High-energy phosphates and tension production in rabbit tibialis anterior/extensor digitorum longus muscles

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High-energy phosphates and tension production in rabbit tibialis anterior/extensor digitorum longus muscles. J. Appl. Physiol. 82(3): 1024–1029, 1997.—The effects of repetitive muscle contraction on energy state and tension production were studied in rabbit tibialis anterior/extensor digitorum longus muscles that had been subjected to 90 days of continuous indirect electrical stimulation at 10 Hz. Anesthetized chronically stimulated and control rabbits were challenged with 15 min of stimulation at 4 and 15 tetani/min. Pi-to-phosphocreatine (PCr) ratio (Pi/PCr) was measured in vivo before, during, and after acute stimulation by 31P-magnetic resonance spectroscopy, and tension was recorded at the same time. Although Pi/PCr was low at rest, it was significantly higher in chronically stimulated muscle than in control muscle (0.20 ± 0.02 vs. 0.05 ± 0.01, P < 0.05). Stimulation of control muscle for 15 min at both 4 and 15 tetani/min induced a significant rise in Pi/PCr, whereas the same conditions in chronically stimulated muscle did not produce any significant departure from initial levels. The tension produced by control muscle fell to 93 ± 3% of its initial value during stimulation at 4 tetani/min and to 61 ± 7% at 15 tetani/min, respectively. In chronically stimulated muscle, on the other hand, tension was potentiated above its initial level at both stimulation rates (135 ± 15 and 138 ± 11%, respectively) and remained significantly elevated throughout each trial. The ability of chronically stimulated muscle to sustain high levels of activity with minimal perturbations in Pi/PCr or decrement in tension is attributable to cellular adaptations that include a well-documented increase in oxidative capacity.

tension; chronic stimulation; 31P-magnetic resonance spectroscopy

CHRONIC LOW-FREQUENCY electrical stimulation of the common peroneal nerve of rabbits induces marked alterations in the structure and performance of the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles. During this process there is an increase in oxidative activity, a decrease in anaerobic glycolytic capacity, and a shift toward expression of slow isoforms of myosin; these changes are reflected in a slowing of contraction speed and a marked increase in fatigue resistance (reviewed in Refs. 25 and 27). Although it is known that alterations in muscle protein composition are brought about by changes in mitochondrial gene number (29, 30), transcriptional (14, 16, 30), and posttranslational processes (14, 30), the signaling pathway that links electrical stimulation to gene expression has not been established. If aspects of the response are functionally driven, changes in muscle metabolism with continuous low-frequency stimulation (12, 13, 20) may reveal candidate signaling factors. Changes in the activities of H+, Pi, phosphocreatine (PCr), and ATP, in particular, could provide clues to possible molecular signaling mechanisms as well as insights into the nature of muscle adaptation to exercise.

The primary goal of this study was to develop and to use a system that would permit muscle tension, phosphate metabolites, and pH to be measured simultaneously in vivo during acute electrical stimulation of the TA and EDL muscles of the rabbit. This muscle group was selected for study because much information is already available about the response of its biochemical and contractile characteristics to chronic stimulation (25, 27) and because there is growing interest in this preparation as a model of muscle plasticity (17). To obtain multiple measurements from each limb, 31P-magnetic resonance (MR) spectroscopy was used as a noninvasive means of assessing intramuscular Pi, PCr, and ATP concentrations, as well as pH. Differences in the contractile and metabolic properties of control TA/EDL muscles and TA/EDL muscles that had been subjected to chronic low-frequency stimulation were explored by challenging them with acute tetanic stimulation. This system resembles a previously described model based on chronically stimulated canine latissimus dorsi muscle (6), but in the present study, tension recording and 31P-MR spectroscopy were performed simultaneously, rather than on successive days. The hypothesis was tested that chronic stimulation would induce changes in TA/EDL muscles that increased the stability of ATP metabolites, indexes of muscle free energy, pH, and tension production during acute tetanic stimulation.

METHODS

Animal preparation for chronic stimulation. Adult male New Zealand White rabbits (2.5–3 kg) were housed individually in a climate-controlled room (18–21°C) and were provided with standard laboratory chow ad libitum. Stimulators and electrodes were implanted under aseptic conditions as described previously (12, 15), except that general anesthesia

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was maintained with 1–3% isoflurane and mechanical ventilation. In each case, stimulation was confined to the anterior compartment of the left hindlimb. Stimulators were activated ~7 days after surgical implantation to allow animals to recover from the stress of surgery. Animal care personnel or the first author of this study palpated the stimulated limbs daily to confirm that the implanted device was functioning satisfactorily. The study design called for a chronic stimulation duration of 90 days to induce a uniform adaptation of the EDL muscle, which appears to occur more slowly than in the TA (5, 24). In two of the five rabbits, chronic stimulation was terminated before 90 days, in one case because of stimulator malfunction and in the other because of dry gangrene of a toe on the unstimulated limb. For these reasons, these two rabbits were excluded from further analysis.

Acute stimulation. The acute-stimulation protocol was performed on TA/EDL muscles that had undergone chronic stimulation, and the right TA/EDL muscles of five rabbits that had not undergone surgery. Before acute stimulation, anesthesia was induced with ketamine (50 mg/kg) and acepromazine (0.5 mg/kg im) and was maintained via muscle relaxation was induced with vecuronium bromide. Muscles were prepared as described above, except that the peroneal nerve was surgically exposed in the mid-calf. After ensuring the integrity of the nerve, two pairs of indifferent electrodes were placed on the ankle and knee muscles to allow tetanic contractions to be achieved by electrical stimulation of the peroneal nerve (Grass S88 stimulator) with the following parameters: pulse frequency 100 Hz; train duration 100 ms; pulse amplitude 10–20 V (supramaximal); pulse duration 2 ms. Muscle tension above resting tension and arterial blood pressure were recorded on a strip-chart recorder. In all experiments, resting muscle tension was constant for the duration of each experiment. At the completion of each experiment, TA/EDL muscles were excised and weighed.

31P-MR spectroscopy. A rectangular surface coil (31P, 8P) tuned to phosphorus (81 MHz) was positioned over the anterolateral lower limb. The cradle was placed in the bore of a superconducting magnet (4.7 Tesla, Oxford Magnetics, Oxford, UK) equipped with Accusar gradients. The magnetic field homogeneity was optimized by manual adjustment of the room temperature shims until PCr line width was ~40 Hz. In initial studies, the volume from which data were collected was localized to the TA/EDL group by means of 31P images acquired with a coil of identical dimensions and orientation but tuned to 200 MHz for 31P detection (block size 256 K, sweep width 40 K, field of view 50 mm, repetition time 200 ms, echo time 10 ms). Positioning of the coil relative to these muscles was found to be reproducible by using only external landmarks that were used for subsequent 31P-MR studies. During 31P-MR spectroscopy, the following parameters were used: sweep width 10 kHz, block size 2,048 points, interpulse delay 0.21 s, and a pulse width ranging from 10 to 15 µs (nominal power 1 kW), which was selected to optimize the signal-to-noise ratio (S/N) of the β-ATP peak, as suggested by Evelhoch et al. (10). The combination of these parameters and 286 scans/spectrum resulted in a 1-min temporal resolution.

Data analysis. One-minute free induction decays were grouped in 5-min signal-averaged blocks before fast Fourier analysis. After line broadening (exponential multiplication of 20 Hz) and baseline correction (GE Omega v. 4.2), the peak areas of Pi, Pi, and Cr were derived for each decay. These partial saturation parameters were expressed as grams above resting tension per unit (g) of wet muscle mass (g) and as a percentage of initial tetanic tension. Intracellular pH was calculated by using the chemical shift of Pi, (23). One-way repeated measures analysis of variance (ANOVA; for normally distributed variables and the Wilcoxon signed-rank test (for non-normally distributed variables) were used to detect significant changes with time within a rabbit over the course of the experiment. Dunnett’s test was used to identify significant departures from the initial condition. Group differences among chronically stimulated, unstimulated, and unoperated control muscles were identified by one-way ANOVA for normally distributed variables and by Kruskal-Wallis one-way ANOVA on ranks for variables with non-normal data distributions. Multiple comparisons were conducted by Student-Newman-Keuls and Dunn’s method for normally and non-normally distributed variables, respectively. Statistics were computed with the use of SigmaStat v. 1.01 (Jandel Scientific, San Rafael, CA). All values are expressed as means ± SE, and a probability of P < 0.05 was accepted as significant.

RESULTS

Muscle weight. The weight of the TA/EDL muscles in chronically stimulated rabbits (3.4 ± 0.3 g) was significantly less than in unoperated controls (5.3 ± 0.4 g; P < 0.05).

Contractile performance. Tension production for the two groups of rabbits is shown in Fig. 1. Tension production of unoperated control muscle was 258 ± 39 g at the onset of 4 tetani/min stimulation and fell slightly during the rest of the stimulation period, reaching 244 ± 37 g at 10 min and 241 ± 37 g at 15 min of stimulation; however, none of these changes were statistically significant. After 20 min of recovery, tension was 218 ± 31 g at the first contraction of 15 tetani/min stimulation. Thereafter, tension decreased to a lower level than during 4 tetani/min, reaching 152 ± 27 g at the final contraction (61 ± 7% of initial tension; P < 0.05). In chronically stimulated muscle,
tension was potentiated above 84 ± 28 g/g to 105 ± 30 g/g in the first 5 min of stimulation at 4 tetani/min (P < 0.05). After 10 min of stimulation, tension was 107 ± 29 g/g, and it had not increased further by 15 min of stimulation (109 ± 25 g/g). At the onset of 15 tetani/min stimulation, tension was 81 ± 20 g/g and increased significantly (P < 0.05) to 119 ± 16 g/g after 5 min, at which level it stabilized for the rest of the stimulation.

Metabolic changes. Figure 2 shows spectra from unoperated control and chronically stimulated muscle during acute stimulation at 15 tetani/min. The peaks of Pi, PCr, and ATP are clearly distinguishable in these spectra. The S/N for the β-phosphate peak of the control spectrum was ~100:1.

pH. At rest, pH in unoperated control muscle was 7.12 ± 0.02. During 4 and 15 tetani/min stimulation, pH decreased significantly, reaching 7.03 ± 0.04 (P < 0.05) and 6.94 ± 0.03 (P < 0.05) after 15 min of stimulation at 4 and 15 tetani/min, respectively (Fig. 3). At rest, there was no significant difference between the pH of chronically stimulated muscle (7.16 ± 0.02) and that of unoperated control muscle. In chronically stimulated muscle, pH did not change significantly from initial levels during or after stimulation in either trial. Although pH tended to be lower in unoperated control muscles during 4 tetani/min stimulation, the difference relative to chronically stimulated muscle was not significant until the fifth minute of stimulation at 15 tetani/min. At all subsequent time points, pH was

Fig. 1. Tension, normalized to muscle mass (g), in response to stimulation at 4 and 15 tetani/min for chronically stimulated rabbit tibialis anterior (TA)/extensor digitorum longus (EDL; ●) muscles and unoperated control muscle (▲). *Significant difference, chronically stimulated vs. unoperated control muscle (P < 0.05).

Fig. 2. 31P-magnetic resonance (MR) spectra at rest and after 15 min of stimulation at 15 tetani/min and difference spectra for unoperated control (A) and chronically stimulated muscle (B). ppm, Parts/million.
significantly lower in unoperated control than in chronically stimulated muscle (Fig. 3).

Indexes of phosphorylation potential. Initial values of $P_i/PCr$ were significantly higher for chronically stimulated muscle ($0.20 \pm 0.02$) than for unoperated control muscle ($0.05 \pm 0.01; P < 0.05$). In chronically stimulated muscle, no detectable change took place in $P_i/PCr$ in response to stimulation at either 4 or 15 tetani/min. This behavior differed markedly from that of unoperated control muscle. During stimulation at 4 tetani/min, $P_i/PCr$ increased, reaching $0.26 \pm 0.03$ at 15 min (Fig. 4). $P_i/PCr$ recovered to initial levels within 15 min ($0.05 \pm 0.01; P > 0.05$). During stimulation at 15 tetani/min, $P_i/PCr$ increased rapidly, reaching $1.68 \pm 0.36$ at 15 min ($P < 0.05$ relative to the initial level).

**DISCUSSION**

In this study, an in vivo model of the rabbit TA/EDL muscle complex was developed in which tension measurement and $^{31}$P-MR spectroscopy could be performed simultaneously. The results corroborate previously reported findings on adaptations in fatigue and phosphatometabolism in chronically stimulated muscles (6) and extend them to changes in force, pH, and $P_i/PCr$ occurring dynamically during an acute challenge.

Sampled volume. Axial images obtained with a proton coil of the same dimensions as those used for acquiring $^{31}$P-MR spectra indicated that the predominant source of signal in unoperated control muscle was a crescent of muscle extending to one-half the depth of the TA. However, the correspondence between the imaged volume and that sampled spectroscopically would not be precise because of differences in B1 field intensity at $^1$H and $^{31}$P frequencies. Furthermore, chronic stimulation reduced the total muscle mass by 50%, and, because the pulse duration used to acquire the spectra was nearly constant, sampling under these conditions would have extended more deeply into the TA/EDL muscle group. In anticipation of this effect and to minimize any resulting inhomogeneity within the sampled volume, chronic stimulation was carried out for 90 days, a period long enough to ensure complete transformation of all the muscles of the anterior compartment. At the same time, the diameter of the surface coil was deliberately limited to 8 mm, with a view to restricting the sampled volume to this compartment. It is therefore unlikely that the spectroscopic results were influenced to any significant degree by inclusion of unstimulated muscle.

Fatigue resistance. The development of fatigue resistance in fast-twitch skeletal muscle that had been subjected to chronic electrical stimulation was first described 20 years ago (26, 28). The ability of the chronically stimulated, or “conditioned,” muscle to maintain a stable tension output during prolonged periods of imposed activity has been confirmed in many subsequent studies in rabbit limb muscles (reviewed in Refs. 25, 27) and in the canine latissimus dorsi muscle (6). Chronically stimulated muscle will eventually show force fatigue but only at work rates very much higher than those that produce fatigue in control muscle (20). Thus, some feature of transformation enables tension to be maintained at contraction rates that normally produce fatigue. Muscle fatigue that occurs in unconditioned muscle during high-intensity contractions of short duration is associated with increases in $H^+$ and...
lactate (2, 11) and in Pi (3). In chronically stimulated muscle, these metabolic changes are minimal. This can be explained by a tighter coupling between ATP supply and demand such that the by-products of ATP hydrolysis (Pi, H+, ADP) remain at resting levels. The increase in anaerobic glycolysis and the associated increase in proton and lactate production rates that can occur in control muscle are averted, resulting in lower concentrations of the end-products of metabolic activity that are capable of inhibiting force production.

Tetanic tension in control and chronically stimulated muscle. In agreement with previous studies in the rabbit (4, 28), chronically stimulated muscle produced significantly less tetanic tension than unoperated control muscle. Because tetanic tension is closely correlated with muscle cross-sectional area (4), this finding is consistent with the lower total mass of the TA/EDL in these experiments and with the reported observation of smaller fiber cross-sectional areas in muscle transformed by continuous electrical stimulation (27).

Muscle energy state. Muscle phenotype is associated with significant differences in fiber energy state. In vitro biochemical analysis and 31P-MR spectroscopy both show that resting slow-twitch muscle has a lower phosphorylation potential than resting mixed or fast-twitch muscle (23). In the present study, P_i/PCr, an inverse index of phosphorylation potential, was significantly higher in chronically stimulated than in control muscle at rest. This is consistent with the predominantly slow-twitch fiber type composition of chronically stimulated muscle that has been reported previously (25, 27). An alternative explanation that deserves discussion is the possibility of muscle injury associated with stimulation because P_i/PCr is known to increase for several days after damaging eccentric contractions (21). There is, however, good evidence to suggest that injury is not a major consequence of chronic stimulation. In experiments conducted on the same muscles, and under conditions identical to those in the present study, Lexell et al. (19) found histological evidence of degenerating fibers amounting to only 3.5% of TA and 10.4% of EDL muscles by volume, respectively. Moreover, this represented a maximum value, attained at 9 days; after 3 wk of stimulation, no evidence of degenerating fibers could be found (18). Thus the higher resting value of P_i/PCr in chronically stimulated muscle is likely to be the consequence of transformation rather than damage to the fibers.

During tetanic stimulation, P_i/PCr increased in unstimulated control TA/EDL muscle but remained unchanged in chronically stimulated muscle. Similar findings have been reported for the chronically stimulated canine latissimus dorsi muscle (6). The increase in P_i/PCr in unoperated TA/EDL during acute tetanic stimulation is due to PCr hydrolysis, which maintains ATP concentration at constant levels (22), and to Pi accumulation. The stability of P_i/PCr in chronically stimulated muscle indicates that the rate of ATP synthesis was closely coupled to the rate of ATP hydrolysis at both 4 and 15 tetani/min. Thus temporal buffering of ATP concentration was not required. Microscopic examination and in vitro assay of chronically stimulated muscle have demonstrated striking increases in mitochondrial density, oxidative enzyme capacity, and capillarity (9, 12, 25, 27). Thus, chronically stimulated muscle appears to be well equipped to synthesize ATP at the high rates necessary to match the energy requirements of demanding contractions. The close coupling between synthesis of ATP and rates of hydrolysis in chronically stimulated muscle is suggestive of differences between control and chronically stimulated muscle in the fraction of maximum metabolic rate that is required to support tetanic stimulation and/or in the mechanisms that regulate metabolic rate, which might include altered sensitivity to ADP and Pi, in vivo (8) or control by NADH (1) or F, adenosine triphosphatase activity (7). Studies designed to investigate these possibilities have yet to be performed.

Conclusion. We have shown that it is possible to monitor muscle tension and intramuscular high-energy phosphate compounds simultaneously in an in vivo model. The system was applied to the TA/EDL muscle complex of the rabbit and used to examine contractile and metabolic adaptations to chronic electrical stimulation at 10 Hz. Muscles stimulated for 90 days had undergone a marked reduction in peak tetanic tension and muscle mass from control levels. However, these muscles showed no evidence of fatigue under conditions of acute tetanic stimulation that produced a substantial decline in the force generated by unconditioned muscles in the contralateral limb and in the limbs of unoperated control animals. Despite these demanding conditions, the transformed muscle was able to maintain stable levels of ATP hydrolysis products and a constant P_i/PCr. This increased capacity for homeostasis may be a direct consequence of an increased oxidative capacity, or it may involve changes in the regulation of oxidative phosphorylation. We believe that these results illustrate the potential of this system for studying factors underlying fatigue resistance, regulation of oxidative phosphorylation in skeletal muscle, and metabolic factors that may signal molecular alterations during continuous low-frequency stimulation.

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