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Spontaneous discharge and increased heat sensitivity of rat C-fiber nociceptors are present in vitro after plantar incision

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Abstract

Postoperative pain is characterized by spontaneous pain at the surgical site and increased pain due to movements. To study postoperative pain mechanisms, we investigated discharge properties of mechano-heat sensitive C-fiber afferents innervating the rat glabrous hindpaw skin 1 day after plantar incision. Behaviors indicating spontaneous pain, heat and mechanical hyperalgesia were present 1 day after incision. Recording of afferents using in vitro glabrous skin-nerve preparation showed that more C-fibers from the incision had spontaneous discharge than control rats. The spontaneously discharging fibers from incised rats had lower heat response threshold compared with fibers without spontaneous activity. In all fibers less than 2 mm from the incision, an increased percentage responded to lower temperatures (35–41 °C), the mean heat response threshold was 3.1 °C less, the stimulus–response function for heat evoked response was shifted to the left and the total number of impulses in response to a 33–48 °C heat stimulus was increased. Heat responses of C-fibers more than 2 mm from the incision, however, were not different from control. The mean mechanical response thresholds, measured by a servo force-controlled stimulator, were not different between groups. The total spikes evoked at supra-threshold mechanical stimulation were not increased in afferents from the incision. In conclusion, 1 day after incision, when behaviors indicating spontaneous pain, heat and mechanical hyperalgesia are present, C-fibers close to incision showed spontaneous discharge and sensitization to heat but not to mechanical stimuli, in vitro.

Keywords: Primary afferent neuron sensitization; Plantar incision; in vitro preparation

1. Introduction

In the perioperative period, patients experience constant and aching pain near the surgical site and/or acute exacerbation of pain due to activities such as coughing, movement, physiotherapy and dressing changes. Although the intensity, duration and quality of pain are variable among patients, they probably share common mechanisms: direct injury, inflammation and release of chemical mediators which sensitize peripheral nociceptive terminals to heat and mechanical stimuli.

The rat model of incisional pain, characterized previously by our lab (Brennan et al., 1996), is strikingly similar to human postoperative pain and the responses of humans to a small incision (Kawamata et al., 2002). As reported in previous studies (Zahn and Brennan, 1999; Zahn et al., 1998, 2002), the mechanisms for postoperative pain are different from conventionally used inflammation-induced pain models since a major component of post-operative pain is the surgical incision-induced pain that results from injuries in the skin, fascia, muscles and small nerves innervating these tissues. It is of considerable interest, therefore, to understand the mechanisms underlying ongoing pain, heat and mechanical hyperalgesia in this animal model.

Zahn and Brennan (1999) reported that the most persistent pain behaviors of the present animal model are largely primary hyperalgesia and the dominant role of peripheral sensitization in incisional pain has been reported (Pogatzki et al., 2002b). In agreement, Hamalainen et al. (2002) and Pogatzki et al. (2002a) showed that Aδ and C-fibers from an incision were sensitized to mechanical stimuli and that the development of mechanosensitivity in mechanically insensitive fibers after an incision may be

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important for the maintenance of hyperalgesia. None of these reports, however, demonstrated heat sensitivity of afferents innervating an incision.

In this study, using the glabrous skin incision and an in vitro glabrous skin nerve preparation (Du et al., 2001), we recorded mechanosensitive C-fiber nociceptors innervating the plantar aspect of rat hindpaw 1 day after incision and compared this to control rats. We determined if afferent fibers were sensitized to heat and mechanical stimuli 1 day after an incision in vitro and made comparisons to behavioral studies.

2. Methods

The experimental procedures were approved by The University of Iowa animal care and use committee and in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals issued by the International Association for the Study of Pain (Zimmermann, 1983).

2.1. Animals and surgery

Adult male, 200–250 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*.

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 (Brennan et al., 1996). Topical and systemic antibiotics were administered.

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

2.2.1. General

The rat glabrous skin-nerve in vitro preparation, modeled as saphenous nerve-skin preparation (Reeh, 1986), has been described elsewhere (Du et al., 2001). In brief, rats were killed with CO_2; the medial and lateral planter 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 (in mM: 110.9 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2So_4, 24.4 $NaHCO_3$, and 20 glucose), which was saturated with a gas mixture of 95% O_2 and 5% CO_2. The temperature of the 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 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).

2.2.2. Recording and stimulation procedures

In this study we concentrated on the mechanosensitive C-fiber nociceptors. The fibers, that had conduction velocity <1.2 m/s and slowly adapting to mechanical stimulation were considered as 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 (5–20 V at 0.2–1 Hz for 0.5–2 ms) 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. After conduction velocity determination, a standard protocol of mechanical stimulation followed by a ramp heat stimulation was done.

2.2.3. Mechanical stimulation

A servo force-controlled mechanical stimulator (Series 300B Dual Mode Servo System, Aurora Scientific, Aurora, Ontario, Canada) (Khalsa et al., 1997; Levy and Strassman, 2002) was used to determine quantitative mechanosensitivity. A flat-ended cylindrical metal probe attached to the tip of the stimulator arm was placed just close to the receptive field so that no force was generated. A computer-controlled ascending series of displacement ramp stimuli was applied to the most sensitive spot of the receptive field at 10-s intervals. The duration of each displacement ramp was 5 s. In pilot experiments, two consecutive ascending series of mechanical stimuli were applied at 5-min intervals until threshold was determined. Threshold of the units were unchanged in 90% of cases suggesting that our stimulus duration and interstimulus interval have little influence on sensitization or desensitization. To deliver suprathreshold stimuli, once threshold was reached, 48 μm more displacement was given. Force generated due to displacement is reported in milli-Newton (mN) units. The stimulator produced a maximum force of 235 mN.

2.2.4. Heat stimulation

After mechanical stimulation, a standard heat ramp was delivered by a feedback-controlled heat stimulator. The receptive field of each unit was isolated with a small metal ring (5 mm internal diameter), which could seal by its own weight. The bath solution within the ring was manually removed with a syringe. A thermocouple was gently placed to measure intracutaneous temperature. A radiant lamp was placed in the translucent area underneath 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 to 48 °C in 15 s. We determined that the temperature of the epidermal side was about 2 °C higher than the corium side. To avoid heat sensitization, heat stimulation was always done at the end of experiment and fibers having receptive fields in the heat-stimulated area were avoided for subsequent recording.

2.3. 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 more than one fiber was present in a recording, data were analyzed only if the spike shapes and amplitudes were different and could be easily discriminated. If a unit discharged at a rate of 0.1 imp/s or more without any intentional stimuli, it was categorized as spontaneously active. The heat and mechanical threshold of a unit was measured as the temperature and force 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 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

A chi-square test was used to compare the percentage of spontaneously discharging C-fibers and percentage of heat sensitive fibers between groups. The response thresholds for heat and mechanical stimuli were compared using Dunnett's multiple comparison test. The stimulus–response relationship for the heat evoked responses was compared using ANOVA. The comparison between heat response threshold of fibers with or without spontaneous activity was compared using an unpaired *t*-test. The total evoked action potentials during each heat or mechanical stimulus 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 $\pm$ SE in the figures.

3. Results

3.1. General properties of C-fiber afferents

Eighty-four afferent fibers were recorded from the medial and lateral plantar nerves; 62 were mechanosensitive C-fibers and 22 were 'unclassified fibers'. 'Unclassified fibers' were recognized during heat (33–48 °C) stimulation. We did not locate receptive fields of these unclassified fibers before or after the heat stimulation procedure; therefore, the conduction velocity of these units could not be measured.

Thirty-five fibers (24 C-fibers and 11 'unclassified fibers') were studied from 16 control rats. Forty-nine (38 C-fibers and 11 'unclassified fibers') were studied in 18 rats, 1 day after plantar incision. During our initial studies, it was apparent that fibers near the incision had different properties than those located outside the incision. For analyses, we subdivided those having receptive fields <2 mm and those having receptive fields >2 mm from the incision. Of 38 C-fibers from incised rats, 15 had receptive fields <2 mm from the plantar incision. The conduction velocity of the incised fibers ranged from 0.1 to 1.1 m/s (0.78 ± 0.27) and those of control fibers ranged from 0.1 to 1.2 m/s (0.74 ± 0.27 m/s). The receptive fields of all fibers were located in glabrous hindpaw skin, as shown in Fig. 1. The fibers had small punctate receptive fields; the area could not be quantified.

3.2. Spontaneous activity

No afferent fibers developed spontaneous activity during testing. A greater proportion of C-fibers from <2 mm from the incision showed spontaneous activity (40%, 6 of 15; $P<0.05$, χ^2-test, Fig. 2) compared to control (12.5%, 3 of 24). An example of a spontaneously active C-fiber from an incised paw is shown in Fig. 2A. The range of activity was 0.1–1.0 imp/s for all spontaneously active fibers. For all 9 spontaneously active C-fibers, there was no difference in the average activity in control vs. incised fibers (0.6 ± 0.3 vs. 0.6 ± 0.3 imp/s). Moreover, the prevalence of spontaneously discharging fibers having receptive fields >2 mm from the incision was not different from control (5 of 23, 21.7%; Fig. 2B). When present, these fibers had a discharge frequency of 0.3 ± 0.3 imp/s.

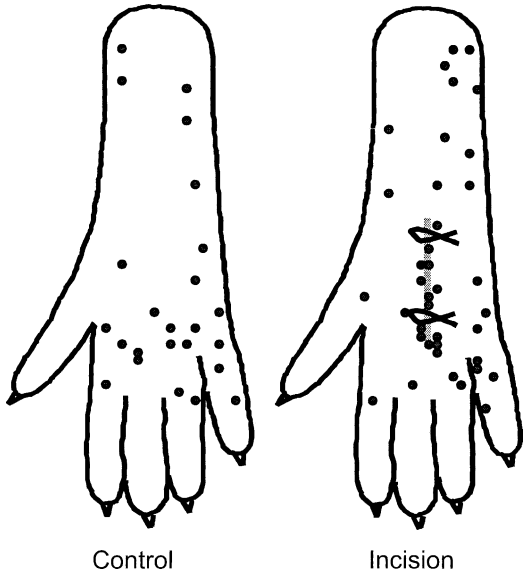

Fig. 1. Distribution of receptive fields of C-fibers for control and incised rats. Each dot represents receptive field of a single C-fiber. The receptive field area was not quantified. The receptive fields of all tested C-fibers were located in glabrous hindpaw skin.

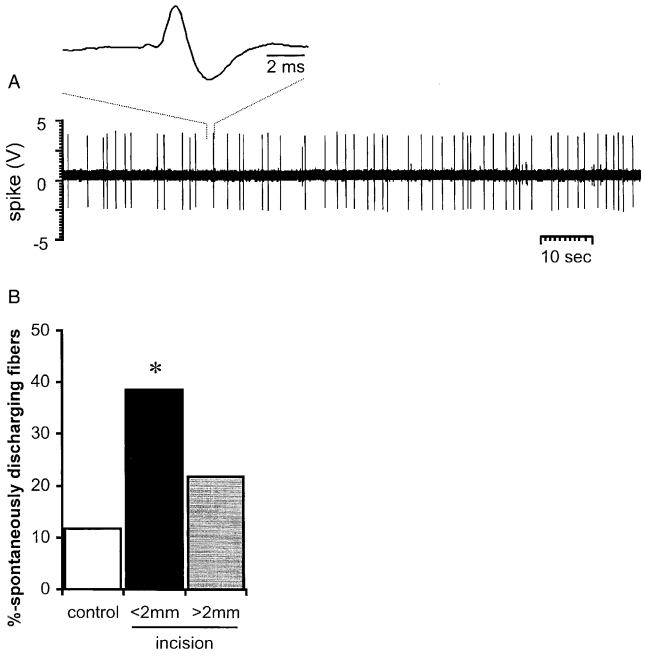

Fig. 2. Spontaneous activity of C-fibers in control rats and 1 day after plantar incision. A: A sample recording of activity from a single C-fiber. The mechanosensitive receptive field of this fiber is adjacent to and includes the incision. The mean firing frequency in this example was 0.5 imp/s. *Inset* displays an action potential of this fiber. B: More mechanosensitive C-fibers, with receptive fields <2 mm from the incision, had spontaneous activity compared to control ($P<0.05$, χ^2-test).

3.3. Response to mechanical stimulation

None of the C-fibers responded to <20 mN force. An ascending series of ramp-displacement stimuli was used to determine response threshold and suprathreshold sensitivity of C-fibers (Fig. 3). Due to variation in tissue compliance, variable displacements were used to generate the same force onto receptive fields.

The distribution and summary of mechanical response thresholds of C-fibers studied 1 day after incision are shown in Fig. 4A and B. The response threshold of control C-fibers ranged from 23 to 235 mN (102 ± 54 mN). In incised fibers having receptive field <2 or >2 mm from the incision, the response threshold ranged 23–188 mN (84 ± 44 mN) and 33–235 mN (92 ± 54 mN), respectively. Nine of the 11 C-fibers (83%) with receptive fields <2 mm from the incision responded to 100 mN or less (Fig. 4B). In the control group, 10 of 19 fibers (53%; Fig. 4B) responded to 100 mN or less; this difference, however, was not significant.

Increased responses were evident in all groups of C-fibers as stimulus intensity was increased to a suprathreshold stimulus, 48 μm displacement from threshold. Fig. 4C shows that there was no difference in response to a suprathreshold displacement among the three groups of fibers. There was no difference in force used to generate 48 μm displacement among the groups.

3.4. Responses to heat stimulation

The threshold temperature to excite C-fibers was less in those C-fibers innervating <2 mm from the incision compared to control. This is illustrated in Figs. 5 and 6A and B. Fig. 5 shows responses to heat of two single C-fibers. The responses shown in Fig. 5A are representative of control C-fibers, that generally began their response to heat stimuli at ~39 °C, whereas Fig. 5B is a representative

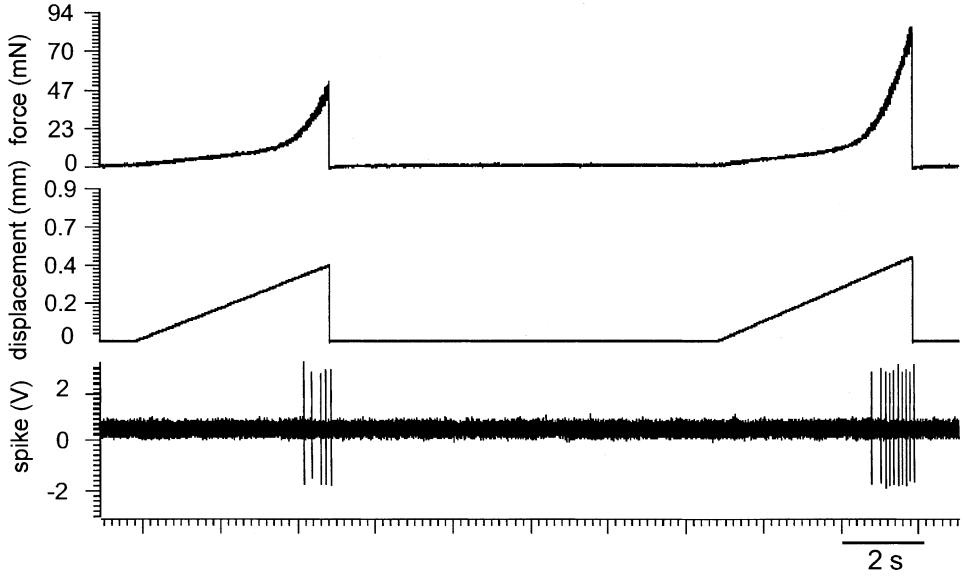

Fig. 3. Responses of a typical C-fiber to the ascending series of ramp-displacement stimuli. The upper trace shows force generated by corresponding displacement stimuli. The responses increased monotonically as stimulus intensity increased. The mechanical threshold was considered as the force that elicited a second action potential or an increase of spontaneous activity by more than 50%.

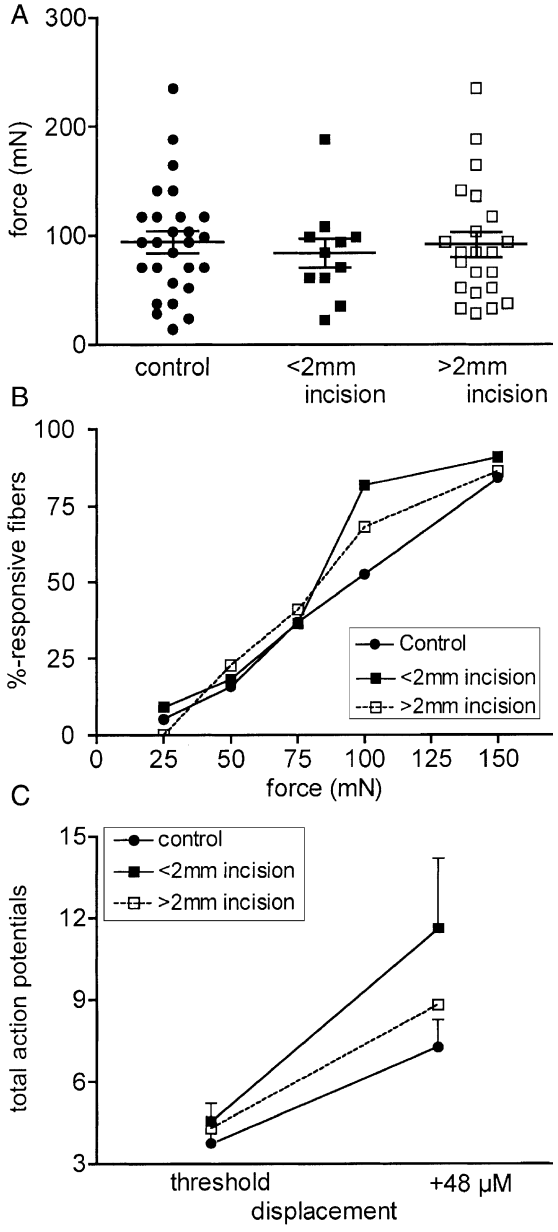

Fig. 4. Incision does not change the mechanosensitivity of C-fibers. (A) Distribution of response thresholds for C-fibers; the lines represent mean response thresholds (±SE) in mN. (B) The percentage of C-fiber mechanosensitivity in control ($n=19$), <2 mm from the incision ($n=11$) and >2 mm from the incision ($n=22$). (C) Comparison of the mean of the total action potentials (±SE) evoked from C-fibers at threshold or suprathreshold stimulation. Total action potentials were determined by counting the total impulses generated during the 5-s stimulation period.

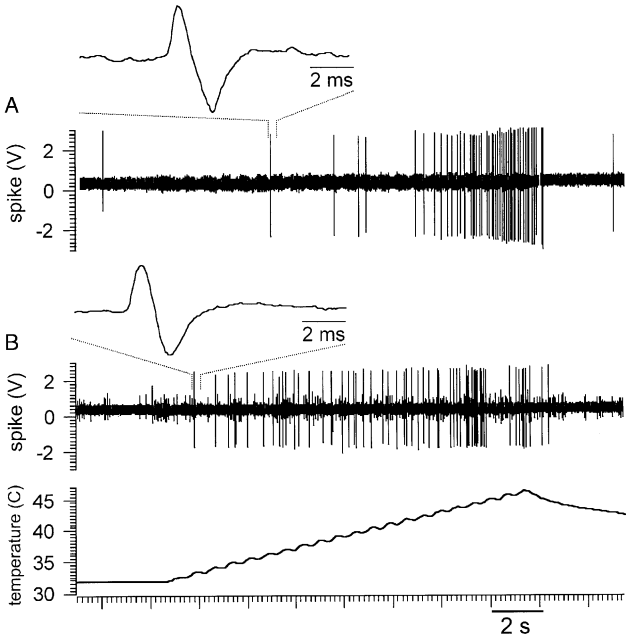

Fig. 5. Decreased heat response threshold of C-fibers from <2 mm from the incision. Records from two single C-fibers innervating control (A) and <2 mm from the incision (B) during 15-s ramp heat stimulation from 33 to 48 °C. *Insets* display single action potential of these fibers.

example of C-fibers innervating <2 mm from the incision. This afferent responded at ~35 °C.

A greater percentage of C-fibers, that had receptive fields <2 mm from the incision, responded to 35–41 °C temperatures compared to controls (Fig. 6A). For example 8 of 13 fibers <2 mm from the incision responded at 37 °C vs. 2 of 17 in control rats ($P<0.005$, χ^2-test).

The scatter plot of threshold temperature of all fibers is shown in Fig. 6B. The mean heat response threshold was significantly less (36.7 °C±3.6) in the C-fiber innervating <2 mm from the incision ($P<0.05$, Dunnett's multiple comparison test) than control (39.8 °C±3.2). Those innervating >2 mm from the incision (39.2 °C±3.7) were not different than control.

The increased heat responsiveness after incision was not only a decrease in threshold temperature but also leftward shift of the stimulus–response function (Fig. 6C). Once fibers reached threshold, they increased their firing rate as the heat stimuli increased. On average, the peak discharge appeared at 47 °C with a diminished response at 48 °C. Fig. 6C shows a stimulus–response relationship for the heat responses in the three groups of fibers. Fibers, that had receptive fields <2 mm from the incision, had significantly increased heat evoked discharges compared to control (Fig. 6C; $P<0.05$, Dunn's multiple comparison test). Those innervating >2 mm from the incision were not different from control.

Since it is not clear which feature of neural responses is more important to the central nervous system to interpret pain, we also compared total evoked action potentials during a 15-s ramp-heat stimulation. As shown in Fig. 6D, a significant difference was seen between control and incised fibers innervating <2 mm from the incision (40±22 vs. 72±41 imp; Dunn's multiple comparison test). There was no difference in total heat evoked action potentials between control fibers innervating >2 mm from the incision (43±47 imp).

A noteworthy relationship was present between spontaneous activity and heat sensitivity of C-fibers (Fig. 7). Among fibers innervating <2 mm from the incision,

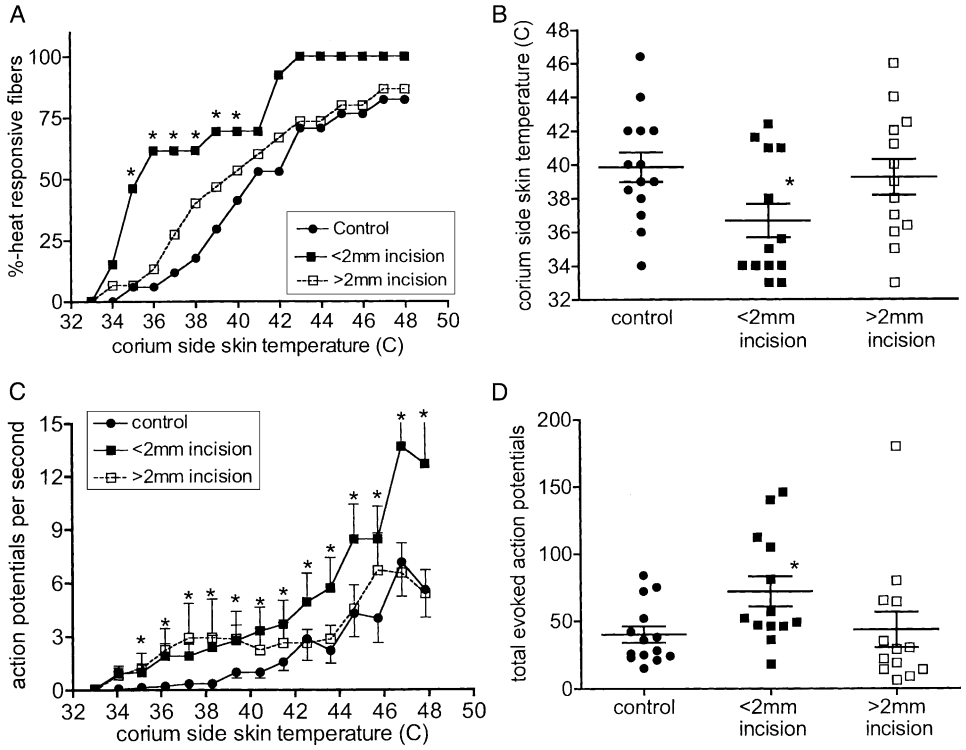

Fig. 6. Incision increases heat sensitivity of C-fibers. (A) The percentage of heat responsive units vs. temperature in three groups. An increased percentage of fibers, which have receptive field <2 mm from the incision, respond to 35–41 °C heat compared to controls ($P<0.03$, χ^2-test). (B) Distribution of threshold temperature for activation of C-fibers between groups. The lines represent mean (±SE)(control vs. <2 mm from the incision, $P<0.05$, Dunnett's multiple comparison test). (C) Leftward shift of stimulus–response function for heat evoked response (bin width 1-s; mean number of spikes±SE). (D) Distribution of total evoked action potentials by heat responsive units (control vs. <2 mm from the incision, $P<0.05$, Dunn's multiple comparison test).

the mean heat response threshold of spontaneously discharging fibers was less than fibers without spontaneous activity (38.3 °C±1.3 vs. 34.1 °C±0.5; $P<0.04$, unpaired t-test). Similarly, spontaneously discharging fibers >2 mm from the incision had lower heat response threshold compared to fibers without spontaneous activity (35.5 °C±1.0 vs. 40.9 °C±1.0). Of three spontaneously discharging control fibers, heat sensitivity was tested in one fiber, which responded at 43 °C.

3.5. Responses of 'unclassified fibers' to heat stimuli

'Unclassified fibers' were the fibers, which could not be identified by mechanical stimulation before the heat-ramp. Obviously, no background activity was present. It is likely that some of these fibers are mechano-insensitive afferents as described previously (Davis et al., 1993; Handwerker et al., 1991). We compared the heat response properties of these unclassified fibers in control and incised rats (Fig. 8). The threshold temperature to excite these unclassified fibers from the incised paw was ~2 °C less than control; but the difference is not significant (Fig. 8A). Of 11 control fibers tested, 4 fibers responded to the 39–40 °C heat stimuli whereas 10 of 11 incised fibers responded at the same termperature (Fig. 8B; $P<0.05$, χ^2-test). The stimulus–response relationship for heat evoked response was not different between control and incision (Fig. 8C). Thus some evidence of heat sensitization was present in these uncharacterized fibers.

4. Discussion

In the present experiments, 1 day after plantar incision, C-fibers were activated in vitro, as evidenced by their spontaneous activity. Lowering of heat response threshold

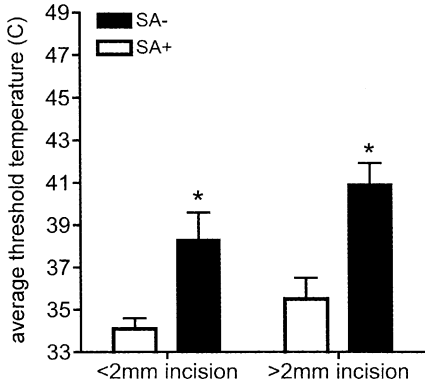

Fig. 7. Heat threshold is less in spontaneously discharging C-fibers from incised rats. Significant differences in the mean heat threshold were evident ($P<0.05$, unpaired t-test) between fibers with or without spontaneous activity.

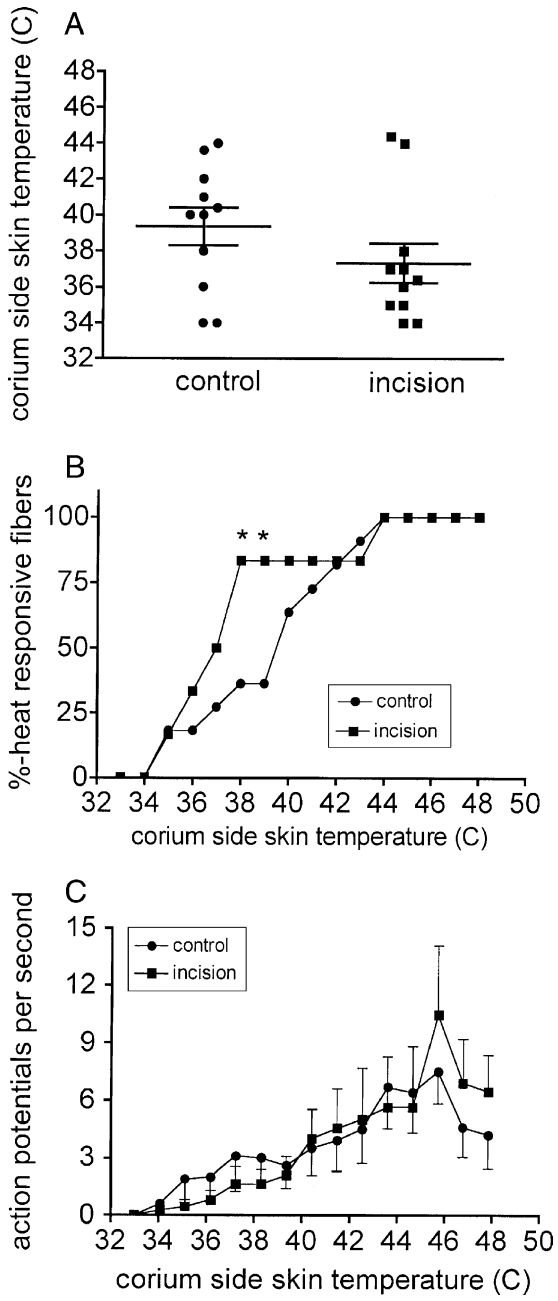

Fig. 8. Heat sensitivity of 'unclassified fibers'. (A) Distribution of threshold temperature for activation of 'unclassified fibers'. Lines represent the mean heat response threshold (±SE). (B) Increased percentage of 'unclassified fibers' from incised rats responded to the 39–40 °C compared to control ($P<0.05$, χ^2-test). (C) No difference was observed in the 'unclassified fiber' responses (bin width 1 s; mean number of spikes±SE) to the ramp heat stimulation.

and the leftward shift of stimulus–response for heat demonstrated heat sensitization occurred. Heat sensitization of C-fibers was localized to <2 mm from the incision. These changes in fiber activity were present at a time when animals showed behaviors indicative of spontaneous pain and heat hyperalgesia (Brennan et al., 1996; Zahn and Brennan, 1999). However, the incision did not change mechanosensitivity of C-fibers, in vitro.

4.1. Spontaneous activity and heat sensitization

Many neurophysiological studies, using both in vivo and in vitro techniques, have shown sensitization of primary afferents innervating inflamed tissue (Andrew and Greenspan, 1999; Banik et al., 2001; Kirchhoff et al., 1990; Kocher et al., 1987; Koltzenburg et al., 1999; Schaible and Schmidt, 1985, 1988). However, most of these studies did not compare neurophysiological data with the observations from animal behaviors. This is largely due to the fact that pain behavior studies were usually performed in the glabrous hindpaw skin and recording of nerve fibers were performed from other areas. To overcome this issue, we performed behavioral and neurophysiological studies in the plantar aspect of rat hindpaw.

Our observation, increased percentage of spontaneously discharging C-fibers from an incision, confirms results from in vivo recording from rats 1 day after incision (Pogatzki et al., 2002a). Pogatzki et al. (2002a) observed that 6 of 15 C-fibers had spontaneous activity 1 day after plantar incision; none had spontaneous activity in the sham group. In that study, 4 of 6 spontaneously discharging C-fibers had activities greater than 15 imp/s. These high activities were not present in vitro suggesting a contribution in vivo of mediators removed from the in vitro preparation.

An interesting finding of the present study is that spontaneously discharging fibers from incised rats typically had lower heat response threshold compared to the fibers without any spontaneous activity. And this was present in all incised fibers irrespective of their receptive field locations. The coexistence of spontaneous activity and heat sensitization supports the notion that release of mediators decreases the heat response threshold of nociceptors to the point at which body temperature becomes an adequate stimulus for excitation (Cesare and McNaughton, 1997; Sorkin and Wallace, 1999). Examples of these mediators are bradykinin and prostaglandins. Both have been shown to sensitize nociceptors to heat stimuli (Lang et al., 1990; Liang et al., 2001; Mizumura et al., 1993) by distinct mechanisms: bradykinin possibly lowers the threshold temperature for activation of vanilloid receptor 1 (VR1) via protein kinase C (Sugiura et al., 2002), whereas prostaglandins have been shown to lower the threshold of activation of a tetrodoxin-insensitive Na^+ channel (Gold et al., 1996). Of note, some of these mediators do not necessarily contribute to incision-induced pain behaviors. For example, recent data shows that bradykinin antagonists do not affect incision-induced heat hyperalgesia (Leonard et al., 2004).

In the present study, heat sensitization in the incised hindpaw preparation was prominent, even evident on a mixed population of fibers, named as 'unclassified fibers'. This group of fibers probably included uncharacterized mechanosensitive, high threshold mechanosensitive and mechano-insensitive Aδ and/or C-fibers. Despite such heterogeneity in the fiber characteristics, more incised

fibers from the uncharacterized group responded at 39–40 °C temperature during ramp heat ramp stimulation.

Many studies that used experimentally induced inflammation (Kocher et al., 1987; Koltzenburg et al., 1999; Szolcsanyi, 1987) or exogenous inflammatory mediators (reviewed by Mizumura and Kumazawa, 1996; Woolf and Salter, 2000) observed sensitization of C-fibers to heat stimuli. Also in humans, C-fibers were sensitized to heat after topical application of capsaicin or mustard oil (LaMotte et al., 1992; Schmidt et al., 1995). It is likely that sensitized C-fibers contribute to heat hyperalgesia observed in the present studies.

4.2. Primary but not secondary heat hyperalgesia

In this study, C-fiber sensitization to heat was localized to the incision site. This is in agreement with a behavioral study reported earlier that withdrawal latency to radiant heat from two remote areas of incision was unchanged from the baseline values (Zahn and Brennan, 1999). Earlier studies characterized secondary hyperalgesia (sensory changes outside the area of injury) as increased pain only to mechanical stimuli (Raja et al., 1984). Secondary heat hyperalgesia was not observed after capsaicin injection (Ali et al., 1996) or noxious heat stimulation (Thalhammer and LaMotte, 1982). Likewise, Campbell et al. (1988) did not observe heat sensitization of nociceptors after a cut injury outside the receptive field although sensitization was evident after direct cut to the receptive field.

4.3. Absence of mechanical sensitization

Kawamata et al. (2002) reported the development of both primary and secondary hyperalgesias to punctate mechanical stimulation after a small experimental incision in human subjects. Behavioral studies after plantar incision (Brennan et al., 1996; Zahn and Brennan, 1999) also showed both primary and secondary mechanical hyperalgesias. However, we could not identify sensitization of C-fibers to mechanical stimuli. This observation complements previous studies from our laboratory (Pogatzki et al., 2002a) that used in vivo recordings from the incised rat. These experiments suggested that the incision significantly lowered the response thresholds and increased peak responses to punctate mechanical stimulation of Aδ-fibers but not C-fibers. In vivo, the mechanosensitive receptive field size of both Aδ and C fibers as greater in incised rats. The receptive fields were much smaller and punctate in vitro and could not be quantified.

Studies found that inflammation lowers heat, but not mechanical response threshold of mechanosensitive cutaneous C-fibers (Banik et al., 2001; Kocher et al., 1987; Koltzenburg et al., 1999; Reeh et al., 1986). C-fibers in tumor-bearing mice did not have decreased mechanical threshold although they had spontaneous activity and were sensitized to heat (Cain et al., 2001). These studies, however, did not utilize a stimulus–response function or suprathreshold mechanosensitivity, which has been reported to be changed after experimental inflammation (Ahlgren et al., 1997; Andrew and Greenspan, 1999; Cooper et al., 1991). In our study, suprathreshold sensitivity of C-fibers from <2 mm from the incision tended to be increased above control, but the difference was not statistically significant.

One reason of apparent controversy among studies reporting mechanical sensitization of nociceptors lies in fiber selection bias, which is probably inevitable in the population study (discussed in Cooper et al., 1991). In the present study we recorded a unique subset of C-fibers that are predominantly heat responsive. It has been suggested that after inflammation heat unresponsive high threshold mechanoreceptors (Cooper et al., 1991) or mechano-insensitive afferents (MIA)s (Davis et al., 1993; Handwerker et al., 1991) are sensitized to mechanical stimuli. MIAs have also been shown to contribute to mechanical hyperalgesia in humans (Schmelz et al., 1994; Schmidt et al., 2000). Using the plantar incision model, Pogatzki et al. (2002a) found that the percentage of MIAs 1 day after plantar incision was less than the control suggesting MIAs, perhaps converted to mechanically sensitive afferents 1 day after incision. Therefore, the absence of mechanical sensitization in our study does not exclude the possibility of the involvement of peripheral sensitization for mechanical hyperalgesia; rather, our data only suggest a minimal role for these C-fibers in mechanical hyperalgesia.

4.4. Significance for postoperative pain

Twenty-eight million people undergo surgery and experience postoperative pain annually in the United States alone (Mangano, 1995). However, few studies looked at the mechanisms involved in incisional pain. Spontaneous activity of C-fibers shown in this study and in Pogatzki et al. (2002a) may provide a basis for ongoing pain that persists after clinical surgery. It is hypothesized that spontaneous activity could sustain central sensitization and thus expanding receptive fields of dorsal horn neurons (Vandermeulen and Brennan, 2000); this might cause secondary mechanical hyperalgesia. In our study, spontaneous activity of C-fibers linked to lower threshold for activation of C-fibers to heat stimuli, suggesting that one of the mechanisms for developing spontaneous activity might be the lowering of nociceptor threshold to the body temperature. This proposition is supported by reports that nociceptor heat sensitivity has a predictive value for assessing individual pain perception during postoperative period (Granot et al., 2003; Werner et al., 2004). However, factors that promote such plasticity of nociceptors after incision are largely unclear. Inflammation increases the heat sensor VR1 expression in dorsal root ganglia (Ji et al., 2002) but the incision does not (Simizu et al., 2003; our unpublished observation). However, a substantial reduction

in plantar incision-induced heat hyperalgesia in VR1 knockout mice (Simizu et al., 2003) proposes the further possibility of the involvement of this ion channel by unknown mechanism. Future studies should reveal molecular mechanisms responsible for developing heat sensitization of nociceptors that provides new opportunities for therapeutic approaches to managing postoperative pain.

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