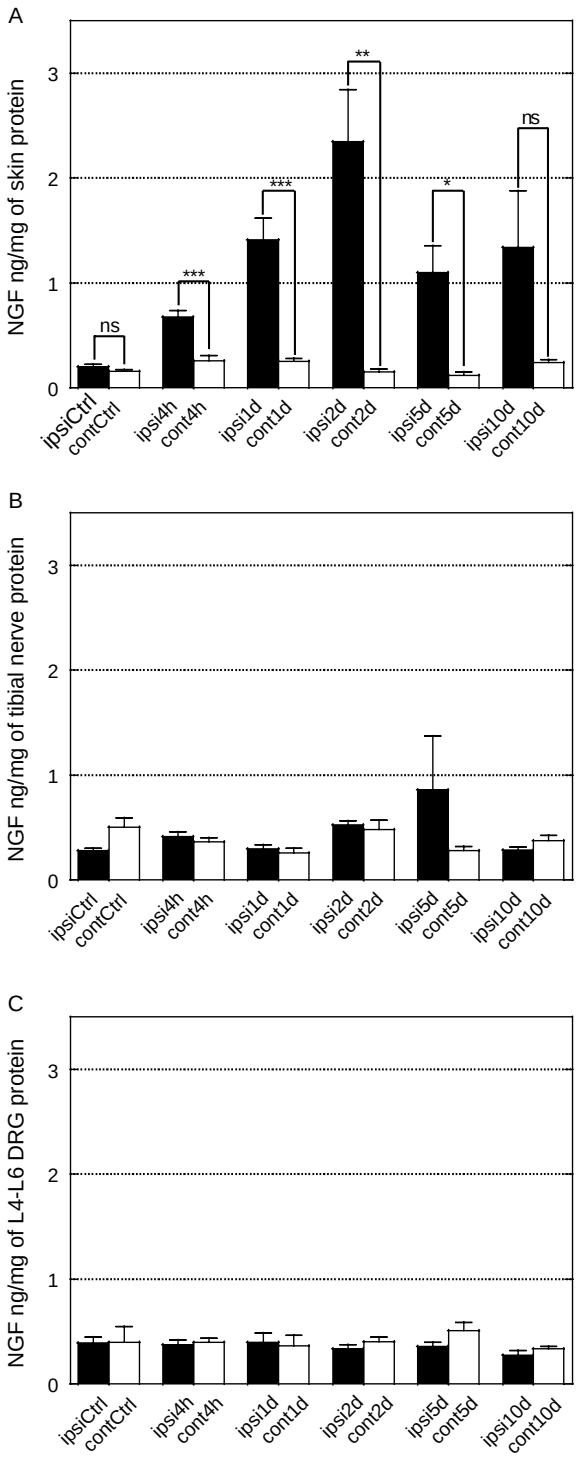

Fig. 1. Temporal expression profiles of nerve growth factor (NGF) protein in the skin, tibial nerve and L4-L6 dorsal root ganglia following incision in the plantar surface of rat hindpaw. Results are shown as the average (±SE) NGF level per milligram of total tissue protein. Incisions were made in the right ipsilateral (ipsi) hindpaw and compared to the contralateral (cont) left side. Unincised rats are used for controls (Ctrl). Ipsi4 h, Ipsi1d, Ipsi2d and Ipsi5d, Ipsi10d represents tissues from the incised right side 4 h, 1 day, 2 days 5 days and 10 days after incision, respectively. Cont4 h, cont1d, cont2d and cont5d, cont10d represents tissues from the unincised left side 4 h, 1 day, 2 days, 5 days and 10 days after incision, respectively. ***, $P<0.001$; **, $P<0.005$; *, $P<0.05$; ipsi vs. cont by unpaired t-test.

incision when compared with IgG treated rats (the time point '0', $P<0.01$, Dunnett's test). The smaller doses (1 and 0.1 mg/kg) of anti-NGF were not effective at this time. On postoperative days 1 and 2, the guarding scores were significantly less in anti-NGF treated rats with all doses used (0.1, 1 and 2.8 mg/kg; $P<0.01$, Dunnett's test). The most effective dose was 2.8 mg/kg anti-NGF, which almost abolished (>80% decrease) guarding pain scores 1-day after incision.

3.2.3. Heat hyperalgesia

The mean withdrawal latency to radiant heat was 26.0±3.6 s before incision. After incision, rats demonstrated a significant decrease in withdrawal latency indicating heat hyperalgesia (Fig. 2(C)). In contrast, the contralateral paws of the same rats did not demonstrate any significant changes. The incision-induced decrease in withdrawal latency was significantly attenuated on postoperative days 1 and 2 in rats treated with 2.8 mg/kg anti-NGF 1-day before incision (Fig. 2(C); $P<0.02$, Mann Whitney test). Lower doses of anti-NGF treatment, however, were not effective (Fig. 2(D)).

3.2.4. Effects of intravenous rat anti-NGF

Since the bioavailability of anti-NGF by intraperitoneal administration is not clear, we anticipated a greater effect by intravenous administration. Thirty minutes before incision, the intravenous administration of 0.3 mg/kg rat anti-NGF (Sigma) or 0.3 mg/kg IgG in small number of rats ($N=3$, anti-NGF and $N=6$, IgG) showed that guarding pain scores decreased in 4 h, and 1-day and 2-days after incision by 24, 60 and 60%, respectively (data not shown). There was no effect on heat hyperalgesia.

3.3. Effect on anti-NGF treatment on sensitization of C-fibers

We previously reported that 1-day after plantar incision, sensitized C-fiber nociceptors were present in vitro as evidenced by their spontaneous activity and sensitization to heat (Banik and Brennan, 2004). In this study, we determined the effect of anti-NGF treatment (2.8 mg/kg) on sensitization of C-fibers. We recorded 24 C-fibers from the medial and lateral plantar nerves of 9 incised rats that received preoperative anti-NGF. Of 24 C-fibers, 12 had receptive fields <2 mm from the plantar incision. These data were compared to the data reported in Banik and Brennan (2004).

In our previous study, we observed that after incision, C-fibers innervating <2 mm from the incision responded to lower temperatures (35–41 °C) by increased total action potentials during a 15-s heat stimulation. We did not see any difference after preoperative treatment of anti-NGF. Fig. 3 shows responses to heat of two single C-fibers. The responses shown in Fig. 3(A) are representative of incised C-fibers, whereas Fig. 3(B) is a representative example of

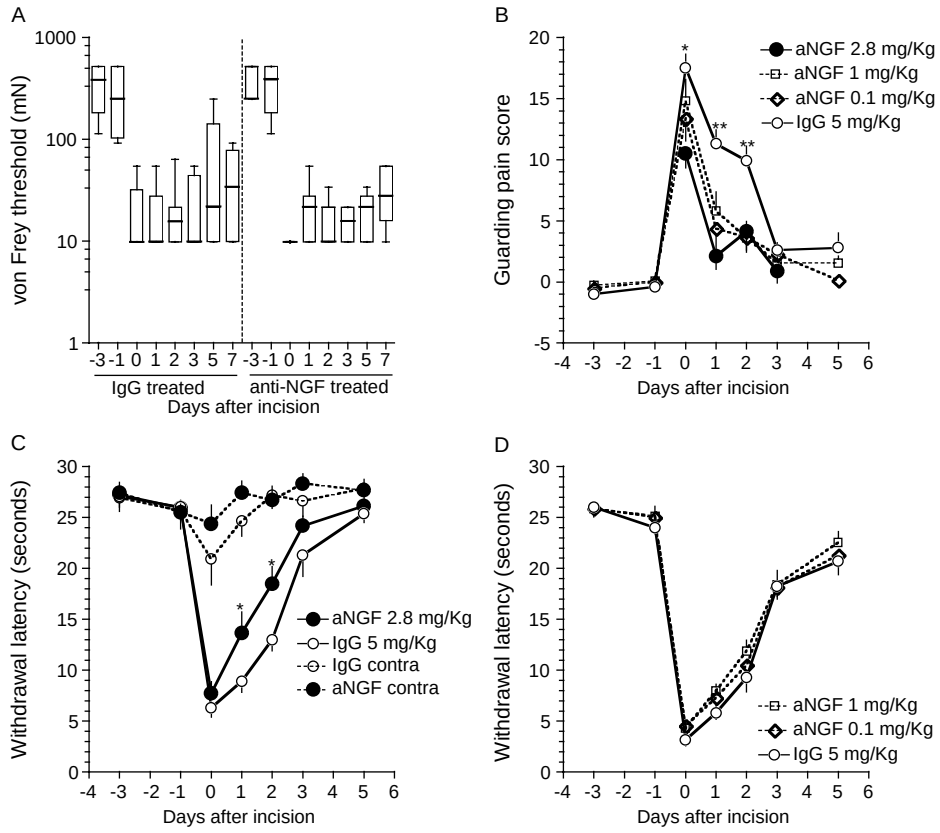

Fig. 2. Effects of anti-NGF on pain behaviors caused by plantar incision. (A): Withdrawal threshold to von Frey filament application. The results are expressed as median (horizontal line) with the first and third quartiles (boxes) and 10th and 90th percentiles. Anti-NGF treated rats received 2.8 mg/kg anti-NGF intraperitoneally one day before incision. (B): guarding behavior (see Section 2) after incision. $*P<0.05$, $**P<0.01$; Dunnett's test. (C) and (D): paw withdrawal latency to radiant heat. $*P<0.05$, Mann Whitney test. The time points '0' mean four hours after incision.

incised C-fibers after anti-NGF treatment. Fig. 4(B) and (C) demonstrated that in vitro incision-induced C-fiber sensitization to heat unchanged in anti-NGF treated rats.

After incision, a greater proportion of C-fibers <2 mm from the incision showed spontaneous activity (40%, 6 of 15) compared to control (12.5%, 3 of 24; Banik and Brennan, 2004). Anti-NGF treatment did not decrease the percentage of spontaneously discharging C-fibers after incision (Fig. 4(A)). In anti-NGF treated rats, 5 of 12 C-fibers (42%) <2 mm from the incision showed spontaneous activity contrasting 3 of 12 fibers (25%) >2 mm from the incision. The range of activity was 0.1–1.0 imp/s for all spontaneously active fibers. Anti-NGF treatment also had no influence in the average activity (0.6 ± 0.3 vs. 0.5 ± 0.3 imp/s).

4. Discussion

The present study demonstrates that hindpaw incision leads to a rapid increase of NGF content in skin and that increased NGF after incision contributes to pain related behaviors; incision-induced guarding and heat hyperalgesia were affected. Responses to mechanical stimuli, however, were not affected demonstrating modality-specific affects on pain processing. Anti-NGF treatment did not influence C-fiber sensitization caused by incision studied in vitro.

4.1. Upregulation of NGF in plantar skin

NGF expression was increased in incised skin from 4 h through 5 days. These data are in agreement with others showing increased NGF expression after punch removal of hairy skin (Matsuda et al., 1998). Previous studies also observed a two to three-fold increase of NGF in the adult rat hindpaw after injection of complete Freund's adjuvant (Woolf et al., 1994) or perineural capsaicin (Saade et al., 2002). In contrast, Constantinou et al. (1994) showed that NGF levels are increased after skin wounding only in neonatal rats but not in adult rats.

Inflammatory mediators have the capability to increase NGF levels in skin tissues as it has been shown that NGF levels were increased in cultured fibroblast or keratinocyte by interleukin-1 (Lindholm et al., 1988), tumor necrosis factor-$\propto$ (Hattori et al., 1996), interferon-γ (Hattori et al., 1994) and histamine (Kanda and Watanabe, 2003). How the increased NGF levels after tissue injury affect pain

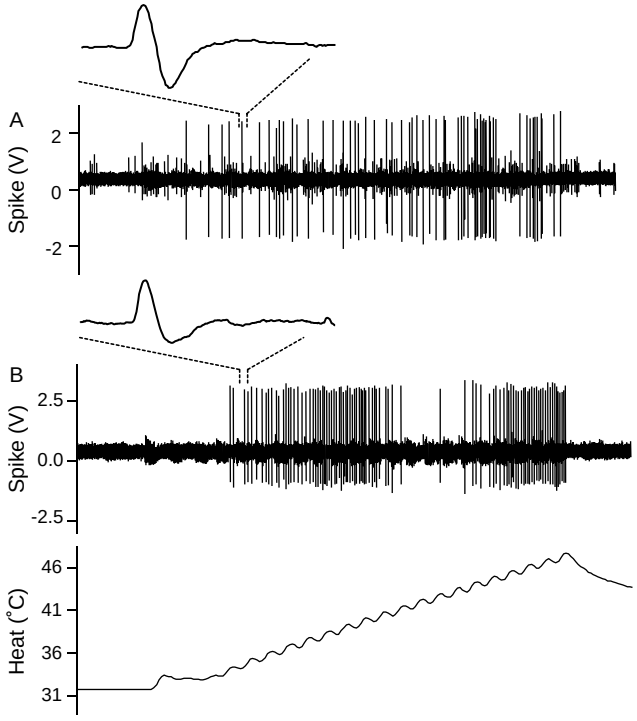

Fig. 3. Effects of anti-NGF on heat sensitization of C-fibers by plantar incision. Records from two single C-fibers from rats received with (B) or without (A) single intraperitoneal administration of 2.8 mg/kg anti-NGF one day before incision. Both of these fibers have receptive fields in less than 2 mm from the incision. Trace below shows 15-s ramp heat stimulation from 33 to 48 °C. *Insets* display single action potential of these fibers.

behaviors is largely enigmatic. One hypothesis is, high levels of NGF in the periphery accumulate in the DRGs by axonal transport (Korsching et al., 1983; Richardson and Riopelle, 1984; Ji et al., 2002) and increase gene expression. However, we did not see an influence of plantar incision on tibial nerve and DRG NGF content. An alternate possibility is, NGF itself might not be retrogradely transported; rather it might activate its receptor TrkA and then activated TrkA by itself or through downstream substrates provides a retrograde signal (reviewed by, Miller and Kaplan, 2001). In agreement, exogenous NGF-induced hyperalgesia developed rapidly in the rodents (Chuang et al., 2001). The acute action of NGF has been demonstrated in cultured DRG neurons where it sensitized capsaicin-evoked response (Shu and Mendell, 2001), and in isolated skin-nerve preparation where it sensitized C- or Aδ-fiber responses to heat (Rueff and Mendell, 1996).

Although the increased NGF levels had dropped from the peak by 5 and 10 days after incision, it was still elevated compared to the contralateral controls. In our data, 5 and 10 days after incision, incision-induced pain behaviors had already been returned to the baseline. This suggests that NGF has a role in acute pain within the first few days after incision. The importance of high NGF levels on and after 5th post-incision days remains open for the future study.

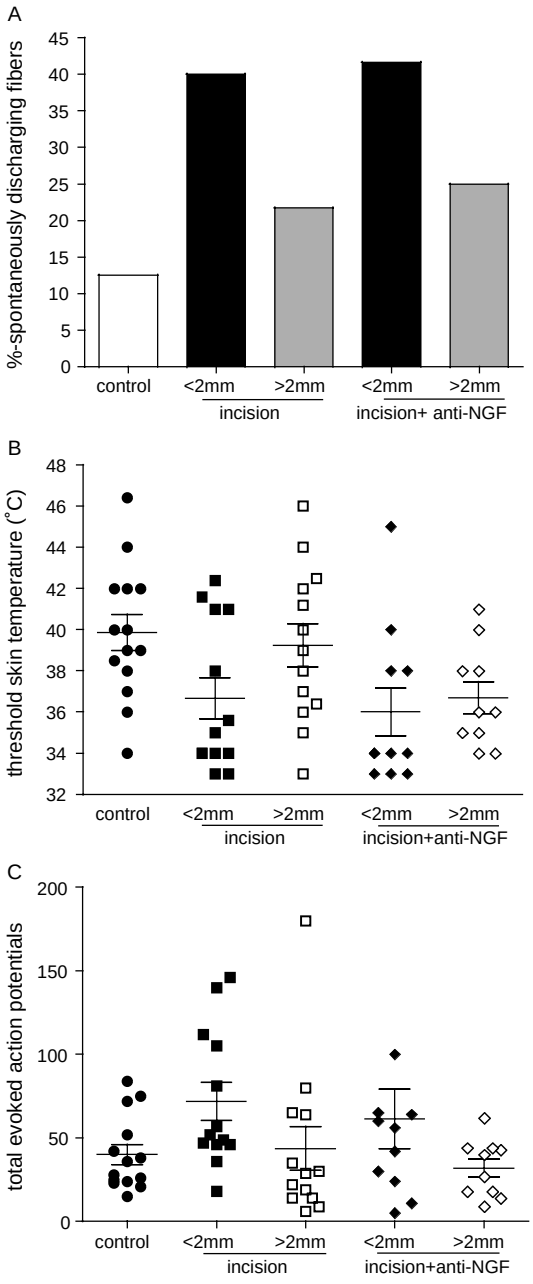

Fig. 4. Effects of anti-NGF treatment on incision induced activation of C-fibers, spontaneous discharge and sensitization to heat, in vitro. One group of rats received single intraperitoneal administration of 2.8 mg/kg anti-NGF one day before incision. (A) Incidence of spontaneous discharge. (B) Distribution of threshold temperature for activation of C-fibers. (C) Distribution of total evoked action potentials by heat responsive units. The lines represent mean ± SE <2 mm and >2 mm mean fibers innervating <2 mm or >2 mm from the incision. A chi square test was used to compare the percentage of spontaneously discharging C-fibers and a non-parametric Dunn's test was used to compare total evoked action potentials. The heat response thresholds were compared using Dunnett's test.

4.2. Anti-NGF attenuates guarding pain scores in a dose dependent manner

The present study, for the first time, shows that a single dose of anti-NGF treatment attenuates incision-induced

guarding pain. The effect is robust and it nearly abolishes guarding pain 1-day after incision (>80% decrease). Moreover, this effect also exists when lower doses of anti-NGF used.

Other studies used guarding pain scores as index for non-evoked pain (Attal et al., 1994; Bennett and Xie, 1988; and Choi et al., 1994). Recent data from our laboratory showed that capsaicin pretreatment significantly attenuated heat hyperalgesia and guarding pain but responses to mechanical stimuli were not affected (Brennan et al., 2002). Likely small diameter afferents contribute a major component of heat hyperalgesia and guarding behavior. In support, in an isolated skin-nerve preparation where blood born mediators are absent, C-fiber afferents innervating the incision show spontaneous activity and sensitization to heat (Banik and Brennan, 2004). However, we did not observe any influence of anti-NGF treatment on altered C-fiber characteristics after incision, in vitro. It is possible that anti-NGF is effective in the presence of mediators that are removed in an in vitro preparation. In agreement, previous studies in vivo (Pogatzki et al., 2002), 1-day after incision, demonstrated that some C-fibers have a very high background activity, greater than that seen in vitro. The degree of sensitization seen in vitro may not be sufficient to detect an effect of anti-NGF.

The guarding pain scores may be, in part, a reflection of behavioral allodynia, i.e. pain induced by gentle stroking (Koltzenburg et al., 1992; Ochoa and Yarnitsky, 1993). Rats lifted their paw or did not bear weight on the paw because touching the mesh floor might be painful. Touch evoked pain has been suggested to be mediated in the central nervous system (Kilo et al., 1994; Koltzenburg et al., 1994). Zahn and Brennan (1999) showed that hindpaw incision leads to sensitization of the dorsal horn neuron as evidenced by their spontaneous activity. Taken together, touching of the hindpaw might cause barrage of more input from mechanoreceptors, which stimulates already sensitized dorsal horn neurons.

NGF produced profound mechanical hyperalgesia in animals (Dyck et al., 1997; Lewin et al., 1993; 1994) and humans (Petty et al., 1994; Svensson et al., 2003) but the underlying mechanisms remain to be elucidated. NGF-induced mechanical hyperalgesia was not blocked by NMDA receptor antagonist or mast cell stabilizer (Lewin et al., 1994). However, Lewin and his colleagues (1992) reported that after exogenous NGF, an increased number of dorsal horn neurons received input from primary afferents. This observation suggests NGF might trigger the development of new synapses or strengthening of weak synapses between central terminals of primary afferents and dorsal horn neurons. This hypothesis, however, could not explain why punctate mechano-allodynia measured by von Frey hair stimulation has not been influenced by anti-NGF treatment or sequestration of endogenous NGF by TrKA IgG fusion molecule (Zahn et al., 2004).

4.3. Anti-NGF induced attenuation of heat hyperalgesia

In contrast to the effect of anti-NGF on guarding pain scores, its effect on heat hyperalgesia was small and only effective at a relatively greater dose. Other studies also agree that blockade of inflammatory heat hyperalgesia requires greater dosages of anti-NGF molecules (Lewin et al., 1994; Sammons et al., 2000; Woolf et al., 1994; Zahn et al., 2004). The heat hyperalgesia that follows after exogenous NGF administration is rapid in onset (within 10 min) and localized to the injection site. It has been proposed that NGF induced degranulation of mast cells and subsequent release of 5-hydoxytryptamine on peripheral terminals of nociceptors may be a mechanism of early onset heat hyperalgesia (Lewin et al., 1994). Rueff and Mendell (1996) confirmed this by showing that acute administration of NGF could sensitize C-fibers to thermal stimuli, in vitro. However, in our study, anti-NGF did not affect heat hyperalgesia immediately after incision. This suggests that other factors produce a dominant role on heat hyperalgesia immediately after incision.

Although anti-NGF treatment significantly attenuated heat hyperalgesia 1-day after plantar incision, it did not prevent sensitization of C-fibers innervating the incision. This is in contrast to Koltzenburg et al. (1999), who showed that sequestration of endogenous NGF by concurrent local administration of TrKA IgG fusion molecule prevented carrageenan induced sensitization of C-fibers. Several possibilities may account for this apparent discrepancy. First, Koltzenburg and his colleagues did not compare their data with the observations from animal behaviors. In our experiments, the difference between anti-NGF treated and the IgG treated group is so small and this difference in animal behaviors might not be detectable measuring heat sensitivity of primary afferents. Second, Koltzenburg et al. (1999) recorded C-fibers after acute administration of carrageenan while we recorded C-fibers 1-day after incision. Lewin and his colleagues (1992) argued that early phase of exogenous NGF induced heat hyperalgesia is mediated by peripheral mechanism while a late phase (7 h to 4 days) is mediated by central mechanisms. This may explain why we did not see a reversal of C-fiber sensitization 1-day after incision. Third, the consequences of tissue injury produced by antigen injection are likely different than that produced by an incision (see introduction).

4.4. Clinical implications

An estimated 28 million surgical procedures are performed in each year in the United States alone (Mangano, 1995). Almost all of these procedures involved some form of pain management, but studies reported that between 47 and 75% of postoperative patients experienced unrelieved pain (Chung and Lui, 2003; Cohen, 1980; Donovon et al., 1987; Klopfenstein et al., 2000; Marks and Sachar, 1973; Warfield and Kahn, 1995). The peculiarity of

post-operative pain is constant aching pain near the surgical site and acute exacerbation of pain added to the basal pain due to activities such as movements, dressing changes etc. Guarding pain scores measured in the present study are either related to ongoing pain or to touch or activity evoked pain in the perioperative period. Therefore, our data that a single preoperative dose of anti-NGF abolishes or attenuates guarding pain scores is intriguing from a therapeutic viewpoint.

Postoperative patients rarely experience heat hyperalgesia. However, it has been suggested that non-evoked pain might be the 'tip of iceberg' of heat hyperalgesia (for review, Sorkin and Wallace, 1999; Reeh and Petho, 2000). The routine practice of cooling in the injured tissue for pain relief is not new and supports the involvement of heat hyperalgesia in non-evoked pain. We have recently been shown that lowering of the heat response threshold of C-fibers might be a mechanism of developing spontaneous activity one day after incision, in vitro (Banik and Brennan, 2004). Therefore, it is tempting to know if blockade of NGF is similarly effective in alleviating non-evoked pain in postoperative patients.

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