Encoding of Compressive Stress During Indentation by Group III and IV Muscle Mechano-Nociceptors in Rat Gracilis Muscle

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Ge, Weiqing, and Partap S. Khalsa. Encoding of compressive stress during indentation by group III and IV muscle mechano-nociceptors in rat gracilis muscle. J Neurophysiol 89: 785–792, 2003. First published November 20, 2002; 10.1152/jn.00624.2002. The mechanical state encoded by group III and IV muscle afferents, putative mechano-nociceptors, during indentation was examined using an isolated muscle-nerve preparation in a rat model. Gracilis muscle and its intact innervation were surgically removed from the medial thigh of the rat hindlimb and placed in a dish containing rodent synthetic interstitial fluid. The tendons of the muscle were coupled to an apparatus that could stretch and apply compression to the muscle. Using a standard teased-nerve preparation, the neural responses of single mechanically sensitive group III or IV afferents were identified. Afferents were classified as mechano-nociceptors on the basis of their graded response to noxious levels of compressive stress (or strain) as well as, in some cases, their polymodal response to noxious thermal stimuli. Mechano-nociceptors (n = 13) were stimulated using controlled compressive stress (10–30 kPa) or strain (40–80%) while simultaneously measuring displacement and force by compressing the muscle between a flat cylinder and a hard platform. Linear regression was used to evaluate the relationships between neural response and mechanical stress, force, strain, and displacement. The mean neural response (threshold: 1.1 ± 0.4 kPa; sensitivity: 0.5 ± 0.1 Hz/kPa; means ± SE) was significantly and substantially more highly correlated with compressive stress than force, strain, or displacement. The data from this study support the hypothesis that muscle nociceptors stimulated by indentation encode compressive stress rather than force, strain, or displacement.

INTRODUCTION

Primary muscle pain, due to acute mechanical stimuli, is based on the stimulation of muscle mechano-nociceptors. The receptive endings of these afferents are associated with many structures in skeletal muscle including muscle extracellular matrix, muscle fibers, and blood vessels (Stacy 1969). The afferents have axons that conduct action potentials in the group III (thinnily myelinated axons) and IV (unmyelinated axons) ranges (Iggo 1960; Lloyd 1943; Painal 1960), functionally analogous to cutaneous Aδ and C mechano-nociceptors. In recordings from single peripheral afferents from cat or rat muscles, nociceptors can respond to single or combinations of natural noxious (tissue damaging) stimuli including: mechanical (Kauffman et al. 1983, 1984; Mense and Meyer 1985; Painal 1960), noxious temperature (Mense and Meyer 1985), and various chemicals including bradykinin (Mense and Meyer 1988), prostaglandin E2 (Schaible and Schmidt 1988), 5-hydroxytryptamine (Mense 1981), and leukotrienes (Hoheisel and Mense 1994). A critical question, not addressed in previous investigations, is what is the relevant mechanical stimulus that activates muscle mechano-nociceptors (Marchettini 1993; Mense 1993; Simone et al. 1997). This question has gone unanswered largely because of the difficulty in quantifying the mechanical state that develops at the receptor ending during either stretch and/or compression.

Using hindlimb muscles (gastrocnemius, soleus, or triceps surae) in the cat, Bessou and Laporte (1958) were the first to systematically examine the responses of group III and IV afferents with compression and/or stretch. They were followed by Painal (1960) and Iggo (1960), who stimulated group III and IV feline muscle afferents (tibialis anterior; and gastrocnemius or soleus, respectively) by squeezing, stretching, and prodding (with a glass rod and/or an algometer). These investigations showed that some, but not all, group III and IV afferents respond to “natural” mechanical stimuli. They also established that some group III afferents responded to innocuous or nonnoxious mechanical loads and, hence, putatively functioned as mechanoreceptors rather than nociceptors. While some group III afferents do respond (weakly) to stretch, their dominant response is to compression (Pinal 1960).

During noxious mechanical stimulation (e.g., a blunt traumatic blow), muscle mechano-nociceptors do not experience the externally applied load (i.e., force or displacement). Rather they experience internally developed local stress (with units of Pascals; related to distribution of force) and/or local strain (a dimensionless quantity; related to relative displacement). In other connective tissues including ligament (Fuller et al. 1991b), joint capsule (Grigg and Hoffman 1982; Khalsa et al. 1996), and skin (Ge and Khalsa 2002; Grigg 1996; Khalsa et al. 1997; Prete and Grigg 1998), mechanically sensitive afferents appear to encode the local stress, or a stress-related quantity, rather (or at least much better) than the local strain. It may be helpful to distinguish the concepts of mechanical stress and pressure and the layman’s use of the latter to mean compression. Stress is a tensor quantity, which in three dimensions has nine components, typically only six of which are independent (Khalsa et al. 1997). Pressure is the mathematically trivial case that describes a state where the stresses along the three Carte-
sian axes are equivalent in magnitude and the shear terms are zero. That is, a pressure occurs when the stress is the same along any arbitrary set of axes. During unconfined indentation (i.e., compression) in vivo, arguably pressure never develops beneath the indenter; rather, a complex state of stress occurs where stresses along the Cartesian axes are typically different and shear stresses are present.

The aim of the current study was to examine what mechanical state was encoded by group III and IV muscle nociceptors during static indentation similar to what can occur during blunt traumas or sustained noxious compression. The working hypothesis, based on previous investigation in skin and ligament (Khalsa et al. 1996, 1997, 2000a), was that the neural response would be more highly correlated with compressive stress than other relevant variables (i.e., compressive force, displacement, or strain). To eliminate confounding variables due to nonlinear geometry and tension developing during indentation, we used an isolated rat muscle-nerve preparation with a flat (i.e., linear) geometry that enabled us to apply compression without developing tension.

METHODS

Isolated muscle-nerve preparation

Experiments were performed using isolated gracilis muscle-nerve specimens that were obtained from the hindlimbs of adult Sprague-Dawley rats (~250 g, either sex), using a university-approved animal protocol. The gracilis muscle is a relatively thin, strap-like muscle, with a relatively uniform geometry (width and thickness over its entire length) and no pennation of its muscle fibers (Crouch 1969; Walker and Homberger 1998). This geometry facilitates establishing similar reference mechanical states (especially of tension). The isolated muscle-nerve preparation was similar to a well-established isolated skin-nerve preparation (Ge and Khalsa 2002; Grigg 1996; Khalsa et al. 1998). This geometry facilitates establishing similar reference mechanical states (especially of tension). The isolated muscle-nerve preparation was similar to a well-established isolated skin-nerve preparation (Ge and Khalsa 2002; Grigg 1996; Khalsa et al. 1998). On the day of an experiment, a rat was anesthetized with pentobarbital sodium (30 mg/kg ip initially, supplemental doses as needed). The hair on the hindlimb was clipped, and the skin of the medial aspect of the right hindlimb was removed, exposing the underlying gracilis muscle. To establish a common reference state for each muscle specimen based on each specimen’s intrinsic elastic state (i.e., approximating 0 tensile stress), the reference configuration was taken as the in vivo (while anesthetized) relaxed length of the gracilis muscle. Hence, there were slight variations of the knee joint angle due to natural variability in joint geometry and muscle length. The superficial muscle fascia was carefully excised, and two small (1 mm diam) markers were glued to the surface of the muscle near the origin and insertion of the muscle. The distance between these markers was measured with a digital micrometer (resolution: 0.01 mm), which established the in vivo reference length of each muscle specimen. Later, once the muscle was hooked to the stretching apparatus, the muscle would be stretched until the muscle closely approximated the reference length (Fig. 1).

The nerve to the gracilis muscle is a small, singular branch of the obturator nerve and always innervates the gracilis muscle via its deep surface. Carefully freeing the intact nerve from its surrounding fascia, the nerve was followed cephalically through the obturator foramen into the abdominal cavity, until a sufficient length was obtained (~5 cm). This nerve was cut proximally, and then the distal gracilis muscle tendon was excised from its insertion into the femur while the proximal tendon was kept intact on its origin from the pelvis, which was excised using bone cutters. The gracilis muscle [mean midsection thickness = 1.01 ± 0.07 (SE) mm, n = 13] and its intact innervation were then placed in a dish where it was superfused with circulated and gassed (100% O2) rodent synthetic interstitial fluid (Koltzenburg et al. 1997). Tabs (2 × 5 mm, 3 tabs per end, total of 6 tabs) were cut into the margins of the muscle tendons and coupled to force transducers mounted respectively on the ends of six linear actuators (Fig. 1). The muscle was then stretched to approximate the in vivo (reference) length. All compressive loads were subsequently applied with the muscle in this reference state.

Mechanical system and measurements

“Pure” compressive loads were applied similarly as previously reported for rat skin experiments (Ge and Khalsa 2002; Khalsa et al.

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**FIG. 1.** A: location of rat gracilis muscle relative to tibia, femur, and pelvis. B: top view—schematic of gracilis muscle suspended in a dish containing synthetic interstitial fluid (SIF). Each tendon tab was coupled by a length of suture to a force transducer (L1–L6) mounted on a linear actuator (A1–A6). A DC servo-motor (g) applied compression to the muscle by actuating a lever arm (f). The nerve (n) was threaded into a separate chamber, filled with mineral oil, for recording. C: side view—a, indenter tip; b, dish; c, muscle; d, hard platform; e, SIF bath; f, indenter arm; and g, rotatory motor.
1997, 2000a). Briefly, compressive loads were applied by indenting the muscle between a flat platform and a hard platform. The hard platform was a $15 \times 17 \times 25$-mm acrylic solid positioned just underneath the muscle. Indenters were acrylic cylinders with radii of 1.580, 2.236, or 3.162 mm. The smallest-diameter indenter was designed such that it would have an area much greater than the area occupied by the receptive ending of a nociceptor. This ensured that shear stress (and strain) that developed around the edge of the indenter, and which was immeasurable, would not confound the “pure” compressive stress created toward the center of the indenter (Khalsa et al. 1997, 2000a). The largest-diameter indenter was designed so that, for a given compressive force, the compressive stress would be four times smaller than that developed for the smallest diameter indenter; and, the middle-diameter indenter was equally spaced between the smallest and largest indenters. Indenters were actuated with a servo-controlled DC motor (which could be operated in either force or displacement control; model 305B, Aurora Scientific, Aurora, Canada) mounted on a three-axis positioning stage (resolution: 0.1 mm and range: 40 mm on each axis). Actuator control and data acquisition (6 tensile loads, which were only evaluated to verify that no change in tension had occurred; 1 compressive load, and 1 compressive displacement) were accomplished via a laboratory computer, A/D and D/A converter (sampling at 500 Hz), and custom software. Compressive stress was calculated from the applied force divided by the cross-sectional area of the indenter. The same compressive stresses could be applied for indenters of different diameter by varying the force; and applying the same compressive forces but varying the indenter diameters would achieve different compressive stresses. For convenience, compressive stress was formulated as a positive value, which is in contrast to the usual convention where tensile stresses are positive and compressive stresses are negative.

Two types of compressive strain were measured to enable examining whether “plastic” deformation of the muscle (i.e., developing a crater at the site of repeated indentation) would affect the statistical correlations between neural response and strain(s). As has been previously described for skin (Ge and Khalsa 2002; Khalsa et al. 1997, 2000), Lagrangian strain was calculated as the change in thickness divided by the original thickness of the muscle before any indentations were performed (i.e., $\varepsilon_L = (L_0 - L) / L_0 \times 100$, where $L_0$ was the original thickness and $L$ was the thickness of the muscle during the indentation). The thickness of the muscle at a given location of indentation was measured in two steps. First, the deep surface of the muscle was taken as the location of the surface of the hard platform. This location was determined by lowering the indenter to the platform until a minimal force (5 mN) was detected. For the immediate thickness ($L_0$), the measurement was made ~15 s before the beginning of each neurophysiological trial were performed. For the immediate thickness ($L_0$), the measurement was made ~15 s before the beginning of each neurophysiological trial were performed. Eulerian strain was calculated as the change in thickness divided by the muscle thickness immediately prior to a given indentation trial (i.e., $\varepsilon_E = (L_0 - L)/L_0 \times 100$). For convenience, both of these strains were formulated such that compressive strains were positive in magnitude. For uniaxial Lagrangian strains ($\varepsilon_L$), as performed in these experiments, displacement and strain were directly proportional (and hence correlated). This was not necessarily the case for Eulerian strains ($\varepsilon_E$), as if the thickness of the muscle changed over time, due to repeated indentations as occurs in skin (Khalsa et al. 1997), then displacement and strain would potentially not be well correlated.

Neuron recording, identification, and classification

Neuron recording, identification, and classification were performed similarly as previously reported for rat skin experiments (Khalsa et al. 2000a). Briefly, the nerve innervating the muscle was threaded from the saline compartment through a hole into an adjacent oil-filled chamber. Bundles of nerve filaments were teased apart until the neural response of single neurons could be discriminated. Neural responses were monitored on a digital oscilloscope with a hardware window discriminator, over an audio speaker, and by a real-time template matching system (Spike2 version 4.1, Cambridge Electronic Design, Cambridge, UK). Neurons, with conduction velocities in the group III (2–20 m/s) and group IV (<2 m/s) range [which were corrected for the room temperature of the saline bath (Petajan 1968)], were initially sought by systematically stimulating the muscle surface with a bipolar stimulating electrode (SS8 stimulator, Grass, W. Warwick, RI). Candidate neurons were then evaluated for mechanical sensitivity by probing their receptive field with a blunt glass rod. The most-sensitive spot (MSS) of a neuron’s receptive field was determined by use of calibrated monofilaments (Stoelting). Conduction velocities were determined by dividing the conduction latency by the length of the nerve from the recording electrode to the MSS. The conduction latency was determined by electrically stimulating the surface of the muscle at the MSS and measuring the action potential latency on the digital oscilloscope (which was triggered by stimulation pulse). The “saturation level” of the neural response was defined as the magnitude of load above which there was no significant increase in neural response or at which the neural response actually decreased for increasing load (Khalsa et al. 1996; Rossi and Grigg 1982). Afferents were examined for their responsiveness to thermal stimuli (37°C: body temperature and 55°C: noxious heat) by placing a servo-controlled thermal probe on their MSS for 5 s and to cold by placing ice chips (0°C) on their MSS for 10 s as has previously been reported to test the heat and cold sensitivity of cutaneous mechano-nociceptors (Khalsa et al. 1997). Afferents were classified as mechano-nociceptors on the basis of their graded response to noxious levels of compressive stress (or strain), as well as, in some cases, their polymodal response to noxious thermal stimuli.

Experimental protocol

Once a suitable afferent was identified, compressive loads were applied at its MSS by first lowering the indenter to the surface of the muscle until a subthreshold contact force (5 mN) was detected. The contact force was maintained for 0.5 s, and then the load was stepped indented to a predetermined load in stress control mode (Fig. 2A) or a predetermined strain in strain control mode (Fig. 2B), maintained for 10 s, and then unloaded gradually (3-s duration). Inter-trial intervals were 3 min to allow the muscle to recover its prestimulus state and to allow the neurons to have similar responses for repeated simulations (Baumann et al. 1986; Ge and Khalsa 2002) (Fig. 3). Ranges of loads were applied to encompass the estimated threshold to estimated saturation level for compression for each neuron, as has been previously described (Ge and Khalsa 2002; Khalsa et al. 1997). Typically, load increments were at 5 kPa for stress control and 10% for strain control. Generally, trials were repeated three times at each compressive stress and strain magnitude. After all the trials were completed for an indenter of a given diameter, then the same loading sequence (i.e., range of compressive stresses) would be repeated for an indenter of a different diameter. The sequence of use of the different indenters was arbitrary. As has been previously reported (Ge and Khalsa 2002; Khalsa et al. 1996, 1997, 2000a), stability of the neural response (NR) was periodically evaluated by repeating an earlier trial and determining if a significant change in NR had occurred. If so, then the trial was excluded and data collection terminated for that neuron.

Data analysis

The neuronal response was characterized by the overall mean frequency by dividing the total number of action potentials by the duration of the constant stimulus (i.e., 10 s). The response at a given load was reported as the mean of all repeated trials (typically 3). The
relationships between the neuronal response and stress, force, displacement, and strain were evaluated by linear regression and Pearson correlation for each variable. As muscle is a viscoelastic tissue, it exhibited creep (increasing displacement during constant stress) or relaxation (decreasing stress during constant strain) during stress- or strain-controlled indentation, respectively (Fig. 2). These values tended toward an equilibrium value at \( \sim 7-8 \) s. Hence, the force and displacement values used to calculate stress and strain, respectively, were taken as the averages of the force and displacement, respectively, for the last 2 s during the 10-s indentation. The slope of the linear regression was used to determine the sensitivity of a neuron to the stimulus (with the metric expressed as \( \text{[Hz/kPa]} \)). ANOVA and Student’s t-test were used to assess significant differences between the Pearson correlation coefficients for each relationship. All means are reported with their SE.

**RESULTS**

Nineteen putative mechano-nociceptors were isolated during successful experiments. Among them, six neurons stopped responding prematurely to the stimulation before sufficient data were obtained for the stress and strain control trials. Hence the results for the stress and strain control trials were based on data from 13 afferents, while results for the inter-trial time experiments (Fig. 3) were based on 3 of the 13 afferents, as well as one of the other six afferents (i.e., \( n = 4 \)). Of the 13 afferents, seven were categorized as group III (mean conduction velocity: 6.2 ± 0.7 m/s) and six as group IV (mean conduction velocity: 1.0 ± 0.1 m/s); and the conduction velocities were consistent with previous reports in cats (Iggo 1960; Mense and Meyer 1985; Painalt 1960) and humans (Marchettini 1993; Simone et al. 1994). Of the total 19 afferents, 5 responded to cold, noxious heat or both, whereas of the 13 afferents, only 2 afferents responded to noxious heat and/or cold (Table 1). Receptive fields for all afferents (\( n = 19 \)) were spot-like in area (\( \leq 1 \text{ mm}^2 \)), almost always singular (only 2 of the 13 afferents had double receptive fields, and the distance between the receptive fields was \( \sim 5 \) mm), and all were located in the muscle proper rather than in either tendon. Whereas all the afferents were initially silent (there was no spontaneous discharge) prior to mechanical stimulation, typically within five trials of compression they all began to have a low-level spontaneous discharge that averaged 0.3 ± 0.05 Hz during the inter-trial intervals, similar to that reported by other investigators (Berberich et al. 1988; Mense and Meyer 1985).

The inter-trial interval of 3 min was based on the time necessary for neurons to have reproducible responses to a given load (Fig. 3). This interval also appeared to have been sufficient to allow the muscle to recover its original thickness, as there was no significant difference between the Lagrangian or Eulerian strains themselves (\( P = 0.95 \)). Hence, for the rest

**TABLE 1. Distribution of afferents responding to mechanical compressions and noxious heat or cold**

<table>
<thead>
<tr>
<th>Afferent</th>
<th>MN</th>
<th>MNC</th>
<th>MNH</th>
<th>MNCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group IV</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Group III*</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Group IV*</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

MN, mechano-nociceptor: sensitive to mechanical stimuli only; MNC, MN + sensitive to cold but not heat; MNH, MN + sensitive to heat but not cold; MNCH, MN + sensitive to cold and heat. * These numbers were based on the total 19 afferents.

FIG. 2. Representative data from 2 trials in which similar compressive strains (displacements) were applied to the gracilis muscle, but using stress (A) or strain (B) control, respectively. Because muscle tissue is viscoelastic, under a constant force (A, stress control mode) it will “creep” and under a constant displacement (B, strain control mode) it will “relax.” Top: indentation force and displacement. Under stress control (A) and using an indenter with a radius of 3.162 mm, the constant compressive stress was 7.7 kPa and during the constant portion of the load, the strain creeped from 25 to 37%. Under strain control (B) with the same size indenter, the constant compressive stress was 37% and during the constant strain, the stress relaxed from 24.8 to 3.2 kPa. Middle: instantaneous frequency calculated from the spike trains of a group IV muscle nociceptor to the respective stimuli. Bottom: time of occurrence of each action potential (AP) of the putative mechano-nociceptor to the stimuli.

FIG. 3. Increasing the inter-trial interval \( \leq 3 \) min resulted in increased mean normalized neural response (NR). The stimulus loads for group IV mechano-nociceptors (\( n = 4 \)) to 10-s compressive trials were midway between threshold and saturation level. Mean NRs for different inter-trial intervals for each neuron were normalized by the maximum NR for a given inter-trial time.
of this manuscript, we make no further distinctions between them and simply report these data as “strain.”

The mean compressive threshold for all mechano-nociceptors was 1.1 ± 0.4 kPa, and although the mean threshold was smaller for group III than group IV afferents, this difference was not significant (P = 0.31; Fig. 4A). The mean compressive sensitivity for all mechano-nociceptors was 0.5 ± 0.1 Hz/kPa, and the difference in sensitivity between group III and IV afferents was not significant (P = 0.25; Fig. 4B).

Under stress control mode, for each neuron, the neural responses to all loads (same stresses with different indenter areas) were correlated to compressive stress, force, strain, and displacement (Fig. 5). On average, for all muscle mechano-nociceptors combined, as well as group III separate from group IV afferents, the neural response was significantly (P < 0.01, ANOVA) and substantially more highly correlated with compressive stress than force, strain, or displacement (Fig. 6). The correlation between the neural response and compressive stress was virtually the same for group III and IV afferents. The differences in the correlations between neural response and force, strain, and displacement for group III and IV afferents were not significant.

The results under strain control mode were similar to that under stress control. For each neuron, the neural response to all loads (same strains with different indenter areas) was correlated to compressive stress, force, strain, and displacement (Fig. 7). On average, for all muscle mechano-nociceptors combined, as well as group III separate from group IV afferents, the neural response was significantly (P < 0.01, ANOVA) and substantially more highly correlated with compressive stress than force, strain, or displacement (Fig. 8). There was no significant difference between the correlation between neural response and stress for group III and IV afferents. The correlation between neural response and stress was slightly higher for strain control ($r^2 = 0.89$) than for stress control ($r^2 = 0.86$), but this difference was not significant (P = 0.46).

**DISCUSSION**

To our knowledge, this is the first report of the encoding of mechanical states (defined by stress and strain) by putative muscle mechano-nociceptors. The data from this study support the hypothesis that both group III and IV muscle mechano-nociceptors stimulated by indentation encode compressive stress rather than force, displacement, or strain. These experiments were designed to eliminate confounding factors typically encountered during in vivo experiments such as nonlinear muscle geometry and simultaneous tension with compression and experimental design issues such as shear loading, and viscoelastic effects due to insufficient inter-trial intervals (i.e., the muscle and/or nociceptor not fully recovering). Hence, the fundamental mechanical state (i.e., stress) encoded by group III and IV muscle mechano-nociceptors appears to be the same as that encoded by a variety of other mechanically sensitive afferents: cutaneous slowly adapting type I (Ge and Khalsa 2002) and type II mechanoreceptors (Grigg 1996), Ruffini afferents in ligament and joint capsule (Fuller et al. 1991a; Grigg and Hoffman 1982, 1984; Khalsa et al. 1996), cutaneous rapidly adapting type II (AB) afferents (Prete and Grigg 1998), and cutaneous A6 and C mechano-nociceptors (Khalsa et al. 1997).

The interpretation of the data from this study must be constrained by the nature of isolated nerve-muscle preparation. Clearly, the lack of blood supply may have affected the responsiveness of the afferents in some fashion. Additionally, the recordings were done in a nonsterile environment and hence bacterial growth in the circulating synthetic interstitial fluid could also have affected the neurons. Nonetheless, no significant change in sensitivity or threshold was observed for a given

![Graph](image-url)
afferent during the typically 3–5 h it took to complete the experimental protocols. Background (spontaneous) discharges were observed at similar rates as has been reported for in situ preparations (Berberich et al. 1988; Mense 1996; Mense and Meyer 1985), and these rates did not change during the recording periods. Further, group III muscle afferents are largely insensitive to ischemia (Paintal 1960), so even if the superfusion of the muscle was insufficient, it would not have likely changed their responsiveness. This suggests that the isolated muscle-nerve preparation reasonably simulated in situ experiments, at least as far as the mechanical responsiveness of the afferents.

While group III afferents terminate in both “free” and corpuscular ends, group IV afferents appear to terminate exclusively in “free” or “fine sensory” endings (Messlinger 1996; Stacey 1969). Despite the potential differences in the morphologies of the group III and IV receptive endings in the current experiments, there were no significant differences observed in their threshold or sensitivity, as has been qualitatively reported previously (Mense 1977). Thus morphological differences do not appear to affect the acute functional response to mechanical stimulation. This would suggest that the morphological differences may be related to other functions, such as chemoreceptivity (for polymodal nociceptors) and/or chemosecretion (Mense 1996; Messlinger 1996).

The compressive thresholds of both group III and IV muscle nociceptors in the current study were relatively low (1.1 kPa) compared with estimates by Paintal (1960) in cat muscle (~16 kPa). Some of the difference may simply be due to species differences (rat vs. other mammals, especially cat), which has also been observed in rat cutaneous nociceptors (Khalsa et al. 1997, 2000a). Another cause of the differences may be due to the methods used to examine the thresholds. The methods used in the current study minimized effects due to nonperpendicular (nonnormal) loading, imprecise and irregular geometry of compressive stimulators (e.g., serrated forceps), potentially confounding interactions between tension and compression, and out-of-plane shear loads. Most or all of these confounders were present in Paintal (1960), as well as other investigations (Franz and Mense 1975). It is possible that the mean threshold of the sampled afferents was low because all these afferents were functioning as mechanoreceptors rather than nociceptors, consistent with reports of the presence of low thresholds in some group III and IV afferents (Bessou and LaPorte 1958; Franz and Mense 1975; Paintal 1960). However, we think this

FIG. 6. Under stress control mode, the mean neural response (mNR) for all muscle nociceptors was more highly correlated with compressive stress than with compressive force (P < 0.01), strain (P < 0.01), or displacement (P < 0.01). For group III afferents, the highest correlation was between mNR and compressive stress (0.86 ± 0.03). For group IV afferents, the highest correlation was between mNR and compressive stress (0.86 ± 0.02).

FIG. 7. Under strain control mode, the NR of a representative muscle mechano-nociceptor to compression using indenters of different radii. The NR was plotted and regressed against compressive stress, force, strain, and displacement. The highest correlation was between the NR and compressive stress. Pearson correlation coefficients are displayed for each relationship. Trials were repeated 3 times, and the error bars represent SEs. In some cases, the SEs were smaller than the symbols.
was unlikely for two reasons. First was simply due to probability, as the ratio of the low- to high-threshold afferents in cat was reported at 1:14 (Franz and Mense 1975). Assuming that this ratio is similar in rat, then the probability was vanishingly small that all the sampled afferents were mechanoreceptors rather than nociceptors. Second, all these afferents continued responding to loads that appeared to be noxious or were at least much greater in magnitude than saturation levels for low-threshold mechanoreceptors (Ge and Khalsa 2002) in rat hairy skin.

The finding that group III and IV muscle afferents encode the same mechanical state during compression as do their cutaneous counterparts, suggests that similar mechanisms may also be operating at the membrane level. In other words, the membrane mechanism that enables cutaneous mechanosensory afferents to transduce mechanical loads in the extracellular matrix (especially collagen fibers) into action potentials may be similarly present in muscle group III and IV afferents. There is strong evidence that mechanosensory ion channels in the receptive ending membrane are fundamental to this process (Garcia-Anoveros et al. 1997; Mannsfeldt et al. 1999), and some mechanosensory channels may be directly connected to the extracellular matrix (Liu et al. 1996). Recently, it has been discovered that a transmembrane protein, integrin α2β1 (which is a receptor for collagen), is expressed in the receptive endings of cutaneous mechanosensory afferents (Khalsa et al. 2000b). Using monoclonal function blocking antibodies to integrin α2β1, the neural response of four types of cutaneous afferents was strongly modulated by blocking the function of integrin α2β1 (Khalsa et al. 2001). Hence, this mechanism may also be involved in muscle group III and IV afferents’ transduction of mechanical stimuli.

In conclusion, putative muscle mechano-nociceptors appear to encode mechanical stress during indentation. Group III and IV afferents responded similarly to the indentation with no significant differences for threshold or sensitivity.

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