Experimental muscle pain reduces initial motor unit discharge rates during sustained submaximal contractions

Dario Farina, Lars Arendt-Nielsen, and Thomas Graven-Nielsen

Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

Submitted 24 September 2004; accepted in final form 28 October 2004

Farina, Dario, Lars Arendt-Nielsen, and Thomas Graven-Nielsen. Experimental muscle pain reduces initial motor unit discharge rates during sustained submaximal contractions. J Appl Physiol 98: 999–1005, 2005. First published October 29, 2004; doi:10.1152/japplphysiol.01059.2004.—The aim of this human study was to investigate the effect of experimentally induced muscle pain on the modifications of motor unit discharge rate during sustained, constant-force contractions. Intramuscular and multichannel surface electromyographic (EMG) signals were collected from the right and left tibialis anterior muscle of 11 volunteers. The subjects performed two 4-min-long isometric contractions at 25% of the maximal dorsiflexion torque, separated by a 20-min rest. Before the beginning of the second contraction, hypertonic (painful; right leg) or isotonic (nonpainful; left leg) saline was injected into the tibialis anterior. Pain intensity scores did not change significantly in the first 150 s of the painful contraction. Exerted torque and its coefficient of variation were the same for the painful and nonpainful contractions. Motor unit discharge rate was higher in the beginning of the nonpainful contraction than the painful contraction on the right side [means ± SE, 11.3 ± 0.2 vs. 10.6 ± 0.2 pulses/s (pps); P < 0.01] whereas it was the same for the two contractions on the left side (11.6 ± 0.2 vs. 11.5 ± 0.2 pps). The decrease in discharge rate in 4 min was smaller for the painful (0.4 ± 0.1 pps) than for the control contractions (1.3 ± 0.1 pps). Initial value and decrease in motor unit conduction velocity were not different in the four contractions (right leg, 4.0 ± 0.1 m/s with decrease of 0.6 ± 0.1 m/s in 4 min; left leg, 4.1 ± 0.1 m/s with 0.7 ± 0.1 m/s decrease). In conclusion, stimulation of nociceptive afferents by injection of hypertonic saline did not alter motor unit discharge velocity but reduced the initial motor unit discharge rates and the difference between initial and final discharge rates during sustained contraction.

MATERIALS AND METHODS

Subjects

Eleven healthy subjects (7 men; age, mean ± SD, 24.2 ± 2.1 yr) participated in the experiment. The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee, and written, informed consent was obtained from all subjects before inclusion.

General Procedures

The subject sat comfortably on a chair with the foot fixed in an isometric force brace incorporating a torque transducer (Aalborg University, Aalborg, Denmark). The ankle was at 90° and the knee joint angle varied between 110 and 130° so that the thigh was in horizontal position. The same procedure was applied in the same experimental session on both legs. The left leg served as a control. The maximal voluntary contraction (MVC) torque in dorsiflexion was recorded three times with 2-min rests in between. The highest MVC measure was the reference for the definition of submaximal induced muscle pain. Stimulation of nociceptive afferents by injection of hypertonic saline decreases discharge rate with an efficacy correlated to the amount of nociceptive input (8).

During sustained contractions, in addition to the modulation of motoneuron discharges, membrane and contractile fiber properties change (e.g., 5, 9). It has been hypothesized that the decrease in discharge rate matches the modifications in motor unit twitch force (the muscular “wisdom” theory) to maintain a constant force output (24). However, when contractile muscle properties are changed by varying temperature or muscle length, motoneuron discharge frequencies do not change accordingly (2, 3). Moreover, with nociceptive input, motor unit discharge rate decreases without modifications in muscle fiber membrane properties (8). Thus decrease in discharge rate may occur independently of modifications in contractile fiber properties mediated by changes in membrane properties.

Although the inhibitory effect of pain-inducing substances on motor unit discharges has been proven in previous work (8, 31), there are no studies that investigated the effect of muscle pain on the contraction-induced modifications of discharge rate over time. This may provide a better understanding of the role of small-diameter afferents in the motoneuron reflex inhibition during sustained contractions. Thus in this human study we analyzed motor unit discharge rates and conduction velocity during submaximal contractions sustained for 4 min with muscle pain experimentally induced by hypertonic saline injection.

During sustained submaximal contractions, motor unit discharge rates decrease (6, 30). The reasons for this phenomenon are still not fully known, although many studies have outlined a role of reflex mechanisms (e.g., 1, 13, 23). Two main reflex mechanisms have been hypothesized as responsible for the decline in motor unit discharge rate. The first is the decreased activity of muscle spindles, which disfacilitates synergistic α-motoneurons. Decrease in spindle activity during sustained submaximal contractions has been observed in humans (23), although an opposite behavior has been reported in cats (21). The second mechanism is related to the inhibitory effect of group III and IV muscle afferents (1, 12), which are excited through chemical stimuli during a sustained contraction (18). A large proportion of small-diameter afferents are nociceptive (26, 27); thus their activity is increased by experimentally proposed nociceptive input.
torque contraction levels. Intramuscular and surface electrodes for EMG detection were mounted after the MVC assessment, as described below.

After 5-min rest, the subject performed a 4-min-long contraction in dorsiflexion at 25% MVC with a real-time feedback on the torque level exerted, provided by an oscilloscope. The subject then had a 20-min rest, after which hypertonic (right leg) or isotonic (left leg) saline was infused. Two minutes after the beginning of the infusion, the subject was asked to perform a 4-min-long contraction at 25% MVC, identical to the previous one. Thus a total of four contractions, two for each leg, were performed by the subject with and without injection of saline. The order of assessment of the two legs was randomized.

Experimental Muscle Pain

Experimental muscle pain was induced by infusion of sterile hypertonic saline (5.8%) into the deep mid portion of the tibialis anterior muscle. Infusion of hypertonic saline was accomplished with a computer-controlled syringe pump (IVAC, model 770) and a 27-gauge needle. A tube (IVAC G30303, extension set with polyethylene inner line) was connected from the syringe to the needle. One 0.5-ml bolus of hypertonic saline was infused in the tibialis anterior muscle at a rate of 45 ml/h; thus infusion took 40 s. The needle was removed at the end of the infusion. The pain intensity was scored by the subject on a 10-cm visual analog scale (VAS) with the lower and upper extremes labeled “no pain” and “most pain imaginable,” respectively. The VAS ratings were sampled by a computer every 5 s for a period of 15 min starting from the beginning of the saline infusion (14). Isotonic saline (0.5 ml, 45 ml/h) was injected into the muscle before the second contraction of the left side.

EMG Recordings

Surface and intramuscular EMG signals were recorded from the tibialis anterior muscle (Fig. 1). The two signals provided information on the motor unit discharge patterns and muscle fiber conduction velocity.

Surface EMG. A linear array of 16 equispaced electrodes (inter-electrode distance 5 mm, bar electrodes 5 mm long, 1-mm diameter) (25) was used to detect surface EMG signals. The array was located between the most distal innervation zone and the distal tendon region. Innervation zone and tendon regions were noninvasively identified in a few test contractions with the use of the array, as described in previous work (7, 8). The skin was slightly abraded and the array held in place by adhesive tape. Surface EMG signals were detected in bipolar configuration and amplified (EMG amplifier, EMG16, LISiN, Prima Biomedical and Sport, Treviso, Italy, bandwidth 10–500 Hz), sampled at 2,048 Hz, and stored by a 12-bit analog-to-digital conversion.

Intramuscular EMG. Four wire electrodes made of Teflon-coated stainless steel (A-M Systems, Carlsborg, WA) were inserted with a 23-gauge needle, 10–20 mm proximal to the surface array top. The wires were uninsulated for ~1 mm at the tip and bent in the insertion needle at different lengths (2–6 mm) to record from as many motor units as possible. The depth of needle insertion was a few millimeters below the muscle fascia. The needle was removed with the wire electrodes left inside the muscle. Intramuscular EMG signals were amplified (DANTEC Counterpoint electromyograph, DANTEC Medical, Skovlunde, Denmark), band-pass filtered (500 Hz–5 kHz), sampled at 20,480 Hz, and stored after 12-bit analog-to-digital conversion.

Signal Analysis

The intramuscular EMG signals were decomposed by a previously described technique (7). The method is based on an amplitude threshold to detected action potentials and on potential classification based on cross-correlation. The four detected channels were decomposed independently, and the results were merged by identifying, from the estimated firing patterns, motor unit activities detected in common by different channels (7). The times of occurrence of the intramuscular

Fig. 1. A 3-s and 500-ms portion of intramuscular (A) and surface (B) EMG signals recorded during a 25% maximal voluntary contraction (MVC) of a subject. Note the propagation of the action potentials along the array in the surface EMG recordings.
action potentials were used as triggers for averaging the multichannel surface EMG signals. The same motor units were analyzed in the two contractions of each leg to avoid masking effects due to different populations of sampled motor units (4). The shapes of the intramuscular action potentials detected in the two contractions were compared by computing the peak of their cross-correlation function. Only motor units that showed intramuscular action potentials in both contractions with a peak of the cross-correlation function higher than 0.85 were accepted for further analysis.

The motor unit discharge pattern was divided in consecutive portions of 20 discharges each. The 20 discharges in each portion were used as triggers for obtaining each averaged surface EMG potential. Thus the surface action potential properties could be assessed over time during the contraction.

Conduction velocity of single motor units was estimated from each averaged surface potential by a multichannel technique previously described (7). Double differential derivations were used for this purpose as obtained by subtracting consecutive bipolar recordings. The channels of the surface array selected for conduction velocity estimation were automatically chosen with the criterion of minimal shape changes, as proposed by Farina et al. (7). Conduction velocity was estimated from the same set of channels in the two contractions of each leg. The maximum of the cross-correlation function between the signals used for conduction velocity estimation was used as an index of signal shape similarity and, thus, of reliability of conduction velocity estimates (10). A threshold of 0.85 was set to the maximum of the cross-correlation function for accepting estimates of conduction velocity. Mean power spectral frequency, peak-to-peak amplitude, and duration [area divided by the peak-to-peak amplitude (29)] of the averaged surface potentials were computed from the subset of motor units for which conduction velocity could be estimated.

Values of VAS and action potential properties at nine time instants, corresponding to the interval 0–240 s (30-s increments) during the contraction, were considered for statistical analysis. At each time instant, the average value of each variable over an interval of 5 s was computed.

Statistical Analysis

Data are presented as means ± SE. In the contractions with injection of hypertonic or isotonic saline, VAS scores were analyzed by two-way repeated-measures ANOVA with the factors injection type (hypertonic or isotonic) and time (nine time instants). Motor unit discharge rate and action potential properties were analyzed by two- and three-way mixed-model ANOVA. The repeated measures were the injection condition (no injection or injection) and time (nine time instants), and the between-group factor was the side (right or left leg). Significances revealed by ANOVA were followed by post hoc Student-Newman-Keuls (SNK) pairwise comparisons. Significance was accepted for P values <0.05.

RESULTS

All subjects were able to sustain the target torque for the 4 min in the four contractions. Exerted torque and its coefficient of variation were not different between the two contractions of each leg. The total number of motor units detected in both contractions was 48 (right leg) and 57 (left leg). Conduction velocity could be estimated, according to the criterion described above, in 35 and 33 motor units of the right and left leg, respectively. The number of double differential channels used for conduction velocity estimation was not significantly different in the two legs (4.6 ± 1.2 channels).

Experimental Muscle Pain

VAS scores depended on the injection type (hypertonic/isotonic saline) (F = 58.6, P < 0.001), time (F = 4.9, P < 0.001), and interaction between these factors (F = 4.1, P < 0.001). Hypertonic saline resulted in higher VAS scores than isotonic saline at the nine time instants (P < 0.001) (Fig. 2). VAS score with hypertonic saline injection did not change in the first 150 s of contraction, after which it decreased (P < 0.05) (Fig. 2).

Motor Unit Discharge Rate

An example of motor unit action potentials detected intramuscularly and, through averaging, at the skin surface for the two contractions of the right leg is shown in Fig. 3. The high similarity of the intramuscular action potentials between the two contractions indicated that most likely the same motor unit was detected.

Discharge rate depended on time and on the three-way interaction among leg, injection type, and time (F > 6.7, P < 0.001). In the three control contractions, discharge rate was higher in the first 30 s than in the rest of the contraction (P < 0.01; Fig. 4). In the painful contraction, discharge rate was higher in the beginning (first time instant) than in the rest of the contraction (P < 0.05). In the first 30 s of the painful contraction, discharge rate was lower than in the corresponding interval of time in the contraction without injection of the same leg (P < 0.01; Fig. 4).

The difference between discharge rate in the beginning and end of the contraction was smaller for the painful [0.4 ± 0.1 pulses/s (pps)] than for the other contractions (1.2 ± 0.1 pps for the contraction without injection of the right leg, 1.3 ± 0.1 pps and 1.2 ± 0.1 pps for the left leg; ANOVA: F = 4.8, P < 0.05; SNK: P < 0.001).

Action Potential Properties

Conduction velocity of single motor units depended on time (F = 26.9, P < 0.001) but not on other factors. It was different for time instants that differed by 60 s or more (P < 0.05; Fig. 5).

![Fig. 2. Mean (solid lines) and SE (dashed lines) of visual analog scale (VAS) scores in the 2 contractions with injection of hypertonic (right leg) and isotonic (left leg) saline. Time t = 0 corresponds to the beginning of the injection. The contractions started 2 min after the beginning of the injection. The 9 time instants used for statistical analysis are shown by black circles. Asterisks indicate the time instants with smaller VAS scores than the first time instant (P < 0.05).](http://jap.physiology.org/inline/fig/1001MOTOR_UNIT_PROPS_DURING_PAINFUL_CONTRACTIONS.html)
Mean power spectral frequency of the surface action potentials (initial value, 85.8 ± 2.5 and 87.3 ± 2.1 Hz for the right and left leg, respectively) decreased over time (by 18.1 ± 1.5 Hz, right leg, and 17.5 ± 1.6 Hz, left leg, in the 4 min; ANOVA: $F = 18.9$, $P < 0.001$) and did not depend on the leg or injection condition. Action potential duration (initial value 9.1 ± 0.6 ms, right leg, and 9.7 ± 0.6 ms, left leg) significantly increased by 2.0 ± 0.8 ms (right leg) and 1.8 ± 0.7 ms (left leg) in the 4 min (ANOVA: $F = 21.5$, $P < 0.001$; SNK: $P < 0.01$) and was not different in the four contractions. Peak-to-peak amplitude of the surface potentials did not change during the sustained contractions and did not differ among the four contractions (113.4 ± 1.9 and 114.7 ± 1.7 μV, for the right and left leg, respectively).

**DISCUSSION**

In this study we analyzed motor unit discharge rate and membrane fiber properties during sustained contractions with
experimentally induced muscle pain. Motor unit discharge rates were lower in the beginning of painful contractions compared with control conditions. Moreover, muscle pain reduced the difference between initial and final discharge rate during sustained contraction. Conduction velocity and surface action potential properties were not affected by pain.

Inhibitory Effect of Nociceptive Afferents

The inhibitory effect of nociceptive afferents has been shown in previous work. In the masseter muscle, Sohn et al. (31) observed that the interpulse interval of motor unit discharge increased with pain with respect to control conditions for various isometric contraction forces. Farina et al. (8) demonstrated that the pain-induced inhibition is correlated to the amount of nociceptive input: the higher the pain scoring, the larger the decrease in discharge rate.

The pain-induced decrease in initial discharge rate observed in this study was rather limited (on average ~0.7 pps in the beginning of the contraction; Fig. 4) but in agreement with the decrease reported by Farina et al. (8) (~1 pps with a bolus of 0.9 ml of hypertonic saline). The decrease in discharge rate due to pain in the beginning of the contraction was smaller than that observed over the 4 min during sustained nonpainful contractions (~0.7 vs. ~1.2 pps). Thus a moderate level of muscle pain had an inhibitory effect on motoneurons smaller than that occurring over time in sustained contractions. This may indicate that feedback from nociceptive afferents is not the only mechanism responsible for the decrease in discharge rate during constant force contractions. Accordingly, Walton et al. (34) observed a greater decline in H-reflex amplitude in endurance-trained than in sedentary individuals during plantar flexion contractions, although neuromuscular adaptation in the trained subjects had probably decreased the production and increased clearance of metabolic substrates. On the other hand, the hypertonic saline may have excited only a small portion of nociceptive afferents, contrary to the case of sustained contractions.

Modulation of Discharge Rates With Sustained Contraction

Excitation of nociceptive afferents reduced the total decline in motor unit discharge rates (Fig. 4), i.e., the difference between initial and final discharge rate. The discharge rates in the beginning of the painful contraction were lower than in the nonpainful condition but their decrease over time was limited; thus after 90 s of sustained contraction they were comparable in the painful and nonpainful conditions. This result is in agreement with a contribution of nociceptive afferents in the decrease of discharge rate during sustained activation (1).

If nociceptive afferents did not play any role in the reflex inhibition during sustained contraction, the total decrease in discharge rate over time would have been similar with or without pain, with a lower discharge rate at the end of the painful than the nonpainful contraction, which was not observed. This is in agreement with previous animal and human studies that proved an involvement of small-diameter muscle afferents in the decrease of motor unit discharge frequencies during sustained contractions (e.g., Refs. 12, 15, 33, 35).

The residual decrease in discharge rate observed during painful contractions could have been due to the activation of small-diameter afferents not excited by the hypertonic saline or to mechanisms not involving nociceptive afferents, such as a decrease in the activity of muscle spindles (22).

Membrane Properties

Experimental muscle pain by itself does not change muscle fiber membrane properties (8), as confirmed by the unaltered conduction velocity found in the beginning of the painful contractions. Accordingly, the surface action potential properties were not altered by pain in the beginning of the contrac-

![Fig. 5. Mean ± SE motor unit conduction velocity at the 9 selected time instants during the 4-min-long contractions of the right and left leg. Time instants separated by 60 s or more resulted in significantly different conduction velocity (P < 0.05).]
tion. Moreover, in this study, we showed that pain did not affect the modifications in muscle fiber membrane properties occurring over time during sustained contractions.

Motor unit conduction velocity decreased during the sustained contractions with a rate of change in the range reported in previous work on the tibialis anterior muscle (7). The decrease in conduction velocity was not affected by pain, and the same was observed for the other action potential properties. The decrease in conduction velocity is mediated by the increase in interstitial potassium concentration (20), as a consequence of the generation of the action potential and the imbalance between the sodium-potassium pumping and the diffusion of potassium outside the fibers (11). This mechanism was probably not altered by the injection of hypertonic saline, at least for the superficial motor units investigated (the saline was injected rather deep into the muscle).

The maintenance of constant force under different control strategies with and without pain was thus not mediated by alteration of fiber membrane properties. However, fiber contractile properties may have changed with pain. Recently, Sohn et al. (32) have observed increased motor unit twitch force with pain. Increased twitch force was also hypothesized by De Luca et al. (6) as occurring in the beginning of sustained, nonpainful contractions to counteract the decrease in discharge rates. If this mechanism was involved, the twitch force increase was probably not due to potentiation, as proposed by De Luca et al., because twitch potentiation is mediated by changes in electrical membrane properties (16, 17, 28), with a consequent increase in conduction velocity (19). Eventual changes in contractile properties (32) may thus have been due to modifications in muscle stiffness, which changes the transmission of motor unit force at the joint.

Other mechanisms that may have been responsible for the maintenance of constant force despite the decreased firing rate in the beginning of painful contractions include changes in the synergistic-antagonist muscle activity, recruitment of additional motor units, and increased motor unit short-term synchronization. The present results allow only the exclusion of an involvement of modifications in muscle fiber electrical membrane properties, whereas the investigation of other potential factors should be addressed in future work.

In conclusion, this study showed that experimentally induced muscle pain changes the motor unit control strategy during maintenance of constant force. Motor unit discharge rates in the beginning of the contraction and their decline over time were reduced by pain. On the other hand, muscle fiber membrane properties underwent the same modifications during the contractions with or without pain. This indicates that nociceptive afferents are probably involved in the decrease in discharge rate with sustained contraction. Moreover, these results indirectly prove that the maintenance of constant force during sustained activation with declining discharge rates is not mediated by changes in muscle fiber electrical membrane properties.

GRANTS
This work has been supported by the Danish Technical Research Council.

REFERENCES


