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FORCE OUTPUT AND ENERGY METABOLISM DURING NEUROMUSCULAR ELECTRICAL STIMULATION: A ^{31}P-NMR STUDY

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ABSTRACT. The purpose of this study was to determine the acute physiologic effects of two electrical stimulation protocols commonly used for muscle rehabilitation. Surface electrodes were used to provide 12 stimulations of the calf musculature. In protocol A the duty cycle was fixed at 1 : 1 (10-second stimulation : 10-second rest); for protocol B it was 1 : 5 (10-second stimulation : 50-second rest). We continuously recorded isometric plantarflexor force in six healthy male subjects during stimulation using a load cell connected to a foot pedal ergometer. Metabolic changes in the stimulated gastrocnemius muscle were monitored in the supine position using ^{31}P-NMR spectroscopy (Phillips 1.5 tesla NMR machine). Relative changes in phosphocreatine (PCr), inorganic phosphate (Pi), and intracellular pH (pH_i) were obtained during stimulation and recovery, using a 1.5 cm RF surface antenna. Over the 12 stimulations, protocol A produced a significantly ($p < 0.001$), greater force decline (protocol A: 30.4 ± 1.3%, protocol B: 13 ± 0.8%); a significantly ($p < 0.005$), greater increase in Pi/PCr (protocol A: 210%, protocol B: 50%); and a significantly ($p < 0.001$), lower pH_i (protocol A: 6.8 ± 0.16, protocol B: 7.03 ± 0.12). We conclude that the shorter duty cycle produces more fatigue throughout the stimulation period, possibly as a result of greater intracellular acidosis and reduced availability of the high energy phosphate PCr. The clinical application of this finding relates to the selection of a stimulation protocol that maximizes strength gains in atrophic vs healthy muscle.

Key words: electrical stimulation, energy metabolism, nuclear magnetic resonance spectroscopy, skeletal muscle.

Clinical studies have shown that electrical muscle stimulation (EMS) can be as effective as voluntary isometric exercise for increasing force production in healthy and atrophied muscle (4, 11, 13, 15). Moreover, its effects are independent of voluntary capacity or motivation. The magnitude of the strength gains with EMS is a function of the training stimulus, measured as absolute force production or as a fraction of maximal voluntary contraction (MVC) during an electrically stimulated contraction (5, 12).

The primary goals of a stimulation protocol for rehabilitation are to maximize force production and minimize fatigue. Although the manipulation of EMS parameters to minimize the decline in force production during repeated contractions is commonly advocated for muscle rehabilitation (1, 6, 20), experimentally validated guidelines for optimal EMS treatment protocols are lacking (12), and duty cycles between 1 : 1 and 1 : 5 have been advocated in the clinical setting (10, 18). Although an EMS duty cycle of 1 : 1 or less results in rapid force decline (11), the mechanism of the muscle fatigue is presently unknown.

The purpose of this study was to investigate the alterations in muscle force output and cellular metabolic responses associated with manipulation of the duty cycle during electrical stimulation of healthy human muscle, using two commonly employed rehabilitation protocols as a basis for comparison. Using ^{31}P-NMR spectroscopy to map key energy metabolites and intracellular pH while continuously monitoring isometric force production, we were able to show that a reduction in PCr and an accumulation of H^+ are associated with greater fatigue in the stimulation protocol using the shorter duty cycle.

METHODS

Subjects

A convenience sample of six healthy, untrained male volunteers (age 26.8 ± 2.1 years, weight 69.3 ± 2.4 kg, height 179 ± 4.1 cm) participated in the study. All subjects provided informed consent.

Electrical muscle stimulation

The muscle stimulating current was supplied by a modified Respond II® neuromuscular stimulator (Medtronic Inc., Canada). This stimulator, permitting the accurate selection of a 40 Hz pulse frequency, provided an asymmmetrical balanced biphasic square wave with a 300 μs pulse width, producing a tetanic contraction at 40 Hz. Once activated, the stimulator reached peak current output in 0.5 seconds. Prior to data collection, pulse frequency, pulse width, waveform, and constant current output (at 3000 ohms) were validated using an oscilloscope. Pilot investigations revealed that new AA alkaline cells provided constant current levels during 25, 10-second contractions within the magnetic field of the NMR machine. New cells were provided for each subject.

Electroconductive gel was applied to two carbon rubber electrodes (7×2 cm). Following skin cleansing with alcohol, the electrodes were applied transversely, approximately 10 cm apart, on the skin over the proximal and distal aspects of the medial head of the right gastrocnemius muscle. This placement produced an isometric plantarflexor force at 40% of an MVC measured using a Kin-Com dynamometer (Chattex Corp.).

Experimental protocol

Once positioned inside the bore of the NMR machine, the subject was provided with an on/off hand switch to control the electrical stimulator. The subjects were trained to switch the stimulator on and off in accordance with an audible buzzer synchronized with the NMR pulse program. Each subject underwent 12, 10-second electrically induced contractions with the order of protocol A or B randomly assigned (Fig. 1). A one-hour rest period was followed by the second protocol. A resting NMR spectrum obtained prior to the second protocol showed that pH, Pi and PCr had returned to pre-stimulation values. Protocol A (1 : 1 duty cycle) used 10-second rest periods; protocol B (1 : 5 duty cycle) used 50-second rest periods (Fig. 1).

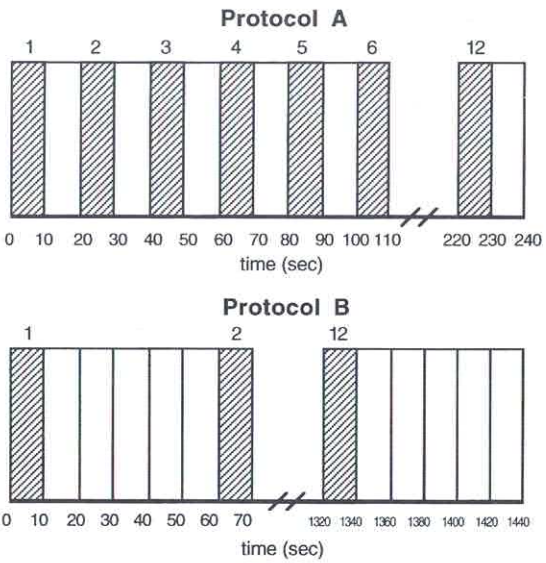

Fig. 1. Experimental stimulation protocol. Each bar in the histogram represents a 10-second block of time. The shaded bars are periods of stimulation; the clear bars are periods of recovery. In protocol A each of twelve 10-second stimulations were followed by one 10-second recovery period. In protocol B each of twelve 10-second stimulations were followed by five 10-second rest periods.

Force measurements

Isometric plantarflexor force measurements during EMS were monitored using a specially fabricated non-magnetic foot pedal ergometer coupled to a load cell by a glassfiber rod. With the subject in supine position, the right foot was placed on the foot pedal. The axis of movement of the ankle joint was aligned with the foot pedal axis, and the foot and knee stabilized with velcro straps.

Prior to data collection, the load cell was calibrated in tension inside the magnetic field using a series of certified weights. A 10-m cable connected the load cell to a 12-bit data acquisition card installed in a microcomputer. This instrumentation enabled continuous (two samples per second) acquisition of tension data with single measurement reliability of ± 0.05 Newtons. Force production data prior to peak tension development were discarded; in all cases this comprised the first second of stimulation.

Nuclear magnetic resonance spectroscopy

^{31}P-NMR data were collected using a 1.5 Tesla Philips Gyroscan NMR system with a 1.0 m bore (access diameter 0.6 m). An oval surface coil with a 2 cm to 7.5 cm minor to major diameter (interrogating a tissue volume of about 15 ml to a depth of the order of 2 cm) was utilized. The long axis of the coil was placed parallel to the two stimulating electrodes, over the medial bulk of the gastrocnemius muscle, and fixed in place with velcro straps and web bandage. Once affixed, the coil was aligned parallel to the longitudinal axis of the magnet.

The pulse program provided a 90° RF pulse of 0.05 ms duration at a frequency of 25.86 MHz. The repetition interval was set a 1000 ms, with 1024 data points collected per repetition. Data were acquired in 10-second bins, with each resulting spectrum being the sum of 10, 1-second acquisitions. The sweep width used in the acquisition was 2 kHz.

Changes in intracellular pH relative to initial resting values were calculated from the chemical shift difference between the center of the Pi and PCr peaks on the NMR spectrum using the following formula:

$$pH = pK + \log_{10}([\delta obs - \delta HPO_4^{2-}]/[\delta H_2PO_4^- - \delta obs])$$

where δobs is the observed Pi chemical shift relative to that of PCr, and the pK is 6.80. $\delta H_2PO_4^-$ and δHPO_4^{2-} represent the chemical shifts at very basic (pH = 10) and very acidic (pH = 4) pH values, respectively (17). This chemical shift difference is used to determine the average relative cellular pH in the tissue under interrogation, with an accuracy of ± 0.05 pH units (17). For the pH calculations in this investigation $\delta HPO_4^{2-} = 3.19$ ppm and $\delta H_2PO_4^- = 5.72$ ppm.

Data analysis

The metabolite levels acquired from the NMR spectra were 10-second averages during contraction and rest. Phosphorus spectra obtained during data collection were processed on a Ramtek workstation interfaced with a Vax (Digital) computer. After Fourier transformation, the relative areas under the resonance peaks were calculated using a Gaussian line-fitting procedure (New Methods Research, Syracuse, N.Y.) and multiplied by the following longitudinal relaxation correction factors (7): (i) $(Pi/PCr) \times 1.27$, (ii) $\{\beta ATP/(PCr + Pi)\} \times 0.328$.

Relative changes in PCr were expressed as the Pi/PCr quotient. Relative pH changes were calculated as described above, while ATP was expressed as $\beta ATP/(PCr + Pi)$.

Although both protocols contained an equal number and duration of stimulation periods (12×10 seconds $= 120$ seconds), the fivefold difference in rest period length between the two protocols naturally resulted in different total application times (protocol A: 240 seconds, protocol B: 1440 seconds, Fig. 1). For this reason data is presented in two formats: (i) changes in dependent variables as a function of stimulation number (without reference to absolute time) and (ii) changes in dependent variables as a function of the total stimulation time of protocol B (the longer protocol).

A two-way (time and group) repeated measures analysis of variance (ANOVA) was applied to the dependent variables (pHi, ATP, force, and Pi/PCr) during work and rest. In addition, changes in tension were compared with changes in Pi/PCr, ATP, and pHi for both protocols.

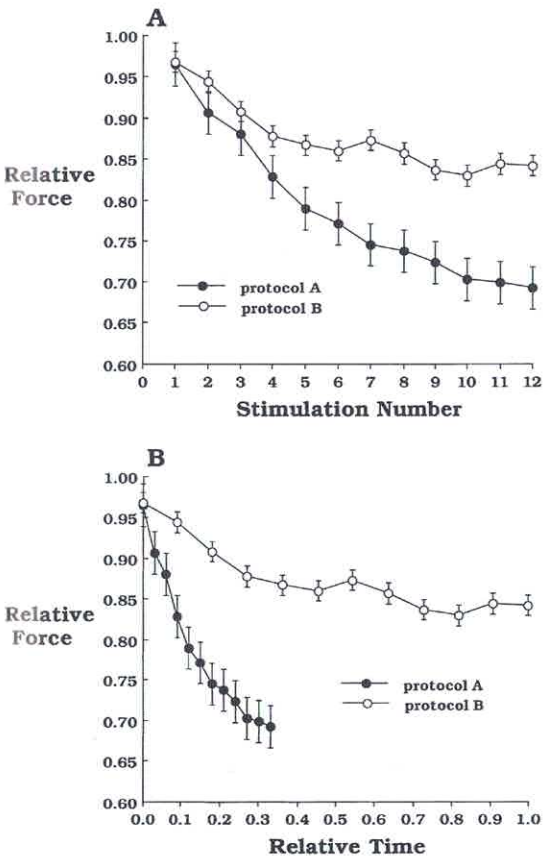

Fig. 2. Force production during stimulation. (A) Percent decline in force relative to the maximum force produced during the protocol. Each data point is an average over 16 separate values obtained during the middle 8 seconds of electrical stimulation in protocol A (10-second stimulation: 10-second recovery) and protocol B (10-second stimulation: 50-second recovery). Values are mean ± S.E. Both protocols exhibited a significant ($p < 0.001$) decrease in force production over 12 stimulations. ANOVA results show that the reduction in force output in protocol A (30%) was significantly greater ($p < 0.05$) than protocol B (13%). (B) Force data from Fig. 2a relative to the total time of protocol B ($1.0 = 1440$ seconds). Values are mean ± S.E.

RESULTS

Changes in muscular force production during both stimulation protocols are shown in Fig. 2. Although both protocols exhibited a significant ($p < 0.001$) reduction in force production over 12 stimulations, the change was significantly ($p < 0.05$) greater in protocol A (30.4 ± 1.3%) compared with protocol B (13 ± 0.8%). Likewise, although both protocols produced a significant ($p < 0.05$) increase in Pi/PCr during stimulation (Fig. 3), Protocol A produced a significantly ($p < 0.01$) greater increase (210%) compared with protocol B (48%). Fig. 4 shows the pHi data during stimulation. Mean resting intracellular pH values were equal at the start of both protocols (7.11 ± 0.01). The

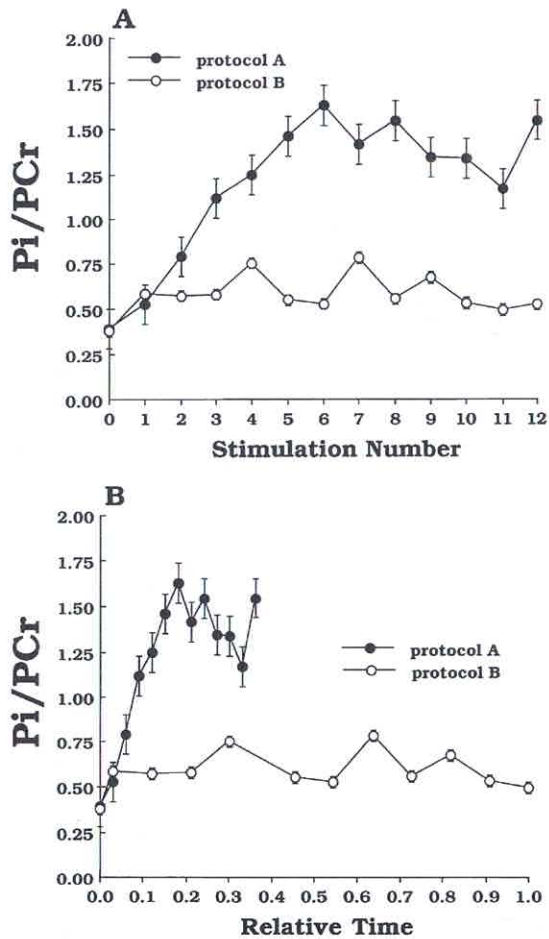

Fig. 3. Pi/PCr during stimulation. (A) Mean ± S.E. changes in Pi/PCr over 12, 10-second stimulations. Protocol A produced a 210% peak increase at stimulation 6, while protocol B produced a 48% peak increase at stimulation 7. Changes in protocol A differed significantly from those in protocol B ($p < 0.01$). (B) Pi/PCr data from Fig. 3a relative to the total time of protocol B ($1.0 = 1440$ seconds). Values are mean ± S.E.

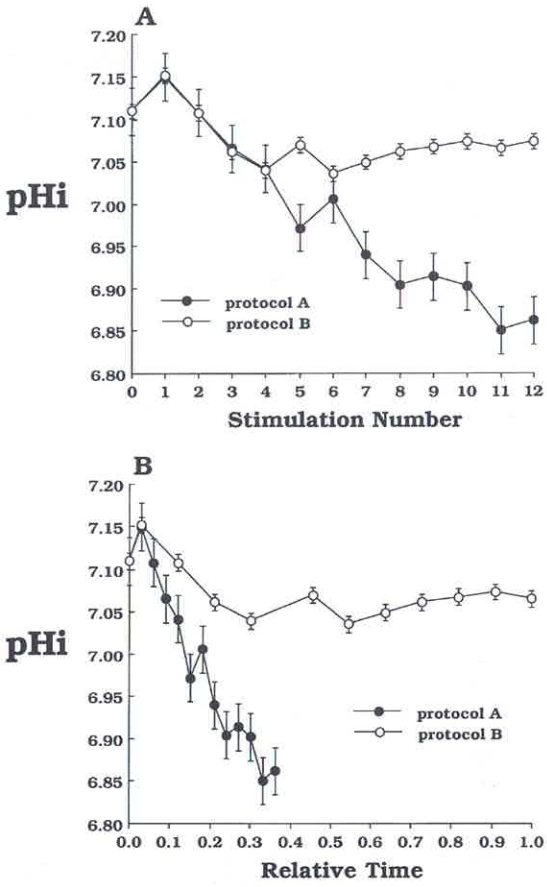

Fig. 4. pHi During Stimulation. (a) Mean ± S.E. intracellular pH change during each 10-second stimulation period in both protocols. The resting pHi is 7.1 ± 0.08. The pHi response to protocol A differed significantly ($p < 0.001$) from protocol B. Following stimulation No. 4, pH in protocol B reaches a plateau, while it continues in a near–linear decline in protocol A. (b) pHi data from Fig. 4a relative to the total time of protocol B (1.0 = 1440 seconds). Values are mean ± S.E.

initial increase in pH is a result of proton consumption during PCr hydrolysis. In protocol A, pHi declined to 6.85 while in protocol B, the lowest pHi value was 7.04. These differences were significant ($p < 0.001$). No significant ($p > 0.10$) changes were found in intracellular ATP concentrations during stimulation.

NMR data acquired during the rest cycles were collected in 10-second bins; protocol A contained a single 10-second bin, protocol B contained five. Pi/PCr in the rest periods between stimulations in protocol A showed almost no recovery compared with the mean values recorded during the previous contraction. In contrast, Pi/PCr in the 10-second rest period just prior to the next contraction in protocol B showed substantial recovery (Fig. 5).

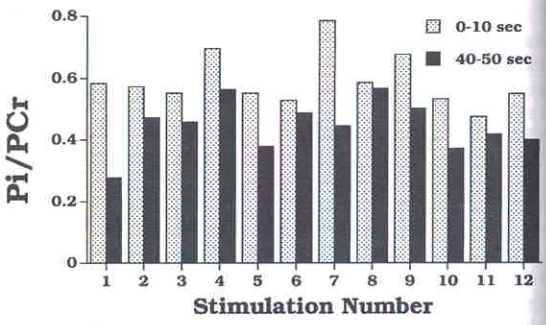

Fig. 5. Pi/PCr during recovery in protocol B. Pi/PCr data collected in the first (0–10 seconds) and last (40–50 seconds) recovery period between each stimulation in protocol B. Compared with protocol A rest periods during which no recovery occurred, substantial recovery of Pi/PCr is seen in protocol B.

DISCUSSION

The two electrical muscle stimulation protocols used in this study produced substantially different metabolic and force responses in the activated muscle despite containing an equal number of contractions and an equal total stimulation time. Protocol A (with shorter rest periods) was associated with significantly greater increases in Pi/PCr and drops in pHi, as well as significantly lower muscle force production values.

The adenosine triphosphate (ATP) requirements during high intensity/short duration contractions, such as the tetanic contractions that result during EMS, are supplied primarily by phosphocreatine (PCr) hydrolysis and lactate production (anaerobic glycolysis). Oxidative phosphorylation in the mitochondria is the main mechanism for ATP resynthesis during recovery (22). Thus, the rate of PCr depletion during short-term, intense work is a measure of anaerobic energy output and the rate of PCr resynthesis during recovery is a measure of oxidative energy output (2). As Fig. 5 shows, sufficient phosphocreatine resynthesis took place during the recovery periods in protocol B to maintain steady-state levels of Pi/PCr after the first contraction (Fig. 3a, Fig. 5). In comparison, Pi/PCr levels did not achieve steady-state until after the sixth contraction in protocol A (Fig. 3a). In addition, pHi stabilized at about 7.05 in protocol B while it continued to drop through the entire stimulation period in protocol A (Fig. 4a). The higher duty cycle and the consequent reduced time for oxidative phosphorylation in the mitochondria in protocol A are associated with a greater drop in pHi, the result of an increased rate of glycolytic activation as a metabolic pathway for ATP synthesis (8, 9). The higher concentration of Pi and ADP in protocol A acts as a potent stimulator of anaerobic

metabolism. The resultant accumulation of H^+ and Pi lead to greater fatigue in protocol A through interference with glycolysis (PFK) and Ca^{2+} binding to troponin (16).

During voluntary work, force is produced by the recruitment of muscle fibers throughout the whole muscle; with EMS a localized maximal contraction is produced. With a voluntary contraction, muscle fiber recruitment occurs through varying the number of motor units activated (spatial recruitment) and/or the force generated by a given motor unit (rate coding), by altering the discharge frequency of the innervating α-motoneuron. The size principal states that CNS activation stimulates slow-twitch fibers first and then, as fatigue increases, the larger FT fibers are gradually recruited. With electrical stimulation, the normal voluntary, physiologic recruitment strategy is disrupted and, instead, all motoneurons in the area of current flow are depolarized, regardless of fiber diameter (14). The stimulation frequency used in this study (40 Hz) produced a uniform, tetanic contraction of all the muscle fibers in the localized volume of muscle stimulated and interrogated by the RF coil. Thus, tetanic EMS contractions likely contribute to a greater increase in lactate production compared with voluntary work at the same intensity.

Muscle fatigue reduces the capability to maintain a training load (11). The 30% decline in force observed during protocol A is similar to the 26% decline reported by Cox et al. who used 10, 10-second EMS contractions, a 1 : 3 duty cycle, but at 100 Hz (3). The 210% increase in Pi/PCr during protocol A is of similar magnitude to the PCr changes reported by Shenton et al. following an investigation of the response of the forearm flexors to EMS (21).

The clinical relevance of the present findings relates to the selection of an appropriate application protocol to maximize strength gains in two different clinical situations. In the case of atrophic or diseased muscle, it is believed the stimulus for strength gain is the total training load while minimizing the development of fatigue (6). Protocol B results in only modest metabolic changes and minimal decrements in force throughout the stimulation period. The longer recovery periods in protocol B permit PCr resynthesis, minimize drops in pHi, result in contractions at a higher intensity relative to maximum, and increase the total training load (force production). In the case of healthy muscle, it is believed that fatigue, in addition to the total training load, is an important stimulus for hypertrophy (19). The present findings show that recovery is insufficient in protocol A to permit any substantial PCr resynthesis, and this, together with the activation of glycolysis, an increase in lactate, and a drop in intracellular pH is associated with substantially greater fatigue. While fatigue may be an important training stimulus in healthy muscle, force production may be more important in atrophic muscle. Although further research is necessary, protocol A may be better for training healthy muscle.

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