Decreasing stimulation frequency-dependent length-force characteristics of rat muscle

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Decreasing stimulation frequency dependent length force characteristics of rat muscle. J. Appl. Physiol. 77(5): 2115–2124, 1994.—Effects of decreasing stimulation frequency on length-force characteristics were determined for rat medial gastrocnemius muscle. The peripheral nerve was stimulated supramaximally with a succession of twitch and frequencies of 100, 50, 40, 30, and 15 Hz. Active peak tetanic and twitch forces and active muscle geometry were analyzed. Optimal muscle length and active slack length shifted significantly (P < 0.05) to higher muscle length by a maximum of 2.8 and 3.2 mm, respectively. Further significant effects were found for distal fiber length and mean sarcomere length of distal fiber (increases) and for fiber angle and aponeurosis length (decreases). Neither muscle length range between active slack and optimal length nor aponeurosis angle was altered significantly. We concluded that decreasing stimulation frequency-dependent length-force characteristics are affected by a complex interaction of length-dependent calcium sensitivity, potentiation of the contractile system, distribution of sarcomere length, and interactions between force exerted and aponeurosis length. Length-dependent calcium sensitivity seems to be a major factor determining the magnitude of the shift of optimal muscle length.

skeletal muscle; medial gastrocnemius muscle; muscle geometry; isometric contraction; electrical stimulation; peripheral nerve; calcium sensitivity; distribution; sarcomere length; potentiation

MUSCLE LENGTH is a mechanical factor modifying muscle force. The interaction between muscle length and force is expressed in length-force characteristics. Investigations dealing with muscle function often study length-force characteristics of maximally activated muscle. In those conditions development of force is achieved by recruitment of all motor units. Another important neurophysiological factor affecting muscle force is stimulation frequency. In 1930 Cooper and Eccles (10) already showed the mechanical response of several cat muscles to stimulation frequencies. Rack and Westbury (26) demonstrated the effects of interaction between muscle length and stimulation frequency on length-force data of fully excited cat soleus muscle, known as stimulation frequency-dependent length-force characteristics. In these studies, discrete constant stimulation frequencies were applied to the nerve. However, variable motoneuron firing frequencies are encountered during muscle activity. For example, during maximal voluntary contractions the initial firing frequency of motor units is high and declines as the contraction sustains (13).

To adjust muscle force in a way more comparable to daily activity, Solomonov et al. (28) reintroduced an electrical stimulation technique that provided submaximal contractions levels by using simultaneous classic 100–120Hz stimulation at the proximal end of the motor nerve and a high-frequency block (600 Hz) at the distal end of the nerve. This stimulation method supplies selective activation of motor units by the size principle: according to their size, small motor units are recruited first and derecruited last and large motor units are recruited last and derecruited first (3). Recently, Huijing and Baan (22) used this stimulation with high-frequency block to study stimulation level-dependent length-force characteristics of rat medial gastrocnemius muscle (GM). Their results showed a shift in optimal muscle length to higher muscle length during blocking, which could be a consequence of different properties of derecruited motor units: it was hypothesized that this shift may be attributed to distribution of sarcomere length with respect to muscle length. However, part of the shift in optimal muscle length could be attributed to stimulation frequency-dependent effects on the length-force characteristics if a lowering of stimulation frequency were caused by the nerve block. Therefore we decided to use a stimulation pulse train with successively decreasing stimulation frequencies during an isometric contraction. Because such a sequence of decreasing firing frequencies also occurs during in vivo muscle activity (13, 19), a description of complex effects will also be relevant for understanding muscle performance during in vivo movement.

The major purpose of the present study was to examine length-force characteristics of rat GM at various decreasing stimulation frequencies during supramaximal stimulation of the peripheral nerve to determine the maximal effect of decreasing stimulation frequency on optimal muscle length. Furthermore, muscle geometry was analyzed to determine whether the interaction effect of decreased force and aponeurosis length contributes to alterations of length-force characteristics. A simple muscle model is presented incorporating distribution of mean sarcomere length of fibers. The purpose of this model is to show that in principle a shift in optimal muscle length during lowering of stimulation frequency can be related in part to the distribution of sarcomere length.

An attending phenomenon evoked by decreasing stimulation frequency is a temporal enhancement of muscle force at low frequencies. Well-known enhancement phenomena are posttetanic potentiation (9) and the catchlike property of skeletal muscle (7). Posttetanic potentiation refers to an augmentation of twitch force and unfused tetanic force during low-stimulation frequency after brief repetitive stimulation. The catchlike property is force enhancement evoked by brief initial high frequency (usually 2 spikes) preceding submaximal stimulation frequencies. Initial firing doublets have also been reported in human muscle (4). In our experimental de-
FIG. 1. Schematic presentation of rat gastrocnemius muscle (GM). Markers were inserted at proximal end of proximal aponeurosis (marker 1), distal end of proximal aponeurosis (marker 2), and distal end of most distal fiber (marker 3). Muscle geometry is represented as tringle with distance between markers 1 and 2 as estimate for active aponeurosis length ($l_{ma}$), between markers 2 and 3 for active fiber length ($l_f$), and between markers 1 and 3 for active muscle length ($l_{mus}$). Fiber angle ($\alpha$) and aponeurosis angle ($\beta$) were calculated according to law of cosines.

creasing stimulation frequency protocol, any force enhancement is manifested mainly in the later part of the contraction and is referred to as potentiation. An additional experiment was performed with both decreasing and constant stimulation frequency protocols to illustrate some unraveling of the complex interaction between stimulation frequency and potentiation.

MATERIALS AND METHODS

Surgical procedure. Six male Wistar rats [mean body mass 301 ± 4 (SE) g] were anesthetized with pentobarbital sodium (initial dose 0.05 g/kg body mass) injected intraperitoneally. Supplementary doses were given intraperitoneally if anesthesia became less deep. The skin of the hindlimb and the surrounding tissue was removed, exposing the medial head of the GM with the blood supply intact. The ischiadic nerve was dissected free and was cut as proximally as possible.

The calcaneus was cut, and the GM was separated from its lateral head and the soleus muscle. The Achilles tendon, attached to a piece of calcaneal bone, was wrapped around a metal hook and was secured with a ligature. In addition, the connection was glued with histoacryl (Braun Melsungen). Subsequently, the hook was connected to a force transducer (Hottinger Baldwin, maximal output error <0.1%, compliance 0.0048 mm/N). The femur was clamped and positioned in a rigid frame such that the muscle was aligned with the force transducer, avoiding torsion of the muscle.

Under observation with an operation microscope (magnification ×10–16; Carl Zeiss) and with help of a small needle, copper wire markers (diam 0.1 mm) were inserted in the muscle at three locations: the proximal end of the proximal aponeurosis (marker 1), the most distal end of the proximal aponeurosis (marker 2), and the distal end of the most distal fiber bundle (marker 3) (Fig. 1).

The muscle was covered with a layer of paraffin oil to prevent it from drying. A thermosensor positioned under the muscle measured muscle temperature, and with a feedback control system using radiant heat the ambient temperature was kept constant at 27 ± 1°C.

Experimental procedure. The ischiadic nerve was stimulated supramaximally using a bipolar silver electrode (constant current 3 mA, width square pulse 100 μs). The distance between the electrodes was 3 mm. A stimulation frequency protocol with variable pulse train was used to achieve gradation in muscle force. The peripheral nerve was stimulated at the following frequencies: twitch and 100, 50, 40, 30, and 15 Hz. A typical force trace resulting from the decreasing stimulation frequency protocol is shown in Fig. 2.

Isometric contractions were performed at different muscle lengths starting near active slack length (a low muscle length at which active muscle force approached 0). The muscle-tendon complex was lengthened with 1.0-mm increments. In the range just below and over optimal length, 0.5-mm increments were used. After lengthening of the muscle to the desired length, the measurements started with recording of passive muscle force during 100 ms in which time the muscle-tendon complex adjusted to the new length (Fig. 2). Subsequently, a twitch contraction was induced; 250 ms after the onset of the twitch contraction, the nerve was stimulated with a succession of five frequencies in decreasing order. For each stimulation frequency, a pulse train of 200 ms was used. After the stimulation sequence, the muscle was brought near slack length. The muscle was allowed to recover for 3 min, and the stimulation protocol was repeated at a different muscle length.

An additional experiment was carried out using the varying stimulation frequency protocol as well as four constant stimulation frequencies (15, 30, 40, and 50 Hz). First, isometric contractions were performed with the decreasing stimulation sequence described above. Subsequently, length-force data were obtained from tetani evoked by a constant stimulation frequency of 15 Hz in a muscle length sequence from low length to a higher length. This procedure was repeated for 30, 40, and 50-Hz stimulation in all cases. The duration of the constant stimulation frequency pulse train was equal to that of the decreasing stimulation frequency pulse train. The magnitude of the force was determined at instants identical to those of the decreasing stimulation frequency protocol (Fig. 2). Recovery time between the isometric contractions was 3 min for the decreasing stimulation frequency sequence and 2 min for the constant stimulation frequencies experiments.

Images of the muscle were recorded with cinemagraphy at 24 frames/s using a 16-mm camera (Arriflex 16SR) with a zoom lens (aperture 10–150 mm). A special-purpose microcomputer controlled timing of events related to generation of the stimulation pattern as well as analog to digital conversion and cinemagraphy.

Data collection and treatment. Muscle force and cinemographic synchronization signals were acquired using a 12-bit

FIG. 2. Example of time-force data of isometric contraction of rat GM. Stimulation protocol used for each measurement is indicated. Muscle force ($F_m$) and geometry were analyzed at instants marked with asterisk. Note that contractions became less fused at stimulation frequency of <50 Hz.
analogue-to-digital converter with a 1,000-Hz sampling frequency (resolution of force <0.05 N). Signals were stored using a second microcomputer.

Images obtained by cinematography were projected onto a translucent screen (magnification X4–5). On the screen, appropriate distances between the markers were measured using a motion analyzer (accuracy 0.05 mm; Dynamic Frame) and were taken as estimates for the proximal aponeurosis length (distance between markers 1 and 2), distal fiber length (distance between markers 2 and 3), and muscle length (distance between markers 1 and 3) (Fig. 1). Note that any effect of curvature of fiber and aponeurosis is neglected by this method. The fiber angle (α) and aponeurosis angle (β) with respect to the line of pull of the muscle were calculated according to the law of cosines.

Active muscle force was estimated by subtracting passive force from total force exerted by the muscle. Data for muscle active force (i.e., peak force) and muscle length data were fitted with a polynomial

\[ y = c_0 + c_1 x + c_2 x^2 + c_3 x^3 + \cdots + c_n x^n \quad (1) \]

where \( y \) represents active muscle force, \( x \) represents active muscle length, and \( c_0, \ldots, c_n \) are fitting constants. Polynomial curve fitting according to Eq. 1 was also used for data concerning the relationships between muscle length, fiber length, and active muscle force. In this case \( y \) represents one of the before-mentioned parameters and \( x \) represents active muscle length. Polynomials that most adequately described the experimental data were selected (see Statistics).

Optimal muscle length was defined as the active muscle length at which the fitted curve for each frequency showed optimal active muscle force within the muscle length range used in the experiment. Muscle slack length was defined as the least active muscle length at which the fitted curve for each frequency approached zero active muscle force. Maximal optimal muscle force was defined as optimal active muscle force for 100-Hz stimulation. Fitted curves were used to calculate mean values of active muscle force, fiber and aponeurosis lengths and angles, and mean sarcomere length of distal fibers for a given muscle length.

Force-frequency relationships were obtained by fitting data for normalized muscle force and stimulation frequency with use of a nonlinear least-squares fitting procedure. The equation fitted to the data was

\[ \frac{y}{y_{\text{max}}} = e^{b_1 + b_2 x} \quad (2) \]

where \( y \) represents active muscle force and \( y_{\text{max}} \) represents maximal active muscle force, both at a given muscle length; \( x \) represents stimulation frequency; and \( b_1 \) and \( b_2 \) are constants selected in the fitting procedure.

Number of sarcomeres. After the length–force measurements, the GM was removed carefully and was fixed (4% formaldehyde, 15% absolute alcohol, and 1.5 mg/l of thymol) for several days. The muscle was kept near optimal length (100 Hz). Subsequently, the muscles were exposed for 4 h to a 20% nitric acid solution to soften the connective tissue, after which they were stored in a 50% glycerol solution for 2–6 days. Under observation through a dissection microscope, the most distal fiber bundle was taken from the muscle. From this bundle, four intact fibers were isolated and prepared on a microscope slide. The number of sarcomeres was determined according to Huijing (20): the number of sarcomeres was counted in 80-μm samples every 800 μm along the length of the fiber. This method provided estimates of mean treated sarcomere length of the distal fiber. Subsequently, the length of the treated fiber was measured using a curvimeter on a projection of the slides (magnification ×24; overhead projector). The mean number of sarcomeres in a fiber was calculated by dividing the treated distal fiber length by the mean sarcomere length of that fiber.

Optimal sarcomere length was defined as that sarcomere length at which force production is expected to be maximal on the basis of optimal overlap between thick and thin filaments. Optimal sarcomere length was obtained from filament parameters electromicroscopically for rat GM (17). Optimal fiber length was estimated by the product of number of sarcomeres and optimal sarcomere length.

Statistics. One-way analysis of variance (ANOVA) (25) was used to select the lowest order of the polynomials for the fitting of the relationships between muscle length, muscle force, and fiber and aponeurosis lengths and angles. One-way ANOVA for repeated measurements was performed to test for the effect of decreasing stimulation frequency on optimal muscle force, optimal muscle length, and active slack length and muscle length range between optimal and slack length and to test for a possible difference between fiber length at optimal sarcomere length and fiber length at optimal muscle length. Two-way (muscle length and stimulation frequency) ANOVA for repeated measurements was used to test the effect of decreasing stimulation frequency on muscle force, fiber and aponeurosis lengths and angles, and sarcomere length in the muscle range between 27.3 and 34.9 mm. Post hoc tests among factor level means were performed using the Bonferroni procedure for multiple pairwise comparisons to locate differences among stimulation frequency with respect to 100-Hz stimulation. A difference at \( P < 0.05 \) was considered significant. Data and fitted curves are presented as means ± SE.

RESULTS

Muscle length-force characteristics. Length-force characteristics of rat GM are shown in Fig. 3. Decreasing stimulation frequency was attended by a decline in active muscle force together with a shift in optimal muscle length to a higher muscle length (Fig. 3A). Note that the progressive shift in optimal muscle length with decreasing stimulation frequency was accompanied by a similar shift in active slack length.

The optimal muscle force ranged from 10.10 N for 100 Hz to 2.57 N for twitch (Table 1). One-way ANOVA for repeated measurements showed a significant effect of decreasing stimulation frequency on optimal muscle force \([F(5,25) = 204.41]\). Post hoc tests showed that optimal muscle force for stimulation frequency of ≤50 Hz was significantly decreased with respect to 100-Hz stimulation. Optimal muscle lengths were observed in the muscle range of 32.11 mm for 100 Hz to 34.89 mm for 15-Hz stimulation, which is an increase of 9% relative to optimal muscle length at 100 Hz (Table 1). Estimates of active slack length were observed in the muscle range of 21.88 mm for 100 Hz to 25.05 mm for twitch stimulation, which is an increase of 14% relative to slack length at 100 Hz (Table 1). One-way ANOVA for repeated measurements showed a significant effect of decreasing stimulation frequency on optimal muscle length and muscle slack length \([F(5,25) = 91.4 \text{ and } 33.0, \text{ respectively}\). Post hoc testing showed that optimal and slack length for ≤40-Hz stimulation were significantly higher with respect to 100-Hz stimulation.

Two-way ANOVA for repeated measurements revealed a significant effect of decreasing stimulation frequency on muscle force \([F(5,265) = 533.2]\). No significant interaction was found between muscle length and stimu-
optimal muscle length and muscle slack length are shifted in such a way that no change in muscle length range between optimal and slack lengths is proved.

To evaluate the effects of potentiation on the length-force characteristics, we compared effects of constant stimulation frequencies with the decreasing stimulation frequency protocol in an additional experiment. Potentiation highly enhanced muscle force at muscle lengths below optimal muscle length (Fig. 4A). For low-stimulation frequencies (15, 30, and 40 Hz), muscle force was enhanced up to 850%. At a muscle length in the range of optimal muscle lengths, potentiation was drastically reduced. Although potentiation was less in this optimal muscle length range, it was still maintained, ranging from 42 to 3% for 15- and 50-Hz stimulation frequency, respectively. A striking difference was shown between decreasing and constant stimulation frequencies in optimal muscle length (Fig. 4B). For 15- and 30-Hz decreasing stimulation frequencies, optimal muscle length was lower than that obtained during constant stimulation frequency. However, for 40-Hz stimulation optimal muscle length was very similar for the two protocols, and 50-Hz constant stimulation frequency yielded a lower optimal muscle length.

We concluded that, because of the decreasing stimulation frequency protocol, muscle force is potentiated, especially at a muscle length below optimal muscle length, whereas at higher muscle length potentiation is present but considerably smaller. Moreover, potentiation affects optimal muscle length.

**Fiber and aponeurosis lengths and angles.** The relationships between the muscle length and the fiber and aponeurosis lengths and angles are shown in Fig. 5. Although two-way ANOVA for repeated measurements showed a significant effect of decreasing stimulation frequency on fiber length \( F(5,265) = 10.2 \), differences in fiber length from that of 100-Hz stimulation could be located only by post hoc testing for 15-Hz and twitch stimulation (Fig. 5A). Within the range of optimal muscle length, the increase in fiber length at 15-Hz stimulation was limited to \( 0.12 \pm 0.19 \) mm with respect to 100-Hz stimulation. We concluded that decreasing stimulation frequency caused significant lengthening of the fiber.

The effect of stimulation frequency on the aponeurosis

![Image](image_url)

**FIG. 3.** Effect of stimulation frequency on length-force characteristics of rat GM. A: active \( F_{\text{mao}} \) (\( F_{\text{mao}} \)) as function of \( l_{\text{mao}} \). B: \( F_{\text{mao}} \) as function of deviation in optimal \( l_{\text{mao}} \) (\( \Delta l_{\text{mao}} \)). Fitted curves of \( F_{\text{mao}} \) are represented as means ± SE \( n = 6 \) for 6 frequency conditions. Vertical dashed lines, optimal \( l_{\text{mao}} \) (\( l_{\text{mao}} \)) range (\( A \)) or \( l_{\text{mao}} \) (\( B \)).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>( F_{\text{mao}, \text{opt}} )</th>
<th>( l_{\text{mao}, \text{opt}} )</th>
<th>( l_{\text{mao}, \text{slack}} )</th>
<th>( \Delta l_{\text{mao}} )</th>
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<tbody>
<tr>
<td>100 Hz</td>
<td>10.10±0.58</td>
<td>32.11±0.52</td>
<td>21.88±0.38</td>
<td>-10.23±0.38</td>
</tr>
<tr>
<td>60 Hz</td>
<td>9.03±0.57</td>
<td>32.41±0.56</td>
<td>24.41±0.50</td>
<td>-10.01±0.32</td>
</tr>
<tr>
<td>40 Hz</td>
<td>7.51±0.62</td>
<td>32.78±0.58</td>
<td>23.27±0.53</td>
<td>-9.51±0.20</td>
</tr>
<tr>
<td>30 Hz</td>
<td>6.02±0.64</td>
<td>33.73±0.54</td>
<td>25.51±0.47</td>
<td>-10.22±0.35</td>
</tr>
<tr>
<td>15 Hz</td>
<td>2.82±0.29</td>
<td>34.30±0.48</td>
<td>24.38±0.35</td>
<td>-8.91±0.36</td>
</tr>
<tr>
<td>Twitch</td>
<td>2.57±0.37</td>
<td>34.30±0.44</td>
<td>25.03±0.59</td>
<td>-9.28±0.34</td>
</tr>
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Values are means ± SE; \( n = 6 \). \( F_{\text{mao}, \text{opt}} \), optimal active muscle force; \( l_{\text{mao}, \text{opt}} \), optimal active muscle length; \( l_{\text{mao}, \text{slack}} \), active muscle slack length; GM, gastrocnemius muscle. * Significant difference (1-way analysis of variance for repeated measurements using Bonferroni post hoc test, \( P < 0.05 \)) with respect to 100-Hz stimulation frequency.
The relationship between mean sarcomere length of the distal fiber and muscle length is shown in Fig. 6A. Sarcomere length was estimated by dividing fiber length by the mean number of sarcomeres (5,877 ± 72; n = 24). Two-way ANOVA showed that stimulation frequency had a significant effect on sarcomere length [F(5,265) = 9.8]. Post hoc testing showed that sarcomere length at 15-Hz and twitch stimulation was significantly increased with respect to 100-Hz stimulation. Within the range of optimal muscle lengths, a range of sarcomere lengths was observed from 2.40 to 2.87 μm. It can be seen from Fig. 6B, a detailed section of sarcomere characteristics, that muscle length at optimal sarcomere length in the distal fiber (2.3 μm) was shifted to the left by ~0.11 and 0.17 mm for 15-Hz and twitch stimulation, respectively.

Estimated optimal fiber length (i.e., product of optimal sarcomere length and number of sarcomeres) was 13.52 ± 0.17 mm (n = 6) and was significantly smaller than fiber length at optimal muscle length at 100-Hz stimulation [14.12 ± 0.22 mm; n = 6; F(1,5) = 8.2]. This fiber length difference is an indication of distribution of mean sarcomere length of the fibers with respect to muscle length.

**Force-frequency relationship.** The effect of muscle length on the force-frequency curve is illustrated in Fig. 7. Normalized muscle force (i.e., muscle force relative to the maximal muscle force at a particular muscle length) decreases for submaximal stimulation frequencies as the muscle shortens. It should be noted that the magnitude of this decrease is determined by two factors: 1) the decrease in force due to the transfer to a length-force curve of a lower stimulation frequency and 2) the magnitude of the shift in optimal muscle length of that curve to higher lengths (Fig. 3A). The latter factor particularly creates the interaction with muscle length: at higher muscle lengths the decrease in force may be less than expected, and at lower muscle lengths the decrease in force will be higher than expected. It should be noted particularly that the shift in optimal muscle length is the result of a complex interaction of several factors, which is analyzed in **DISCUSSION**.

The fitting constants for the sigmoid muscle force-stimulation frequency curves describing the experimental data are shown in Table 2, as this information may be quite useful for muscle modeling.

**DISCUSSION**

In this study we showed that length-force characteristics of fully recruited rat GM muscle are affected by a protocol of decreasing stimulation frequency. An impor-
tant feature of the decreasing stimulation frequency dependent length-force characteristics is the reciprocal relationship between stimulation frequency and optimal muscle length (Fig. 4B).

In natural movements, decreasing firing frequencies are very common. For example, Hoffer et al. (19) showed recordings of in vivo motor unit firing frequencies in walking cats and demonstrated that decreasing firing frequencies generally occur. Therefore, it is essential to have two types of experimental evidence: 1) evidence of the net effects of decreasing stimulation frequency, as provided in the present study, involving possible interaction between stimulation frequency effects and potentiation and 2) detailed studies on the isolated effects of these phenomena to quantify the importance of individual effects and possible interactions for the net effect.

Below we focus the discussion on such effects. However, other phenomena, such as effects of changes in muscle geometry and effects of possible sarcomere length distribution with respect to muscle length, must be considered as well for a more detailed analysis of muscle functional capabilities during submaximal activation.

**General effects of decreased force: interaction between fiber and aponeurosis lengths and angles.** Decreasing stimulation frequency causes muscle force to decrease. Considering the fact that aponeurosis and fiber are connected in series and given the elastic properties of the aponeurosis, any decrease in force will cause a shortening of the aponeurosis. For any given muscle length a shortening aponeurosis will cause an increased fiber length. Furthermore, in pennate muscle angular effects can reduce fiber lengthening by changing the fiber angle. Hence, the effect of decreasing force on fiber length is determined by a complex interaction between muscle force, elastic properties of intramuscular aponeurosis, and angular effects (37). In the present study, we found small increases in fiber length as the stimulation frequency was lowered and the force dropped. As a consequence, optimal sarcomere length should be reached at shorter muscle length (leftward shift of ~0.11-0.17 mm).

This is expected to happen to all fibers and leads to a shift in optimal muscle length to lower muscle length as muscle force declines with decreasing stimulation frequencies. However, the experimentally determined length-force characteristics show a net shift to increased muscle length, i.e., in the opposite direction. Therefore,
LENGTH-FORCE CHARACTERISTICS OF RAT MUSCLE

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15 Hz
20 25 30 35 40
*loo Hz *50 Hz *40 Hz
B
*30 Hz -0-15 Hz -twitch

FIG. 6. A: actual sarcomere length (l), as function of lmax. Optimal lmax (lmax: 2.3 \mu m), lmax of 100-Hz stimulation (32.11 mm), and lmax of 15-Hz stimulation frequency (34.89 mm) are indicated by horizontal dashed line and 2 vertical dashed lines, respectively. Box indicates section enlarged in B. B: detail of lmax-lmax relationship. Note that lmax is reached at muscle length lower than lmax. Fitted curves of lmax for 6 frequency conditions are represented as means ± SE (n = 6).

these fiber length changes cannot explain the stimulation frequency-dependent length-force characteristics found experimentally in this study.

Effects of stimulation frequency per se. A dependence of optimal muscle length on stimulation frequency was already reported for cat soleus muscle by Rack and Westbury (26): a higher optimal muscle length was found for lower stimulation frequencies. Constant stimulation frequency of 5 and 35 Hz shifted optimal muscle length ~5.0 mm. Our additional experiment showed qualitatively similar effects for rat GM (Fig. 4B). Optimal muscle length shifted to a maximal of ~4.0 mm (i.e., optimal muscle length difference between 15- and 50-Hz constant stimulation frequency). Rack and Westbury hypothesized that such effects could be related to alterations of the structure of the T tubules at short muscle length, leading to decreased inactivation of the contractile elements. However, an important factor that should be regarded is the functional consequence of changes in calcium concentration within the fibers. Calcium is the intracellular messenger linking membrane depolarization of the T tubules to activation of the contractile elements, i.e., actin and myosin filaments. Decreasing stimulation frequency leads to fewer action potentials conducted by the peripheral nerve. This reduces the frequency of membrane depolarization of the T tubules. Consequently, the release of calcium from the sarcoplasmic reticulum into the sarcoplasm decreases and influences force development (5, 24). In addition, length-dependent calcium effects are well known in skeletal and cardiac muscle (e.g., see Refs. 11, 31, 32). These sarcomere length effects have important consequences. Stephenson and Wendt (30) showed in rat extensor digitorum longus muscle skinned fibers that sarcomere optimal length shifts ~0.5 \mu m to higher sarcomere lengths at the 20% level of full calcium activation. Several other experimental observations support such a shift in optimal sarcomere length. In frog muscle, Close (8) showed that optimal sarcomere length for twitch stimulation is shifted to higher sarcomere length with respect to tetanic stimulation. Furthermore, decreasing calcium concentration and thus activation levels achieved by administration of

TABLE 2. Fitting constants of force-frequency relationships of rat GM for five muscle lengths

<table>
<thead>
<tr>
<th>Muscle Length, mm</th>
<th>b₀</th>
<th>b₁</th>
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<tbody>
<tr>
<td>26</td>
<td>-4.49</td>
<td>0.11</td>
</tr>
<tr>
<td>28</td>
<td>-3.47</td>
<td>0.10</td>
</tr>
<tr>
<td>30</td>
<td>-2.99</td>
<td>0.10</td>
</tr>
<tr>
<td>32</td>
<td>-2.47</td>
<td>0.09</td>
</tr>
<tr>
<td>34</td>
<td>-2.11</td>
<td>0.09</td>
</tr>
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</table>

b₀ and b₁, values of fitting constants of Eq. 2.
dantrolene led to higher optimal muscle lengths in mouse extensor digitorum longus muscle (33). Dantrolene is a drug that interferes with the release of calcium from the sarcoplasmic reticulum. For skinned rabbit gracilis muscle fibers, optimal sarcomere length is shifted ~0.4 μm when the fibers are exposed to a low-calcium medium (11). Thus, optimal sarcomere length of skinned fibers as well as intact fibers depend on calcium activation level. In addition, sarcomere length at which the ascending limb of the sarcomere length-force relationship reaches zero force is increased for a lower calcium concentration in rabbit skinned muscle fibers (1). If we assume that the shift in optimal sarcomere length (0.5 μm; Ref. 30) found for the 20% calcium level is comparable to our low-stimulation frequencies (15 Hz and twitch), we can estimate the magnitude in optimal muscle length shift that can be ascribed to this phenomenon. If we substitute that value in Fig. 6A, we see that for an increase in sarcomere length from 2.3 to 2.8 μm the muscle length would be increased by ~3.0 mm. Note that the magnitude of the calcium effect is on the same order as the total net effect found experimentally. Therefore, we can conclude that the calcium effect is a major contributor to the shift in optimal muscle length.

Effects of distribution of mean sarcomere length with respect to muscle length. A striking observation in this study is that optimal sarcomere length is reached in distal fibers at muscle length lower than optimal length for 100-Hz stimulation (~0.6 mm below optimal muscle length for 100-Hz stimulation; Fig. 6B). In contrast, other investigations in our laboratory showed that optimal sarcomere length was reached at muscle lengths higher than optimal muscle length (18, 38). Because large individual variation was reported for the same muscle from different animals (34), these contrasting findings could well be related to individual variation. For maximally active muscle, any deviation in sarcomere length at optimal muscle length from optimal sarcomere length is taken as an indication of a distribution of mean sarcomere lengths of the fibers of a muscle with respect to muscle length (18, 21, 34, 38).

For rat GM, the existence of a distribution of sarcomere length is supported by experimental data obtained from twitch and tetanic contractions of motor units (16). Such evidence is also available for cat muscles (2, 23, 29). In several muscles the muscle length at which motor unit optimal length occurs is related to motor unit type (2, 14). Larger and faster units seem to exert their optimal

FIG. 8. Modeled effect of distribution of $l_m$ with respect to $l_{max}$ on $l_{max}$. A: force-frequency relationships for slow- and fast-type motor units (MU) [according to Einsiedel and Luff (12)]. B: high stimulation frequency activates all fiber populations maximally. C: low stimulation frequency reduces force of fiber populations with short optimal $l_m$ (i.e., short $l_m$). D: muscle force-length curve estimated by linear summation of fiber force ($F_f$) for high stimulation frequency (high SF) and low stimulation frequency (low SF). Muscle length ($\Delta l_m$) is function of deviation from active slack length. Note that with decreasing stimulation frequency $F_m$ is reduced and $l_{max}$ is shifted to higher muscle length.
force at lower muscle lengths than do smaller and slower units. Faster motor units are more susceptible to a decrease in force because of lowering stimulation frequency than are slower motor units (12, 16), as illustrated in Fig. 8A. Interaction between this phenomenon and the size-related distribution of sarcomere length should lead to effects on the muscle length at which optimal muscle force is exerted. To illustrate this effect, we modeled a parallel fibered muscle. The constructed parallel fibered muscle consisted of several groups of fibers with distributed sarcomere length with respect to muscle length (Fig. 8B). We assigned fiber populations with low optimal fiber length (i.e., low sarcomere length) to fast-twitch motor units and populations with high optimal fiber length (i.e., high sarcomere length) to slow-twitch motor units. Because the whole fiber population is recruited at a high stimulation frequency, fiber force is maximal for all fibers (Fig. 8B). At low stimulation frequency, all fibers are also recruited; however, fiber force of fast-twitch units is reduced (Fig. 8C). Muscle force is obtained by linear summation of fiber forces. For high and low stimulation frequency, this is illustrated in Fig. 8D. Note that low stimulation frequency shifts the optimal muscle length to higher muscle length. Note also that the active slack length is not shifted, resulting in an increased muscle range between optimal and slack lengths.

Effects of potentiation. Because of the short successive sequence of decreasing stimulation frequencies, muscle force is potentiated during low frequencies in our experiments. In several preparations of cat and rat muscles, force enhancement was demonstrated for whole muscle (6, 9) and motor units (7, 27). Muscle force potentiation can be ascribed to posttetanic potentiation, the catchlike property of the skeletal muscle, or both. Posttetanic potentiation decays exponentially (time course in minutes) in rat fast-twitch muscle (9). Burke et al. (7) showed that the catchlike property is rather diverse in cat motor units. The response to the initial high frequency followed by submaximal frequencies ranged from 500 ms to 5 s for fast-twitch and slow-twitch GM motor units, respectively. Considering the difference in time constants of these potentiation effects [minutes vs. (milli)seconds], it is likely that two separate mechanisms may be active. It is conceivable that both mechanisms affected our results to some extent. To our knowledge, no data are available on the potentiation effects on length-force characteristics of rat GM. Our additional experiment indicates that effects of potentiation, as apparent from differences between the results of the decreasing stimulation and constant stimulation frequency protocols, may be substantial. For example, a difference in the magnitude of the maximal shift in optimal muscle length to higher muscle length was encountered (Fig. 4B). Therefore, potentiation per se is likely to cause a shift in optimal length to lower muscle lengths. Further experimental work is indicated to illuminate the effects of potentiation on muscle length-force characteristics.

Functional consequences for in vivo movement. In this study it is shown that in situ length-force characteristics are related to the frequency of stimulation. The functional significance of this finding for in vivo daily activity needs to be addressed. The combination of the results of Woltjer et al. (35) relating muscle length change to joint angle changes and of Gruner et al. (15) describing hindlimb joint angles during in vivo locomotion indicates that the muscle length range studied in the present study agrees with the length range encountered during rat locomotion. During the stance phase, the muscle length range is relatively small and is below or near the optimal muscle length range as determined in this study. In contrast, the greatest length change is encountered during the swing phase with muscle lengths ranging from below to well above optimal muscle length (i.e., optimal muscle length range). Therefore, our results have some significance for in vivo movements. In general, our results indicate that if muscle shortening were accompanied by decreasing firing frequencies it would mean that, for the length range below optimal muscle length range, the resulting drop of force would be larger than expected without these frequency effects. It should be noted that these firing frequency effects are not usually included in modeling of human movement (36). The additional decrease in force would allow a greater release of energy stored in elastic tendons and aponeuroses. During in vivo movements, stretch is often accompanied by increasing activation (due to both increased recruitment and firing frequency). Electromyographic records of the rat step cycle show that electrical activity is very high during stretch at the beginning of the stance phase (i.e., E2 phase with eccentric muscle action) (15). The subsequent shortening phase (E3 phase with concentric muscle action) showed moderate to low electromyographic activity. Thus, our findings could indicate that more elastic energy would be released.

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