Mechanical and Heat Sensitization of Cutaneous Nociceptors After Peripheral Inflammation in The Rat

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Andrew, David and Joel D. Greenspan. Mechanical and heat sensitization of cutaneous nociceptors after peripheral inflammation in the rat. J. Neurophysiol. 82: 2649–2656, 1999. Tissue injuries commonly cause an increase in pain sensitivity, so that normally painful stimuli become more painful (hyperalgesia), and those usually associated with nonnoxious sensations evoke pain (allodynia). The neural bases for these sensory phenomena have been explored most extensively using heat injuries and experimental arthritis as models. Heat sensitization of cutaneous nociceptors is observed after burns, and sensitization of articular afferents to limb movements occurs after knee joint inflammation. These are likely to be peripheral mechanisms of hyperalgesia. Others, using different models of peripheral inflammation, have only rarely found mechanical sensitization of cutaneous nociceptors. In general these studies have failed to evaluate suprathreshold mechanical sensitivity, which has led to the concept of enhanced spinal cord processing (“central sensitization”) serving as the neural substrate for mechanical hyperalgesia. In the current experiments, the mechanical and heat responses of cutaneous nociceptors supplying the glabrous skin of the rat hindpaw were studied 16–24 h after induction of acute inflammation with complete Freund’s adjuvant. Single-fiber recordings were made from nociceptors in the sciatic nerve of barbiturate-anesthetized animals, and their responses compared with those obtained from nociceptors tested identically in normal animals. Nociceptors were characterized by the following: 1) graded mechanical stimuli (5–90 g) delivered with probes of tip area of 1 and 0.1 mm², 2) their adaptive responses to 2-min mechanical stimuli at three intensities, and 3) their responses to graded heat stimuli (40–50°C). Forty-three nociceptors were studied in the inflamed state; 20 were A fibers, and the remainder were C fibers. Mechanical thresholds, determined with calibrated monofilaments, were not significantly different from controls. Sensitization to suprathreshold mechanical stimuli was observed for both A- and C-fiber nociceptors, although it was greater for the A fibers. Similarly, sensitization during testing of adaptive properties of A- and C-fiber nociceptors was seen, although it was limited to the dynamic (initial) and not the static (plateau) phase of the response. Heat sensitization was observed in 25% of A-fiber nociceptors, but the responses of C fibers to heat were depressed. Other indicators of neuronal sensitization, such as spontaneous activity and expanded receptive fields, were also observed. It was concluded that the mechanical hyperalgesia caused by peripheral inflammation could be explained by nociceptor sensitization. Central mechanisms cannot be completely ruled out as contributing to such hyperalgesia, although their role may be much smaller than previously envisaged.

INTRODUCTION

The effects of tissue damage and inflammation are characterized by increased pain sensitivity, so that the curve that describes the relationship between stimulus level pain intensity is shifted leftward. If the shift is sufficiently large, there are two behavioral consequences: 1) painful stimuli become more painful (i.e., hyperalgesia), and 2) previously nonpainful stimuli such as light touch become painful (i.e., allodynia). Both central and peripheral sensitization in nociceptive pathways have been implicated in the generation of enhanced pain sensitivity (for review see Dubner and Ruda 1992). Recent studies of the effects of injectable or topical agents that evoke an inflammatory response (e.g., carrageenan, complete Freund’s adjuvant, turpentine), have produced apparently conflicting data on the contributions of peripheral and central sensitization after injury. There is no doubt that physical injuries such as burns cause threshold reductions and increases in suprathreshold discharge of heat-sensitive cutaneous nociceptors (LaMotte et al. 1982; Meyer and Campbell 1981). However, until recently, only afferents supplying deep tissues (muscle: Berberich et al. 1988; joints: Schaible and Schmidt 1985, 1988) have been shown to respond to inflammatory agents with increases in mechanosensitivity. In contrast, several studies of cutaneous afferents have reported heat, but not mechanical, sensitization of C-fiber nociceptors (Kocher et al. 1987; Reeh et al. 1986) or that cutaneous nociceptors innervating inflamed tissue were indistinguishable from controls supplying uninflamed skin (Hylden et al. 1989; Woolf and MacMahon 1985). Inflamed cutaneous nociceptors that were spontaneously active in the absence of stimulation have been reported by Kocher et al. (1987) and by Hylden et al. (1989). All of these studies have used threshold reductions to indicate sensitization, but reliance on threshold data alone to predict mechanical sensitization of nociceptors is prone to false negatives (Cooper et al. 1991). Therefore, failure to observe nociceptor sensitization to mechanical stimulation may be because suprathreshold sensitivity was not studied.

We have therefore reinvestigated the effects of inflammation on cutaneous nociceptor sensitivity, by using sufficiently sophisticated stimuli to identify sensitization. The inflammatory model we have used is unilateral injection of complete Freund’s adjuvant (CFA) into the plantar surface of the rat’s hindpaw. This model has been subject to extensive behavioral characterization (Hylden et al. 1989, Iadarola et al. 1988a, 1988b) and causes intense edema and hyperalgesia in the injected paw within a matter of hours. Animals treated this way maintain their weight, display normal grooming behavior, and provided they can guard the affected limb, do not appear to be in inescapable pain (Iadarola et al. 1988a,b). Statistical comparisons were made with a similar sized population of noc-
ceptrors supplying the uninflamed rat hindpaw (Andrew and Greenspan, 1999).

METHODS

Experiments were performed on 18 male Sprague-Dawley rats (300–400g; Harlan, Indianapolis, IN) under institutionally approved protocols.

Induction of inflammation

Subcutaneous injection of CFA (Sigma) was used to induce acute inflammation of the right hindpaw. The animals were anesthetized with 5% Isoflurane in O2, and 150 μl of a 1-mg/ml solution of CFA was injected into the paw over 30 s. The animals were returned to their cages and allowed to regain consciousness. To limit discomfort of the animals after the injection, the ethical guidelines of the International Association for the Study of Pain were followed (Zimmerman 1983). The animals were housed singly in cages, the floors of which were covered with soft bedding, and the survival period was kept as short as possible.

Animal preparation and nociceptor identification

Sixteen to 24 h after CFA injection, the animals were prepared for a terminal experiment. At this time heat and mechanical hyperalgesia are near maximal (Hylden et al. 1989; Iadorola et al. 1988b). Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg; Nembutal, Abbott, North Chicago, IL) and surgically prepared for recording the activity of single hindpaw nociceptors with glabrous skin receptive fields, as described in the companion article (Andrew and Greenspan, 1999). Nociceptors were identified in fine filaments of sciatic nerve by their response to noxious (squeezing), but not nonnoxious (brushing, gentle pressure), mechanical stimuli. By using these criteria, it is possible that nociceptors with very low mechanical thresholds were discarded. Nociceptor-receptive fields, conduction velocity, mechanical threshold, and tissue compliance were determined, as described in the companion article (Andrew and Greenspan, 1999).

Evaluation of nociceptor mechanical and heat sensitivity

Nociceptor responses to short-duration graded mechanical stimuli applied with probes of contact areas of 1 and 0.1 mm² were recorded as described in the companion article (Andrew and Greenspan, 1999), as were adaptive responses to long-duration (2 min) mechanical stimuli. Heat sensitivity was investigated by applying discrete stimuli with a contact thermal stimulator (probe tip area 1.1 cm²; Taylor et al. 1993). Ramp-and-hold (rise time 2.0 s, hold time 5.0 s, interstimulus interval 25 s) stimuli in the range of 40–50°C were delivered in 2°C steps, from an adapting skin temperature of 35°C. Heat thresholds were defined as the temperature that evoked a single impulse from a fiber. When a stimulus evoked more than 1 impulse and was preceded by a subthreshold stimulus, threshold was defined as the mean of the sub- and suprathreshold temperatures. For spontaneously active units, thresholds were defined as the stimulus intensity (force or temperature) that increased the firing rate of a unit by greater than twice the standard deviation of its ongoing discharge frequency.

Data analysis

Electrophysiological data were sampled and digitized as described in the companion article (Andrew and Greenspan, 1999). Responses of spontaneously active units were corrected for background activity. Responses of inflamed nociceptors to graded mechanical stimuli were compared with those of controls using three-factor repeated-measures ANOVA (RM ANOVA), with the three factors being unit type (control or inflamed), stimulus intensity, and probe size and the repeated measures being stimulus intensity and probe size. Tissue compliance and responses to heat were compared across fiber types using two-factor RM ANOVA. Tukey’s test was used to compare groups with one another if ANOVA revealed a significant-factor effect.

Statistical differences between the adaptation of nociceptors supplying normal and inflamed skin were determined by resolving the time course of individual responses into two constants, a and b, using the two-parameter single-exponential-decay relationship $y = ae^{-bx}$, that relates firing rate (y) to time (x). With this function, a is proportional to the peak firing rate (the “dynamic” phase of the fiber’s response), and b is proportional to the rate of response decline (the “static” phase of the response). For each stimulus intensity, comparisons between these constants for control and inflamed fibers were made with the Mann-Whitney rank sum test. For all statistical tests, $P < 0.05$ was considered significant.

RESULTS

General properties of inflamed nociceptors

Recordings were made from 43 nociceptors with receptive fields on the glabrous skin of the hindpaw; 20 were A fibers, and the remainder were C fibers. The conduction velocities of the A fibers ranged from 5.3–34.8 m/s (17.1 ± 9.2 m/s, mean ± SD), those of the C fibers were between 0.7 and 1.2 m/s (1.0 ± 0.1 m/s). Mean monofilament threshold of the A-fiber nociceptors was 756 kPa (764 ± 278 kPa, median ± SD; range 520–1639), and that of the C fibers was 933 kPa (930 ± 308, range 520–1639). The median threshold values of nociceptors supplying inflamed tissue were not significantly different from those of controls ($P > 0.1$ for both A- and C-fiber nociceptors, Mann-Whitney rank sum test). Although the areas of receptive fields were not precisely measured by planimetry, eight of the units in inflamed paws had receptive fields that were much larger than those that could be accounted for solely by the increase in paw volume that inflammation causes (Fig. 1). Fourteen of the units were spontaneously active.
(9 C fibers and 5 A fibers), their rates of ongoing activity were between 0.1 and 1.3 imp/s (0.7 ± 0.3 imp/s, mean ± SD).

Responses to mechanical stimulation

Although there were no significant differences between the monofilament thresholds of units supplying normal or inflamed skin, there was significant sensitization to mechanical stimuli (Fig. 2A and C). Although inflamed A-fiber nociceptors were considerably more variable in their responses than their control counterparts, they were significantly more responsive to suprathreshold mechanical stimuli than were controls for both the 1 (P < 0.003, 3-factor RM ANOVA) and 0.1 mm² (P < 0.04, 3-factor RM ANOVA) probes. There was also a significant interaction between fiber type (control vs. inflamed) and stimulus intensity (P < 0.008, 3-factor RM ANOVA) and also between fiber type and stimulus intensity and probe size (P < 0.005, 3-factor RM ANOVA). The C-fiber nociceptors were significantly more responsive than controls for the 0.1 mm² probe (P < 0.05, 3-factor RM ANOVA), but not for the 1 mm² probe (P > 0.14, 3-factor RM ANOVA). There were no significant interactions among factors for the C-fiber nociceptors.

Adaptation of nociceptor discharge

Stimuli of intensity 25, 50, and 100 g delivered for 2 min with a probe of tip area 0.1 mm² were used to investigate nociceptor mechanical adaptation. Single exponential decays were fitted to individual responses and the peak firing rate and decay rate computed. The R² values (mean ± SD) for these fits for control and inflamed A fibers, respectively, were 25 g: 0.35 ± 0.16, 0.58 ± 0.25; 50 g: 0.40 ± 0.17, 0.67 ± 0.17; and 100 g: 0.47 ± 0.19, 0.66 ± 0.14. Those for the C fibers were 25 g: 0.31 ± 0.18, 0.38 ± 0.19; 50 g: 0.56 ± 0.15, 0.48 ± 0.26; 100 g: 0.47 ± 0.19, 0.66 ± 0.16. The adaptational properties of inflamed A- and C-fiber nociceptors are shown in Fig. 3, and the best-fit exponential function parameters are shown in Fig. 4. For the A-fiber nociceptors, sensitization was observed in the peak of the dynamic phase of responses (P < 0.05, Mann-Whitney rank sum test), but not during the response decay (P > 0.3, Mann-Whitney rank sum test) for all three stimulus intensities. The proportion of A-fiber nociceptors that exhibited rapidly adapting (A-HT[R]: RA) responses to tonic mechanical stimuli declined from 50% in controls to 30% after inflammation (P < 0.04, χ² test). The C-fiber nociceptors were also sensitized during the dynamic phase of the response, but only at the highest intensity tested (100 g; P < 0.05, Mann-Whitney rank sum test). There were no significant differences between the normal and inflamed units in their rates of adaptation at any of the stimulus intensities tested (P > 0.4, Mann-Whitney rank sum test).

In addition to nociceptor sensitization during the dynamic phase of responses to long-duration mechanical stimuli, 15 units (12 C fibers, 3 A fibers) displayed bursting activity during
stimulation. This was observed only rarely in control units and seldom exceeded more than two or three pairs or triplets of action potentials during a stimulus (see Fig. 2 in Andrew and Greenspan, 1999). An example is shown in Fig. 5. Despite the observation that nociceptor bursting was dramatically increased during inflammation, there was no significant relationship between stimulus intensity and any of the following burst characteristics that were measured: number of bursts per stimulus \( (P > 0.1, \text{ANOVA}) \), latency to first burst \( (P > 0.3, \text{ANOVA}) \), interburst interval \( (P > 0.8, \text{ANOVA}) \), peak firing rate within a burst \( (P > 0.9, \text{ANOVA}) \), or the number of impulses in a burst \( (P > 0.5, \text{ANOVA}) \).

Inflammatory effects on skin compliance

Skin compliance was determined by measuring probe displacement during testing a unit with graded mechanical stimuli, to investigate whether changes in skin stiffness could account for the changes in nociceptor mechanosensitivity after inflammation. There was no significant difference between the compliance of control and inflamed A-fiber–receptive fields \( (P > 0.1, \text{ANOVA}) \), latency to first burst \( (P > 0.3, \text{ANOVA}) \), interburst interval \( (P > 0.8, \text{ANOVA}) \), peak firing rate within a burst \( (P > 0.9, \text{ANOVA}) \), or the number of impulses in a burst \( (P > 0.5, \text{ANOVA}) \).

Heat-evoked responses

Of 20 control C-fiber nociceptors, 16 responded to heating in the range tested. Threshold was \( 42.5 \pm 1.9^\circ C \) (mean \( \pm \) SD; range \( 40–45^\circ C \)), and the units monotonically increased their firing rate as stimulus intensity increased (Fig. 7A). Of 20 control A-fiber nociceptors only 1 responded to heat, and it did so with a single impulse at the highest temperature tested (50\(^\circ C\)). This negligible sensitivity of rat A-fiber nociceptors to noxious heat has been reported previously (Lynn and Shakahbeh 1988). Inflammation increased the proportion of A fibers that were heat sensitive (5 of 20 tested), but not significantly so \( (P > 0.1, \text{Fisher exact test}) \). Their thresholds were in the range

![FIG. 4](http://jn.physiology.org/) Relationship between the constants \( a \) and \( b \) (see METHODS) derived from individual responses from each nociceptor after tonic mechanical stimulation of 25, 50, and 100 g with a probe tip area of 0.1 mm\(^2\). Significant differences \( (P < 0.05, \text{Mann-Whitney rank sum test}) \) between control and inflamed nociceptors (*) were evident in the peak firing rates of A-fiber nociceptors at each intensity tested (A) and of C-fiber nociceptors at the highest stimulus intensity (B), but not during response adaptation for either A- or C-fiber nociceptors (C, D).
43–48°C (45.2 ± 1.9°C), and they were significantly more sensitive than controls (P < 0.04, Mann-Whitney rank sum test on responses to 50°C; Fig. 7B). Of 23 C-fiber nociceptors tested after CFA-induced inflammation 19 responded; the proportion of heat-sensitive C-fibers was not significantly different between groups (P = 1, Fisher Exact test). Mean heat threshold of the C-fibers supplying inflamed skin was 42.1°C (range 40–45 ± 1.9°C), which was not significantly different from that of controls (P > 0.6, unpaired t-test). After inflammation, the heat-evoked responses of C-fibers were significantly reduced (Fig. 7A; P < 0.05, 2-factor RM ANOVA). None appeared to be sensitized to heat, and a group of fibers with very weak heat responses seemed to cause the population response to be depressed (Fig. 7, C and D).

**FIG. 5.** Example of the response of a single nociceptor (conduction velocity 1.0 m/s; monofilament threshold 520 kPa) supplying inflamed skin to stimulation of its receptor with a probe of 0.1 mm² at 100-g intensity. Examples of some of the bursts are shown on an expanded time scale.

**FIG. 6.** Force-displacement plots for control (A) and inflamed (B) cutaneous nociceptors in the rat’s glabrous hindpaw. Inflamed C-fiber nociceptors had significantly more compliant receptive fields than did controls (P < 0.05, 2-factor repeated-measures [RM] ANOVA). Bars indicate SD. Each point is the mean of 20 fibers.
DISCUSSION

In the present experiments we have described mechanical and heat sensitization of glabrous skin nociceptors in the rat’s hindpaw after acute inflammation. Mechanical sensitization of nociceptors by agents evoking an inflammatory response has previously been demonstrated only for deep tissue afferents (Berberich et al. 1988; Schaible and Schmidt 1985, 1988), and for goat palate mechanonociceptors (Cooper et al. 1991). The failure of other studies (Hylden et al. 1989; Kocher et al. 1987; Reeh et al. 1986; Woolf and MacMahon 1985) to show mechanical sensitization could be used to infer that mechanical sensitization is peculiar to mechanonociceptors supplying specialized tissues. However, these studies relied solely on mechanical thresholds as a measure of mechanical sensitization, and as reported by Cooper et al. (1991), mechanical sensitization is not necessarily associated with significant reductions in nociceptor thresholds. Our experiments show first, in agreement with Cooper et al. (1991), that mechanical thresholds are inadequate predictors of sensitization and second, mechanical sensitization was observed for cutaneous nociceptors with both A- and C-fiber conduction velocities, including mechanohot nociceptors.

Heat sensitization was evident for A-fiber but not for C-fiber nociceptors that showed significantly depressed responses. This reduction in mean responsiveness per fiber was unexpected because inflammation-induced heat sensitization of rat hairy skin nociceptors has been described previously (Kocher et al. 1987). However, our experiments were performed on units supplying glabrous skin, where burn injuries in monkey have been shown to sensitize A-fiber nociceptors but to desensitize C-fiber nociceptors to heat (Campbell and Meyer 1983; Campbell et al. 1979; Meyer and Campbell 1981). Because our study was performed several hours after inflammation induction, it is not possible to state with certainty that the reduced responsiveness of C-fibers to heat was caused by receptor desensitization, as shown by Campbell and Meyer’s (1983) experiments. An alternative interpretation of these results is that weak heat sensitivity developed in previously heat-insensitive receptors. Therefore, the total nociceptor input to the spinal cord would be increased, despite a reduction in the average firing rate per nociceptor. A similar finding has been made in man by Schmidt et al. (1995) after topical application of mustard oil or capsaicin to the receptive fields of nociceptors that were initially heat insensitive.

The enhancement of A-fiber nociceptor heat sensitivity after peripheral inflammation is similar to that reported in monkey after burns (Meyer and Campbell 1981). Given that the rat A-fiber nociceptors show almost no heat sensitivity, even after repeated stimulation (Lynn and Shakhanbeh 1988), the development of heat sensitivity implies upregulation of heat-transducing elements within the cell membrane. Whether some of the other observations on sensitized nociceptors are due to changes in ion channel expression is unclear. Mechanical sensitization of nociceptors in hairy skin is an effect of several lipoxygenase products, such as (8R, 15S)-dihydroxyicosa(5E-9, 11, 13Z)tetraenoic acid and leukotriene B₄ (Martin et al. 1988; White et al. 1990; see also Kress and Reeh 1996; Levine and Tiawo 1994 for recent reviews), and could be due to chemical modulation of receptor sensitivity. Similarly, the bursting behavior seen during long-duration mechanical stimulation could either be due to chemomodulation, or the incorporation of new ion channels into the receptor membrane.

Evidence was also obtained that under control conditions parts of the terminal arborization of nociceptors were unresponsive but became responsive after inflammation. This conclusion was based on the observation that fibers supplying inflamed skin had larger receptive fields than that of the largest control unit encountered. It is unlikely that these larger fields are false positives due to the increase in size of the inflamed paw, because many of the units (35/43) were of a size similar to control nociceptors. Nociceptor-receptive field expansion...
has been described after burns in monkeys (Thalhammer and LaMotte 1982); after sustained, strong, blunt pressure in rats (Reeh et al. 1987); and after topical mustard oil or capsaicin application in humans (Schmelz et al. 1994). The short time course of receptive field expansion in monkeys (22 min) suggests some sort of local sensitizing phenomenon, perhaps due to inflammatory mediators, to improved stimulus-receptor coupling because of edema (Cooper 1993) or even to strengthening of ephaptic junctions between coupled nociceptors (Schmelz et al. 1994).

We have obtained electrophysiological evidence of a peripheral basis for mechanical hyperalgesia after inflammation: The stimulus-response functions of nociceptors supplying inflamed skin were significantly steeper than those of controls. Also, the proportion of A-fiber rapidly adapting nociceptors was reduced after inflammation. Coupled with the development of nociceptor bursting to tonic stimuli, the afferent inflow into the spinal cord is greatly increased, and as such these peripheral changes are likely to be mechanisms of mechanical hyperalgesia. Thus, the idea that mechanical hyperalgesia is solely mediated at the spinal level seems no longer tenable. In contrast, mechanical allodynia is likely to be mediated through sensitization of spinal cord neurons receiving convergent nociceptive and mechanoreceptive inputs (Torebjörk et al. 1991). However, it is still possible that some inflamed nociceptors may have very low mechanical thresholds and would not have been evaluated in the present study. Heat sensitization was observed for a small proportion of A-fiber nociceptors, and the population response to heat of heat-sensitive C-fiber nociceptors was significantly reduced. This reduced C-fiber response could be interpreted as meaning such fibers have little role in heat hyperalgesia, despite its being prominent behaviorally in this model (Hylten et al. 1989; Iadarola et al. 1988a). However, the total input to the spinal cord would be increased if, as we suspect, some previously heat-insensitive nociceptors display weak heat sensitivity after inflammation. Other possible explanations for heat hyperalgesia include the following: 1) enhanced sensitivity of heat-specific nociceptors, which we would have been unable to detect with our unitary identification criteria and that have not previously been described in rat; and 2) mechanical hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. J. Neurophysiol. 66: 212–227, 1991.

References


