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Neuropathic Pain-like Alterations in Muscle Nociceptor Function Associated with Vibration-induced Muscle Pain

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Abstract

We recently developed a rodent model of the painful muscle disorders induced by occupational exposure to vibration. In the present study we used this model to evaluate the function of sensory neurons innervating the vibration-exposed gastrocnemius muscle. Activity of 74 vibration-exposed and 40 control nociceptors, with mechanical receptive fields in the gastrocnemius muscle, were recorded. In vibration-exposed rats ~15% of nociceptors demonstrated an intense and long-lasting barrage of action potentials in response to sustained suprathreshold mechanical stimulation (average of 2635 action potentials with frequency of ~44 Hz during a 1 minute suprathreshold stimulus) much greater than has been reported to be produced even by potent inflammatory mediators. While these high-firing nociceptors had lower mechanical thresholds than the remaining nociceptors, exposure to vibration had no effect on conduction velocity and did not induce spontaneous activity. Hyperactivity was not observed in any of 19 neurons from vibration exposed rats pretreated with intrathecal antisense for the IL-6 receptor subunit gp130. Since vibration can injure peripheral nerves, and IL-6 has been implicated in painful peripheral neuropathies, we suggest that the dramatic change in sensory neuron function and development of muscles pain, induced by exposure to vibration, reflects a neuropathic muscle pain syndrome.

Introduction

Muscle pain is not a clinical diagnosis but rather a constellation of syndromes with considerable symptomatic overlap that develop in association with repetitive strain or trauma (e.g., muscle spasticity, overuse, vibration exposure) and/or unusual postures [47,57,84]. These syndromes are associated with occupational, sport and musical activities, and include ‘cumulative trauma disorders’, ‘repetitive motion disorders’, and ‘hand-arm vibration syndrome.’ Because of persisting pain or ease of precipitating recurrence or exacerbation, patients with these syndromes often cannot return to their original occupations or activities of daily living [67]. Lack of knowledge concerning cellular mechanisms underlying muscle pain [40,58,76] is an important obstacle to improving rehabilitation strategies and treatment of muscle pain, for which currently available methods are often inadequate [28,57]. While current research on muscle pain has focused intensively on the effects of muscle and tendon strain [8,24], and the role of inflammatory mediators [21,22,34], the underlying mechanisms specifically

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responsible for nociceptor sensitization and chronic muscle pain are only beginning to be addressed.

We have recently shown that exposure to vibration in the rat produces mechanical hyperalgesia in skeletal muscle in the hind limb, and enhances subsequent vibration- or inflammatory mediator-induced muscle hyperalgesia [22]. The effect on muscle nociceptor function of intrathecal administration of antisense for IL-6 receptor, which has been implicated in neuropathic pain [7,42,50,54] was also evaluated. In the present study, we explored the changes in the function of sensory neurons innervating the gastrocnemius muscle after induction of muscle pain by vibration exposure and the role of IL-6 in observed changes.

Methods

Animals

Adult male Sprague Dawley rats weighing 250 to 300 g (Charles River, Hollister, CA) used in these experiments were housed in the Animal Care Facility at UCSF, under environmentally controlled conditions (7 am to 7 pm lights on; 21–23°C) with food and water available *ad libitum*. Animal care and use conformed to NIH guidelines; the UCSF Committee on Animal Research approved all experimental protocols.

Mechanical Vibration

The hind limbs of rats were vibrated with a Digital Vortex Genie II laboratory vortex mixer (Fisher Scientific, Waltham MA), which has a variable speed motor with a real-time digital readout of vibration speed. Rats were anesthetized with 3% isoflurane in oxygen and one hind leg affixed to the platform of the vortex mixer with Micropore® surgical tape (Fisher Scientific) so that the knee and ankle joint were both at 90°, without rotational torque on the leg. The leg vibration parameters were: frequency 50±5 Hz, amplitude 2.5 mm, acceleration 175 m/s², velocity 55.5 cm/s and displacement 5-mm; the vibration frequency is within the range produced by hand-held power tools (35–150 Hz) [68]. Isoflurane-anesthetized rats were exposed to a single session of 15-min vibration.

Single fiber recording for muscle afferents

The *in vivo* single fiber electrophysiology technique employed was similar to that used previously in our recordings from cutaneous afferents [16]. Rats were anesthetized with sodium pentobarbital (initially 50 mg/kg, i.p., with additional doses given throughout the experiment to maintain areflexia), their trachea cannulated, and heart rate monitored.

Animals were positioned on their right side and an incision made on the dorsal skin of the left leg between the mid-thigh and shank. The biceps femoris muscle was partially removed to expose the sciatic nerve and gastrocnemius muscle. The edges of the incised skin, fixed by a metal loop, provided a pool that was filled with warm mineral oil, immersing the sciatic nerve and gastrocnemius muscle. Neural activity was recorded from single fibers in the sciatic nerve, cut proximal to the recording site to prevent flexor reflexes during electrical stimulation of the muscle. Fine fascicles of axons were then dissected from the distal stump, and placed on a recording electrode. Single units were first detected by mechanical stimulation of the gastrocnemius muscle with a small blunt-tipped glass bar. Bipolar stimulating electrodes were placed and held on the center of the receptive field, of the muscle afferent, by a micromanipulator (MM-3, Narishige, Japan). Conduction velocity of each fiber was calculated by dividing the distance between the stimulating and recording electrodes by the latency of the electrically evoked action potential. All recorded muscle afferents have conduction velocities in the range of type III (12%) or type IV (88%) fibers. Mechanical threshold was determined with calibrated von Frey hairs (VFH; Ainsworth, London, UK) and defined as the lowest force

that elicited at least 2 spikes within 1 s, in at least 50% of trials. Sustained (60 s) suprathreshold (10 g) mechanical stimulation was accomplished by use of a mechanical stimulator that consisted of a force-measuring transducer (Entran, Fairfield, NJ, USA), which was held by a micromanipulator (BC-3 and BE-8, Narishige) on the center of the receptive field for 60 s. Neural activity and stimulus onset and termination was monitored and stored on a Windows OS computer with Micro 1401 interface (CED, Cambridge, UK) and analyzed off-line with Spike2 software (CED).

Measurement of hyperalgesia

Mechanical nociceptive thresholds were quantified using a Chatillon digital force transducer (model DFI2, Amtek Inc., Largo, FL). Rats were lightly restrained in an acrylic holder that allows for easy access to the hind limb. A 6 mm diameter probe, which preferentially stimulates muscle [63], attached to the transducer, was applied to the gastrocnemius muscle to deliver an increasing compression force. The nociceptive threshold was defined as the force, in Newtons, at which the rat withdrew its hind leg. Baseline withdrawal threshold was defined as the mean of 2 readings taken at 5-min intervals. Each hind limb is treated as an independent measure and each experiment performed on a separate group of rats. All behavioral testing was done between 10 am and 4 pm.

Antisense oligodeoxynucleotide administration

The method for intrathecal oligodeoxynucleotide (ODN) injection has been described previously [2-4,20,22,38,39,64-66]. Briefly, for ODN injections, rats were anesthetized with 3% isoflurane, and a 30-gauge needle inserted into the subarachnoid space on the midline, between the L4 and L5 vertebrae. ODN (80 µg/10 µl) was slowly injected. This procedure was repeated so that ODN was administered on 3 consecutive days. Control animals received injections of mismatch ODN.

To delineate a contribution of IL-6 signaling in sensory neurons by intrathecal administration of ODN antisense to the IL-6 receptor signal transducing molecule, glycoprotein 130 (gp130), a subunit of the IL-6 receptor signaling complex was employed in these experiments. The antisense ODN sequence, 5'-TCC TTC CCA CCT TCT TCT G-3', was directed against a unique sequence of rat gp130. The corresponding GenBank accession number and ODN position within the cDNA sequence are M92340 and 1834–1852, respectively [83]. The mismatch ODN sequence, 5'-TAC TAC TCA CAT TCA TCA G-3', corresponds to the gp130 subunit antisense sequence with 6 bases mismatched (denoted by bold letters). The antisense- and mismatch 19-mer ODN for gp130 were purchased from Invitrogen (San Francisco, CA). The dose of ODN, 80 µg, was based on prior dose-response studies [74].

Statistics

Group data are expressed as mean ± SEM of n distinct observations. Statistical comparisons were made by a 2-tailed Student's t-test (for 1 or 2 independent populations) or by 1-way ANOVA for comparing multiple treatments, using Prism software. A Chi-square was performed to determine the effect of IL-6 antisense on the percentage of fibers exposed to vibration that developed hyperactivity. $P < 0.05$ was considered statistically significant.

Results

Vibration markedly increases nociceptor response

To assay the effect of the vibration protocol that produces mechanical hyperalgesia in skeletal muscle, on responsiveness of muscle afferents, a suprathreshold (10 g) 60 s stimulus was applied to their receptive fields. Figure 1a (inset) shows that the mean number of action

potentials evoked during the sustained 60 s stimulus was significantly higher for nociceptors from vibration-exposed rats compared to those from control rats ($P=0.014$, Student's *t*-test). A scattergram of number of action potentials produced by individual fibers (Figure 1a), indicates the existence of extremely high-firing frequency nociceptors (firing frequency >1500 spikes/60 s) not seen in sensory neurons from control rats. These high-firing nociceptors in vibration-exposed rats had a greater than 10-fold higher number of action potentials compared to controls (2635 ± 349 vs. 216.8 ± 49.9 ; during the 60 s stimulus Figure 1b). There was no significant difference between the number of action potentials in the other nociceptors (<1500 spikes/60s) in the vibration-exposed rats and those in control rats (282.5 ± 38.7 vs. 316.8 ± 49.9 , respectively, $P=0.298$). Based on these findings further analyses were performed separately for the extremely high-firing frequency nociceptors and the remaining nociceptors. Recordings of responses to suprathreshold (10 g) stimulus in a C-fibers from control, and non high-firing nociceptors (<1500 spikes/60 s) and extremely high-firing frequency nociceptors (>1500 spikes/60 s) are shown in Figure 1c.

Increase in action potential firing is sustained

The histograms for firing frequency over time for sustained 10 g stimulation of muscle nociceptors from naïve and normal frequency vibrated rats were not significantly different ($P>0.005$, one-way ANOVA). The high-firing frequency muscle afferents, however, had significantly greater numbers of action potentials at all time points over the full 60 s stimulus period (one-way repeated measures ANOVA, with Bonferroni post hoc test, $P<0.05$ high-frequency vs. both control and normal frequency, Figure 2).

Low threshold in high-firing nociceptors

The mechanical threshold of high-firing nociceptors from vibration-exposed rats (0.30 ± 0.06 , $n=10$) was significantly lower than for normal firing frequency (0.88 ± 0.09 , $n=64$) nociceptors and those from control (non-vibrated) rats (1.11 ± 0.11 , $n=40$) (one-way ANOVA with Kruskal-Wallis test, both $P=0.0006$, Figure 3A and B). However, there was no significant difference between the vibration-exposed high-firing frequency nociceptors and those from rats not exposed to vibration ($P>0.05$).

No effect on conduction velocity

The average conduction velocity of control (1.25 ± 0.11 m/s) and vibration-exposed high (1.57 ± 0.27 m/s) and normal firing frequency (1.44 ± 0.10 m/s) nociceptors were not significantly different (one-way ANOVA $P=0.349$; data not shown).

gp130 antisense prevents development of high-firing nociceptors

Injection of ODN antisense to the gp130 subunit of the IL-6 receptor ($80\text{ }\mu\text{g}/20\text{ }\mu\text{l}$, intrathecal) for 3 days prior to vibration, significantly reduced the magnitude of vibration-induced acute hyperalgesia compared to rats receiving mismatch ODN ($P<0.0001$; Figure 4 inset). Furthermore, we observed no high-firing frequency nociceptors in animals pretreated with gp 130 antisense, ($n=19$, $P<0.05$, one-tailed Chi squared, Figure 4), the response to 10 g stimulation in the vibrated antisense group was not significantly different from the normal firing vibrated group ($P>0.05$, ANOVA with Scheffé's post hoc test). Thus, treatment with gp130 antisense prevented both the vibration-induced decrease in behavioral mechanical threshold, as well as the vibration-induced emergence of high-firing frequency sensory neurons.

Discussion

In this electrophysiology study of a rat model of hind limb vibration-induced muscle pain [22], we observed the emergence of stimulus-evoked very high-frequency muscle nociceptors that had both markedly enhanced and prolonged response to sustained mechanical stimulation, and significantly lower mechanical threshold than non-high-firing nociceptors and nociceptors in control rats not exposed to vibration. We know of no prior report of such mechanically evoked sustained very high-frequency activity in primary afferent nociceptors under any clinical or experimental condition. While we do not know what the mechanical threshold of the high-firing fibers was prior to vibration, the fact that they all had very low thresholds after vibration suggests that these fibers may even contribute to spontaneous pain after vibration exposure.

That the high-firing nociceptors have very low mechanical thresholds (one-third that of the other two groups of nociceptors), but conduction velocities no different than non-high-firing nociceptors or nociceptors in control rats, supports the suggestion of an abnormality located in the distal terminals of the sensory axon.

Another unique feature of the response of the high-firing nociceptors is the finding of largely unattenuated activity during sustained mechanical stimulation. Nociceptors usually demonstrate a dramatic adaptation to persistent stimulation [60] with a decay time constant of 2-3 s [59,71,81]. Mechanisms involved in transmission as well as transduction have been suggested to explain this adaptation, including rate of recovery from inactivation of sodium channels [79], cytoplasmic calcium concentration [80] and changes in mechanical coupling in transduction [23,51]. Lack of slowing of conduction velocity supports the suggestion of an attenuation in the transduction processes [45,49,72]. While at the present time the mechanism mediating response adaptation, to a sustained stimulus, is unknown, its elucidation may provide an important clue to the pathophysiology of vibration-induced muscle pain.

We also observed that spinal intrathecal administration of antisense to an IL-6 receptor subunit (gp130), which decreases gp130 in sensory neurons [75], prevented the development of both hyperalgesia and the emergence of the high-firing frequency nociceptors induced by exposure to vibration. We have previously shown that IL-6 produces mechanical hyperalgesia in skeletal muscle [21], and the current study suggests that the emergence of the high-firing frequency of muscle nociceptors produced by vibration exposure is also IL-6 receptor-dependent. It is likely that the source of IL-6 in our protocol is from Schwann cells, macrophages or epineural fibroblasts, since IL-6 production is increased in these cell types in a low-force repetitive motion rat model [1], and tail vibration increases IL-6 levels in tail arteries [44]. We hypothesized that the electrophysiological changes we observed following vibration are qualitatively different from those produced during inflammatory hyperalgesia, and that the emergence of extremely high-firing neurons is consistent with neuropathic pain. However, while these changes are prevented by gp130 antisense administration they are not simply due to locally released IL-6 since administration of IL-6 does not produce this effect in nociceptors in joints [10] or muscle (unpublished observations). Taken together with an extensive literature for a role of IL-6 in neuropathic pain [7,42,50,54] and animal models of neuropathic pain [5, 26,41,48,56], these data further support the suggestion of a role of peripheral neuropathy in vibration-induced muscle pain.

Chronic muscle pain is a common clinical problem in individuals exposed to vibration (e.g., hand-arm vibration syndrome) [17,29,35]. While characterized by blanching of the fingers, cold intolerance, deep pain and peripheral neuropathy in the arm and hand [12,13,27,29,61, 82], descriptions of the muscle pain associated with vibration exposure have not been reported to contain features characteristic of neuropathic symptoms (e.g., burning and lancinating [6,

31]). Of note, however, in studies where similar noxious stimuli have been applied to cutaneous and muscle tissue, distinct sensory qualities are reported [11,15], indicating that it may be possible to elicit diagnostically useful, distinctive symptoms from patients with neuropathic muscle pain.

The quality of muscle pain is often described as tearing, cramping or pressing, poorly localized and demonstrating referral, while cutaneous pain is more likely to be described as stabbing, burning, or cutting in quality, more well-localized and non-radiating [58]. In this regard, patients with well-defined peripheral neuropathies may report muscle pain. For example, patients with beriberi neuropathy report muscle tenderness but cutaneous burning pain [43], and patients with mitochondrial neuropathies report muscle pain and cramps, but cutaneous paresthesias and dysesthesias [25]. Similar co-morbidities have also been reported with chemotherapy neuropathies [53,62,70] and Fragile X syndrome [69]. In addition, cold hypersensitivity is both a frequent symptom of vibration exposure [9,12,13,29] as well as a common feature of some forms of peripheral neuropathy [18,27,73]. Finally, vibration is well established to produce peripheral neuropathies in both animals [14,30,33,36,52,55] and humans [9,19,29,32,37,46,77,78]. Thus, failure to have well-described neuropathic muscle pain syndromes may be due, in part, to patients being asked to use descriptors commonly employed for neuropathic pain in the cutaneous domain.

In summary, we have found that in rats exposed to vibration there emerges very high-firing frequency nociceptors comprising ~15% of muscle nociceptors, with normal conduction velocity, but extremely low mechanical nociceptive thresholds compared to the other nociceptors in vibration-exposed rats and nociceptors in control rats. This effect of vibration on nociceptor function was abrogated by a decrease in gp130, an IL-6 receptor subunit, needed for IL-6 receptor function, in muscle nociceptors. Taken together with known effects of vibration to induce peripheral neuropathy and the role of IL-6 in peripheral neuropathy, we suggest that vibration induces neuropathic muscle pain. The low mechanical nociceptive threshold and increased response to sustained mechanical stimuli of these high-firing nociceptors is likely to produce a sensitization of spinal nociceptive circuits and account for the behavioral hyperalgesia seen in rats exposed to hind paw vibration [22]. Future studies will be needed to elucidate the underlying cellular and molecular mechanisms for these changes in mechanically-evoked muscle nociceptor activity.

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Abbreviations

ODN	Oligodeoxynucleotide
IL-6	interleukin-6
gp130	glycoprotein 130
ANOVA	analysis of variance
SEM	standard error of the mean

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Figure 1.

a) The response of nociceptors to sustained (60 sec) (10 g) stimuli, showing individual fibers (main figure) and mean values (inset) in naïve and vibration-exposed rats. The responses of the nociceptors from the group exposed to vibration ($n = 74$) were significantly higher than those of control rats ($n = 40$, $P=0.014$, Student's *t*-test). An unexpected group of fibers with >1500 spikes/60 s, suggest the emergence of an unusually high-firing frequency population of nociceptors in vibration-exposed rats.

b) The high-firing nociceptors (>1500 spikes/60 s) in vibration-exposed rats had an ~10-fold higher number of action potentials compared to nociceptors from control rats (2635 ± 349 vs. 216.8 ± 49), and non-high-firing nociceptors (<1500 spikes/60s) in the vibration-exposed rats (282.5 ± 38.7). There was no significant difference in response of neurons to suprathreshold stimulation in control and non-high-firing frequency nociceptors from vibrated rats ($P=0.298$).

c) Single-unit C-fiber recordings of action potentials evoked by a 10-g stimulus in control and high-firing nociceptors in vibration-exposed rats.

Figure 2.

The time course of the average responses of nociceptors during the 60 s suprathreshold stimulation, for naïve, normal and high-firing nociceptors; bin width is 1 sec.

Figure 3.

- a)** While the mechanical thresholds of muscle nociceptors in control and normal firing frequency vibration-exposed rats was not significantly different, high-firing nociceptors in vibrated rats had significantly lower mechanical threshold than normal firing frequency nociceptors from vibrated rats, or nociceptors from control rats ($P < 0.001$, one-way ANOVA).
- b)** Scattergram of the mechanical thresholds for individual muscle nociceptors.

Figure 4.

A. Injection of ODN antisense, but not mismatch for the gp130 subunit of the IL-6 receptor significantly reduced the magnitude of vibration-induced mechanical hyperalgesia comparison to rats receiving mismatch ODN.

B. In animals pretreated with gp130 antisense, no high-firing frequency nociceptors were observed in response to a sustained (60s) suprathreshold (10 g) stimulation ($P < 0.05$, one-tailed Chi squared).