Force-frequency relationship and potentiation in mammalian skeletal muscle

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Macintosh, Brian R., and Janine C. Willis. Force-frequency relationship and potentiation in mammalian skeletal muscle. J Appl Physiol 88: 2088–2096, 2000.—Repetitive activation of a skeletal muscle results in potentiation of the twitch contractile response. Incompletely fused tetanic contractions similar to those evoked by voluntary activation may also be potentiated by prior activity. We aimed to investigate the role of stimulation frequency on the enhancement of unfused isometric contractions in rat medial gastrocnemius muscles in situ. Muscles set at optimal length were stimulated via the sciatic nerve with 50-µs duration supramaximal pulses. Trials consisted of 8 s of repetitive trains (5 pulses (quintuplets) 2 times per second or 2 pulses (doublets) 5 times per second) at 20, 40, 50, 60, 70, and 80 Hz. These stimulation frequencies represent a range over which voluntary activation would be expected to occur. When the frequency of stimulation was 20, 50, or 70 Hz, the peak active force (highest tension during a contraction—rest tension) of doublet contractions increased from 2.2 ± 0.2, 4.1 ± 0.4, and 4.3 ± 0.5 to 3.1 ± 0.3, 5.6 ± 0.4, and 6.1 ± 0.7 N, respectively. Corresponding measurements for quintuplet contractions increased from 2.2 ± 0.2, 6.1 ± 0.5, and 8.7 ± 0.7 to 3.2 ± 0.3, 7.3 ± 0.6, and 9.0 ± 0.7 N, respectively. Initial peak active force values were 27 ± 1 and 61.5 ± 5% of the maximal (tetanic) force for doublet and quintuplet contractions, respectively, at 80 Hz. With doublets, peak active force increased at all stimulation frequencies. With quintuplets, peak active force increased significantly for frequencies up to 60 Hz. Twitch enhancement at the end of the 8 s of repetitive stimulation was the same regardless of the pattern of stimulation during the 8 s, and twitch peak active force returned to prestimulation values by 5 min. These experiments confirm that activity-dependent potentiation is evident during repeated, incompletely fused tetanic contractions over a broad range of frequencies. This observation suggests that, during voluntary motor unit recruitment, derecruitment or decreased firing frequency would be necessary to achieve a fixed (submaximal) target force during repeated isometric contractions over this time period.

It is well documented that there is enhancement of twitch amplitude during repeated low-frequency stimulation (staircase) and after tetanic contraction (posttetanic potentiation). These increases in twitch active force, which result from prior activity, are collectively referred to as activity-dependent potentiation or postactivation potentiation.

It could be argued that twitch potentiation as a consequence of prior activity is irrelevant with respect to voluntary motor activity, since skeletal muscle is not normally activated with a single pulse. Voluntary recruitment is thought to occur with frequencies of stimulation, which result in incompletely fused tetanic contractions (3–5, 27). When one considers that the mechanism of activity-dependent potentiation is either increased sensitivity of the contractile apparatus to Ca\(^{2+}\) (13, 30) or increased magnitude of Ca\(^{2+}\) transients for each activating pulse (9), it could be anticipated that activity-dependent potentiation would be evident during incompletely fused tetanic contractions. The magnitude of such potentiation should be less when initial contraction amplitude approaches maximal isometric force, since maximal isometric force is not affected by either increased Ca\(^{2+}\) sensitivity or increased myoplasmic Ca\(^{2+}\) concentration.

There is only one study that has systematically investigated the magnitude of potentiation observed with different frequencies of stimulation (32). In that study, it was noted that potentiation after tetanic stimulation in mouse extensor digitorum longus at room temperature was not evident for frequencies of stimulation at and above 20 Hz. This frequency of stimulation, however, does not represent an upper limit for potentiation because there are published records that show potentiation at 37°C with intermittent contractions at 40 Hz in rat gastrocnemius muscle (26, 28), rat respiratory muscles (22), cat tibialis posterior muscle (2), and cat gastrocnemius muscle (7, 8). It seems appropriate to reinvestigate the relationship between frequency of stimulation and activity-dependent potentiation in a muscle preparation at 37°C in order to see if the pattern of stimulation (frequency and duration for each train) affects the magnitude of potentiation that is observed.

The purpose of this study was to systematically evaluate the presence of potentiation during incompletely fused tetanic contractions. It was hypothesized that potentiation would be evident over a broad range of frequencies and that the magnitude of potentiation would decrease as the plateau of the force-frequency relationship is approached. This should be the case if the force-frequency relationship is analogous to the force-pCa relationship, as indicated by measurements of mean free intracellular Ca\(^{2+}\) concentration in intact...
single fibers of skeletal muscle (1). Considering that active force is dependent on the duration of stimulation (number of activating pulses) as well as the frequency, it was considered appropriate to evaluate enhancement of force during repeated stimulation trains of different frequencies and durations. In this way, it could be determined whether frequency of stimulation was an important consideration in limiting enhancement or whether the relative magnitude of initial peak active force was more important. Peak active force is defined as the difference between rest tension and the highest tension achieved during a contraction.

METHODS

Muscle preparation. Sprague-Dawley rats (246-428 g) were given standard rat chow and water ad libitum and kept in a room with a 12:12-h light-dark cycle. The animals (n = 15) were anesthetized with an intramuscular injection of ketamine and xylazine (100 mg/ml each) mixed 85:15, respectively (1 ml/kg), which resulted in a surgical level of anesthesia for the duration of the experiment. The left medial gastrocnemius muscle was surgically isolated (15, 16), leaving the vascular connections and muscle origin intact. The sciatic nerve was isolated from surrounding connective tissues for 1–1.5 cm and cut proximally. The calcaneus was severed, leaving a piece of bone attached to the Achilles tendon. The s oleus, lateral gastrocnemius, and plantaris muscles were dissected free, leaving only the medial gastrocnemius muscle attached to the Achilles tendon. A ligature was tied tightly around the leg midway between the ankle and the knee, and the distal half of the leg was removed. A dissecting probe was inserted longitudinally into the medulla of the tibia, and a drill bit was placed perpendicularly into the femur. Both the probe and the drill bit were rigidly fixed to the myograph base to anchor the origin of the medial gastrocnemius muscle.

Once the leg was fixed in the myograph apparatus, the medial gastrocnemius muscle was secured via the Achilles tendon to a tension transducer (Grass FT 10 with blue springs; maximum load 10 kg). The transducer was mounted on a rack-and-pinion device and oriented to measure the tension in line with the pull of the muscle. The connective tissue between the skin and the muscle was disrupted, and the loosened skin was pulled up around the sides to form a bath, which was filled with warmed mineral oil. Rectal and oil bath temperatures were monitored (YSI thermoprobes) and kept at 36.5–37.5°C with radiant heat.

The distal stump of the cut sciatic nerve was placed across a pair of stainless steel hook electrodes (3 mm separation) and stimulated with square pulses (50 μs) with a Grass model S88 stimulator. Maximal voltage was tested and found to always be <1 V. Subsequent stimulation was 3 V throughout each experiment. Output of the tension transducer was visually displayed on a computer monitor following analog-to-digital conversion at 2,000 Hz and stored for later analysis.

Procedures. Muscle length was adjusted with the rack-and-pinion device to yield maximal developed tension for double-pulse (delay = 5 ms) contractions, optimal length. A conditioning stimulus (200 Hz for 500 ms) was then used to assess the stability of all connections. The muscle optimal length was reset with double pulses 5 min after the conditioning tetanic contraction. This 5-min rest was sufficient to allow all potentiating effects due to the conditioning tetanic contraction to dissipate. Once the optimal length was re-determined, all further stimulation was conducted at that length.

A set of three tetanic contractions was performed (250-ms duration at 100, 200, and 250 Hz) at both the beginning and the end of the experiment to determine maximal force production. A 2-min rest was permitted between consecutive tetanic contractions. Each rat was randomly assigned to one of two groups. One group (n = 7) was stimulated with two-pulse trains (doublet) at 20, 40, 50, 60, 70, and 80 Hz every 0.25 s for a total of 8 s. The second experimental group (n = 8) received stimulation with five-pulse trains (quintuplet) at 20, 40, 50, 60, 70, and 80 Hz every 0.5 s for a total of 8 s. In this study, contractions elicited with quintuplet trains of stimulation will be referred to as quintuplet contractions and contractions elicited with doublet trains of stimulation will be referred to as doublet contractions. The rationale for using doublet and quintuplet stimulation was to separate the effects of frequency from relative force as determinants of the magnitude of potentiation. It is known that a shorter duration train at a given frequency (doublet vs. quintuplet) will result in a smaller peak active force. If frequency of stimulation is a factor limiting the magnitude of potentiation, then doublet and quintuplet contractions at a given frequency should have the same magnitude of potentiation. The order of the test frequency was randomized for each animal. In all cases, a total of 80 pulses were delivered over the course of 8 s. This common feature of the patterns of stimulation (80 pulses over 8 s) was anticipated to elicit a similar magnitude of single-pulse (twitch) potentiation following the intermittent stimulation. This anticipated result assumes that the Ca2+ transient, and therefore the activation of myosin light-chain kinase, would be similar for each activating pulse, regardless of the frequency or duration of each train of pulses.

Data collection. Data (2,000 Hz for 8 s) was initiated 0.5 s before the start of the 8 s of intermittent stimulation. Contractions for analysis were extracted from the data file at the start of intermittent contractions (0 s) and at 1-s intervals up to 7 s. Analysis of contractions consisted of measuring the force developed (rest vs. peak) in response to the first stimulating pulse of a train (first response) as well as the highest developed force achieved in a given incompletely fused tetanic contraction (peak active force). The peak active force was usually obtained with the last stimulating pulse of a train (see Fig. 1).

Each set (8 s) of doublets or quintuplets was followed by a 5-min recovery period during which single twitches were obtained at regular intervals. These twitch contractions were used to evaluate the magnitude and time course of twitch potentiation resulting from the preceding 80 pulses of stimulation. The twitch is the smallest unit of contractile activation and should therefore be the most sensitive measure of potentiation. This series of contractions would permit evaluation of the assumption (above) that a similar magnitude of “potentiating factors” (presumably light-chain phosphorylation) would be achieved with each of the stimulating patterns employed. The sequence of stimulation resulting in 8 s of intermittent, incompletely fused tetanic contractions was repeated in each animal up to five additional times, such that data for up to six stimulation patterns (frequencies) were obtained from each animal. This design reduced the total number of animals required for this study but precluded the possibility of obtaining biochemical measurements specific for the various stimulation conditions. To test for the presence of fatigue, twitch contractions were obtained at the start and the end of the experiment.

RESULTS

Analysis of twitch contractions to detect fatigue showed that twitch amplitude was relatively constant.
over the course of these experiments. The mean value for twitches at the start of the experiment was 2.16 ± 0.2 (SE) N compared with the mean value at the end of the experiment of 2.10 ± 0.2 N. There was no significant difference (P > 0.1). This indicates that there was no fatigue during these experiments.

Stimulation with two or five consecutive pulses at frequencies ranging from 20 to 80 Hz resulted in contractions with varying degrees of summation (see Fig. 2) with peak active force equal to 27 ± 1% and 73 ± 4% of maximum (200- or 250-Hz tetanic contraction) active force for doublet and quintuplet contractions, respectively, when measured at 80 Hz. The extent of summation was much less with doublet stimulation than with quintuplet stimulation, when the stimulation frequency was >40 Hz.

Repeated stimulation with quintuplet trains twice per second for 8 s resulted in a pattern of change in peak active force that was strongly dependent on the frequency of stimulation (Fig. 3A). Figure 3A shows that peak active force increased progressively at 20 Hz. The increase in peak active force appeared to be delayed when stimulation was 40 Hz. At 50 and 60 Hz, peak active force actually decreased before showing enhancement. The pattern at 70 Hz was similar to the one at 40 Hz but with less potentiation; at 80 Hz, peak active force remained fairly constant. Post hoc statistical evaluation focused on potential changes in peak active force from 0 to 1 s and again at 7 s. Two-factor ANOVA showed significant interaction, which indicates that differences across time vary, depending on the frequency of stimulation. The two-way ANOVA was followed by one-way ANOVA and Newman-Keuls test at each frequency. Significant differences were seen from 0 to 1 s at stimulation frequencies of 50, 60, and 70 Hz. Peak active force at 7 s was significantly different from that at 0 s with stimulations of 20, 40, 50, and 60 Hz (Table 1). Peak active force at 7 s was significantly different from that at 1 s for stimulations of 20, 40, 50, 60, and 70 Hz. Figure 3B–D, illustrates examples of quintuplet contractions at 20, 50, and 70 Hz, respectively, at 0, 1, and 7 s for intermittent quintuplet trains.

Repeated stimulation with double pulses, five times per second for 8 s, resulted in increased peak active force regardless of the frequency of stimulation (see Fig. 4), as indicated by the absence of significant interaction (time by frequency of stimulation, P > 0.5) but significant main effects for time (0 vs. 7 s, P < 0.05). This analysis reveals that the differences across time (0 vs. 7 s) are the same for all frequencies of stimulation. Figure 4, B–D, illustrates examples of contractions with 20-, 50-, and 70-Hz stimulation, respectively, at 0, 1, and 7 s during intermittent doublet contractions. The magnitude of potentiation with doublet contractions was similar to the potentiation for quintuplet contractions when compared with a similar initial (0 s) peak active force (see Fig. 5).
In addition to analyzing the peak active force during intermittent contractions at different stimulation frequencies, the force developed in response to the first pulse of a train (first response) was also measured. The magnitude of this response is independent of the frequency of stimulation within the train and should reflect the same attributes as measurement of a twitch contraction. Figure 6 illustrates the progressive change in first response over 7 s of intermittent quintuplet (Fig. 6A) and doublet (Fig. 6B) stimulation. Two-factor ANOVA (time and frequency) showed no significant interaction (P > 0.1) and no significant main effect of frequency (P > 0.1). This analysis reveals that the increase over time for the first response was the same for any frequency of stimulation. To further evaluate the divergent changes in first response and peak active force for quintuplets, the ratio of peak active force to first response at 0, 1, and 7 s was calculated.

Table 2 presents the results of this analysis. In all cases (i.e., at all frequencies of stimulation), the ratio of peak active force to first response decreased across the 7 s of repetitive contractions. Furthermore, the decrease in the ratio from 0 s to 1 s was significant at all frequencies of stimulation. This ratio was also significantly decreased at frequencies of stimulation of 20, 40, 50, and 80 Hz. No further decrease in the ratio from 1 to 7 s was seen for 50- and 60-Hz stimulations; this probably relates to the fact that, from 0 s to 1 s, peak

Table 1. Peak active force before and after 7 s of intermittent contractions

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Time</th>
<th>Quintuplets</th>
<th>Doublets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 s</td>
<td>7 s</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>14.55 ± 1.50</td>
<td>21.44 ± 5.00*</td>
<td>13.94 ± 1.01</td>
</tr>
<tr>
<td>40</td>
<td>24.99 ± 2.94</td>
<td>31.20 ± 4.00*</td>
<td>17.90 ± 0.90</td>
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<tr>
<td>50</td>
<td>42.10 ± 4.40</td>
<td>49.60 ± 6.70*</td>
<td>22.77 ± 1.25</td>
</tr>
<tr>
<td>60</td>
<td>45.50 ± 4.80</td>
<td>51.50 ± 4.80*</td>
<td>25.37 ± 2.42</td>
</tr>
<tr>
<td>70</td>
<td>61.10 ± 7.50</td>
<td>63.30 ± 7.10</td>
<td>25.28 ± 2.42</td>
</tr>
<tr>
<td>80</td>
<td>61.50 ± 4.80</td>
<td>64.24 ± 3.80</td>
<td>26.82 ± 1.11</td>
</tr>
</tbody>
</table>

Values are means ± SE. Peak active force is presented as a % of maximum (obtained from tetanic contractions at 200- or 250-Hz stimulation frequency). Quintuplets refer to intermittent contractions, 2 per second with 5 stimulating pulses per train delivered at the corresponding frequency for each contraction. Doublets refer to intermittent contractions, 5 per second with 2 stimulating pulses per train, delivered at the corresponding frequency for each contraction. *Significant difference between 0 s and 7 s.
active force decreased significantly and, from 1 s to 7 s, peak active force increased considerably.

The total number of activating pulses delivered over the 8 s of repetitive stimulation was the same (80 pulses), regardless of the stimulation frequency within each train or whether each train had two or five pulses. After the 80 pulses in 8 s, twitch potentiation and dissipation of potentiation were monitored. Figure 7 illustrates the time course of change in twitch active force during the 5 min following the repetitive stimulation for the cumulative data. All data were combined because there was no significant effect of (prior) frequency on the magnitude of twitch potentiation observed (P > 0.5) and no effect of doublet vs. quintuplet (P > 0.15). Table 3 presents the relative twitch potentiation, which was estimated to be present at 0 s of recovery, and the time constant for decay of potentiation for each stimulation condition. ANOVA showed no significant interaction (number of pulses per train by frequency, P > 0.5) and no significant main effects (number of pulses per train, frequency of stimulation P > 0.5). Therefore, the magnitude of twitch potentiation and the time course of dissipation of potentiation during the recovery period were not affected by the pattern of stimulation during the 8 s of repeated trains of pulses.

DISCUSSION

Three key observations have been made in this series of experiments: 1) potentiation is evident during brief, intermittent contractions for stimulations up to 70 Hz with five pulses per train and up to stimulation frequen-
Fig. 6. Active force of the first response (active force developed due to the first pulse of the train) across 7 s of intermittent stimulation with trains at 20, 50, and 70 Hz for quintuplets (A; n = 8) and doubles (B; n = 7), showing the progressive increase in equivalent twitch response, regardless of frequency of stimulation. This increase in the first response is also evident in individual contractions presented in Figs. 3 and 4. SE bars are omitted for clarity and for most cases are within the size of the symbol. t = Time.

Fig. 7. Twitch active force was measured at discreet times during the recovery period following 8 s of intermittent contractions (80 pulses total). Twitch amplitude clearly decreased exponentially. All data were combined because there was no difference between quintuplets and doubles or between the various frequencies. SE bars are omitted because they fit within the symbol. Regression equation: %potentiation = 58.578e^0.0114t; r = 0.997.

Table 2. Ratio of peak active force to first response for quintuplet contractions

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Time 0s</th>
<th>Time 1s</th>
<th>Time 7s</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1.15 ± 0.03</td>
<td>1.12 ± 0.02*</td>
<td>1.04 ± 0.01†</td>
</tr>
<tr>
<td>40</td>
<td>1.93 ± 0.13</td>
<td>1.61 ± 0.07*</td>
<td>1.46 ± 0.08†</td>
</tr>
<tr>
<td>50</td>
<td>2.96 ± 0.18</td>
<td>2.29 ± 0.15*</td>
<td>2.10 ± 0.16*</td>
</tr>
<tr>
<td>60</td>
<td>3.92 ± 0.38</td>
<td>2.88 ± 0.14*</td>
<td>2.48 ± 0.07*</td>
</tr>
<tr>
<td>70</td>
<td>4.15 ± 0.22</td>
<td>3.61 ± 0.19*</td>
<td>2.79 ± 0.09†</td>
</tr>
<tr>
<td>80</td>
<td>4.36 ± 0.16</td>
<td>3.96 ± 0.12*</td>
<td>2.98 ± 0.06†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference from results at 0 s (P < 0.05). †Significant difference from results at 1 s and from results at 0 s (P < 0.05).

Table 3. Frequency independence of twitch potentiation and time constant for decay of potentiation

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Potentiation, %</th>
<th>Time constant, min</th>
<th>Potentiation, %</th>
<th>Time constant, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>63.8 ± 5.5</td>
<td>1.5 ± 0.1</td>
<td>75.1 ± 4.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>40</td>
<td>59.7 ± 8.6</td>
<td>1.5 ± 0.1</td>
<td>71.5 ± 3.7</td>
<td>1.4 ± 0.04</td>
</tr>
<tr>
<td>50</td>
<td>62.0 ± 3.8</td>
<td>1.5 ± 0.1</td>
<td>71.4 ± 4.6</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>60</td>
<td>65.3 ± 5.3</td>
<td>1.6 ± 0.1</td>
<td>73.1 ± 3.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>70</td>
<td>61.3 ± 7.9</td>
<td>1.5 ± 0.1</td>
<td>71.6 ± 4.9</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>80</td>
<td>64.2 ± 5.4</td>
<td>1.6 ± 0.1</td>
<td>73.6 ± 4.7</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Doublet stimulation was with 2 pulses at the corresponding frequency, delivered 5 times per second for 8 s. Measurements were made on twitch contractions during the 5-min period of recovery after the 8 s of repetitive stimulation. Quintuplet stimulation was with 5 pulses at the corresponding frequency delivered 2 times per second for 8 s. Potentiation was estimated from curve-fitting twitch contractions during 5 min of recovery using the equation \( F_t = F_a + F_p \times e^{-bt} \), where \( F_t \) is active twitch force at any time \( t \) (in min) after the repetitive stimulation, \( F_a \) is the active twitch force asymptote (as \( t \) approaches infinity), \( F_p \) is the increment of active twitch force above \( F_a \) which would occur at \( t = 0 \), and \( b \) is a rate constant. Potentiation was calculated as \( F_p/F_a \times 100 \). Time constant for exponential decay was estimated as \( 1/b \) and represents the time needed for active force to decrease from \( F_p \) to \( F_a \) in \( 1/e \) of \( F_p \), where \( e = 2.71828 \).
decay of potentiation was independent of the pattern of stimulation during the 8-s period (Table 2). These data provide support for the notion that all factors that may influence the twitch (positive and negative) were apparently independent of prior stimulation frequency and were not substantially different for doublets vs. quintuplets when 80 pulses were delivered in 8 s. This conclusion is further supported by the observation that the first response was potentiated with a similar time course regardless of the pattern of stimulation.

The time course of recovery of twitch force following a completely fused tetanic contraction is apparently faster (16, 19) than is evident here for twitch contractions following intermittent incompletely fused tetanic contractions. Considering that twitch amplitude has been reported to decrease to the pretetanic level by 120 s and to less than the pretetanic active force by 240 s after a tetanic contraction (19), the more rapid recovery may be a consequence of superimposed fatigue depressing the contractile response after a tetanic contraction. In our experiments, the twitch contraction did not decrease to an amplitude less than the control (pre-repeated stimulation) level during the 5-min recovery period. This observation supports the notion that fatigue was not a factor in the experiments reported herein. This is in contrast to the observations of Grange and Houston (12), who observed a depressed contractile response in human quadriceps muscles measured during brief stimulation at 10 Hz after a 60-s maximal voluntary contraction. This depression was followed by a subsequent enhancement of response at 10 Hz, showing a delayed potentiation, which was apparently unmasked by rapid recovery from fatigue. Grange and Houston did not see potentiation of contractions elicited with 20- or 50-Hz stimulation, but the fatigue that was evident may have masked potentiation that otherwise might have been evident.

Clearly, the magnitude of potentiation during repeated, intermittent brief contractions is independent of frequency of stimulation within the range of frequencies evaluated in this study when 80 pulses are delivered in 8 s. The double-pulse stimulation elicited considerable potentiation regardless of the frequency of stimulation. However, Fig. 5 illustrates that the magnitude of potentiation may be limited by the initial peak active force. This limitation is predictable, based on the possible mechanisms of potentiation. It is generally accepted that activity-dependent potentiation is primarily due to increased Ca\(^{2+}\) sensitivity of the contractile mechanism, resulting from regulatory light-chain phosphorylation. Skinned fiber experiments confirm that the magnitude of potentiation for a given level of regulatory light-chain phosphorylation decreases at higher levels of initial (before increases in regulatory light-chain phosphorylation) peak active force (25, 31). This is also consistent with increased potentiation, which is evident in intact cells in which Ca\(^{2+}\) release is depressed by either dantrolene (23) or fatigue (17), and with the observation that limited potentiation is observed at room temperature in mammalian muscle stimulated at relatively low frequencies of stimulation (32).

Further evidence that the magnitude of potentiation is dependent on the peak active force before the intervention can be seen in a paper by Brown and Loeb (6) in which substantial potentiation is reported for frequencies of stimulation from 30 to 43 Hz. These authors observed a fivefold increase in twitch active force, and in one example show a 50% enhancement of contractions in response to a 43-Hz train. This large twitch enhancement may be due to the low twitch-to-tetanus ratio (0.06) reported in that study.

Although the observations described above are consistent with the theory that potentiation results from increased sensitivity to Ca\(^{2+}\), they do not rule out other possible mechanisms. For example, enhanced Ca\(^{2+}\) release could also cause twitch potentiation and would permit greater enhancement at low initial Ca\(^{2+}\) concentrations than at high initial concentrations.

The literature provides us with knowledge concerning the impact of repetitive stimulation on myosin light-chain phosphorylation (14, 17, 20, 21) and the corresponding associated twitch enhancement (13, 19, 29). Assuming that the progressive rise in the first response represents enhanced twitch contraction due to regulatory light-chain phosphorylation and assuming that light-chain phosphorylation does not decrease during the quintuplet contractions, it can be concluded that the decrease in peak active force of the quintuplet contractions at 50 and 60 Hz from 0 to 1 s is due either to a transient decrease in peak activation (lower peak Ca\(^{2+}\) concentration) or to a fast-acting (on/off?) decrease in Ca\(^{2+}\) sensitivity. Known factors that could affect a decrease in Ca\(^{2+}\) sensitivity include increases in H\(^+\) concentration and inorganic phosphate concentration (10, 11). It seems unlikely that these factors would occur more rapidly or have a greater effect on contractions obtained with 50-Hz stimulation than on those obtained with 70- or 80-Hz stimulation; therefore, it is unlikely that there was a rapid decrease in Ca\(^{2+}\) sensitivity during these quintuplet contractions. Furthermore, it has been reported that this effect of low pH is decreased at physiological temperatures (24, 33). Therefore, it seems reasonable to conclude that the transient decrease in peak active force during the intermittent quintuplets at 50 and 60 Hz was due to transient failure to achieve as high an intracellular Ca\(^{2+}\) concentration during these contractions, compared with the initial contraction and possibly the later ones. This conclusion will need additional research to be verified, but a potential mechanism is proposed below.

The transient decrease in peak active force, which was observed with quintuplets at 50, 60, and 70 Hz, was not observed at other stimulation frequencies. If the theory presented above is correct (the transient decrease is due to decreased peak myoplasmic Ca\(^{2+}\) concentration), it would appear that this situation is avoided when the frequency of stimulation is above or below this range. It would appear from close examination of Fig. 3C that the decrease in peak active force at 1 s is associated with accelerated relaxation, leading to...
forces for quintuplet contractions certainly complicated the pattern of enhancement is different at different frequencies for voluntary motor unit recruitment strategies. It is known that repetitive stimulation results in decreased twitch half relaxation time and increased peak rate of relaxation (15, 18). The mechanism for the accelerated relaxation is not known, but, if it is due to faster uptake of Ca$^{2+}$ by the sarcoplasmic reticulum, a lower peak myoplasmic Ca$^{2+}$ concentration would occur.

The ratio of peak active force to first response provides additional evidence that Ca$^{2+}$ transients change during the sequence of repetitive stimulation, at least at 50 and 60 Hz. Assuming that myosin light-chain phosphorylation is the primary factor contributing to enhancement of the twitch (first response), it would be expected that peak active force would be enhanced to a lesser extent than the first response. This would occur because myosin light-chain phosphorylation is thought to alter the sensitivity to Ca$^{2+}$ (13, 25) and therefore has a greater relative effect at low levels of activation than at high levels of activation. However, because the ratio of the peak active force to first response decreased over the first second and then did not change further, whereas the first response continued to rise in amplitude over this time, we suggest that something in addition to phosphorylation of the regulatory light chains contributes to the pattern of change in peak active force.

These experiments have confirmed the following hypothesis: potentiation was evident over a broad range of stimulation frequencies, and the magnitude of potentiation decreased as the peak of the force-frequency relationship was approached. Considering that voluntary recruitment of motor units occurs with frequencies that elicit incompletely fused tetanic contractions, these observations have important implications for voluntary motor unit recruitment strategies. When potentiation is increasing, motor unit derecruitment or decreased firing frequency must occur if the task requires repeated submaximal isometric contractions with a specific target force. Alternatively, if duration of contraction was not an issue, voluntary activation could reach the same target force by using fewer pulses per train, as activity-dependent potentiation enhances the response per activating pulse. The fact that the pattern of enhancement is different at different frequencies for quintuplet contractions certainly complicates this situation.

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