Stretch-induced enhancement of mechanical work production in frog single fibers and human muscle

Yuichi Hirano, and Naokata Ishii. Stretch-induced enhancement of mechanical work production in frog single fibers and human muscle. J. Appl. Physiol. 83(5): 1741-1748, 1997.—The relations between the velocity of prestretch and the mechanical energy liberated during the subsequent isovelocity release were studied in contractions of frog single fibers and human muscles. During isometric contractions of frog single fibers, a ramp stretch of varied velocity (amplitude, 0.02 fiber length; velocity, 0.08–1.0 fiber length/s) followed by a release (amplitude, 0.02 fiber length; velocity, 1.0 fiber length/s) was given, and the amount of work liberated during the release was measured. For human muscles, elbow flexions were performed with a prestretch of varied velocity (range, 40°; velocity, 30–180°/s) followed by an isokinetic shortening (velocity, 90°/s). In both frog single fibers and human muscles, the work production increased with both the velocity of stretch and the peak of force attained before the release up to a certain level; thereafter it declined with the further increases of these variables. In human muscles, the enhancement of work production was not associated with a significant increase in integrated electromyogram. This suggests that changes in intrinsic mechanical properties of muscle fibers play an important role in the stretch-induced enhancement of work production.

cross bridges; prestretch; work output; eccentric contraction; countermovement

When contracting muscle is stretched (prestretch) and subsequently allowed to shorten, it exerts larger mechanical work than it does without prestretch. This effect of prestretch has been regarded as important in various movements of organisms, including movements of humans. Indeed, movements seen in vivo are often associated with the forced lengthening (biomechanically termed as eccentric action) before the shortening (concentric action) of muscles (11). However, studies with isolated muscle preparations and those on whole body movements have so far led to separate interpretations for the mechanisms underlying such an enhancement of mechanical performance.

Both whole muscle preparations and single muscle fibers of the frog maintain tension higher than that determined by their length-tension relations when they are stretched and kept at stretched lengths (1, 12, 13). Accordingly, releasing them either isotonically or isokinetically from the stretched state gives rise to larger velocity or force, respectively, resulting in an upward shift of the force-velocity relations (8, 13, 28). In particular, experiments with isometric releases followed by stretches of single fibers (13, 29) have shown the stretch-induced increase in steady-state shortening velocity, which was determined after a complete elastic recoil of the series elastic component (SEC). These studies strongly suggest that stretching the contracting muscle causes an improvement of the ability of the contractile element (actin-myosin cross bridges) to generate force, even though the molecular mechanism of such an improvement is not fully understood.

On the other hand, muscle contractions in situ are subjected to more complex mechanical environment. Muscles have much longer SEC than do single fibers. The major portion of SEC is located in the tendon and may play an important role in the storage of elastic energy and thereby make the movement energetically more efficient (2, 17). Because muscle fibers generate larger force (eccentric force) against the forced lengthening during isometric and isotonic contractions, it is plausible that the SEC stores an extra elastic energy through the course of lengthening and then liberates it during the subsequent shortening under smaller load (3, 10). However, it should also be noted that the overstretched SEC may diminish the length change of muscle fibers and consequently reduce the work done by the muscle fibers themselves (4, 15). In addition, the level of motor-unit recruitment can be modified by neural reflex; it can be either facilitated (stretch reflex) or depressed on stretch through actions of muscle proprioceptors.

Therefore, in human movement, the mechanical advantage of an eccentric counteraction (stretching the muscle) before a concentric action may be the enhancement related to various mechanical events occurring at various stages of contraction. However, mechanical advantage of eccentric counteraction has been so far interpreted mainly in terms of the role played by the SEC (3, 7, 22), although the enhancement of mechanical performance in the interacting cross bridges has become evident by studies with single fibers. In this particular context, it should be noted that the enhancement of the ability of single fibers to generate force has been shown to depend on the rate of prestretch in a manner that the enhancement is lowered with the increase in the rate of prestretch (26). Thus, in the present study, we applied a similar stretch-release protocol with varied stretch velocity to contractions of both frog single muscle fibers and human muscles (elbow flexors) to see how the stretch-induced enhancement of mechanical performance within muscle fibers is related to contractions in situ.

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METHODS

Preparation of frog single fibers. Single muscle fibers were dissected from the tibialis anterior muscles of Rana japonica in Ringer solution with the following composition (in mM): 115 NaCl, 1.8 KCl, 2.5 CaCl₂, 10 tris(hydroxymethyl)aminomethane-maleate, pH 7.2. A pair of small clips of aluminum foil (16) was tied to both tendons. The connectors were attached close to the fiber insertions, so that the length of tendinous material between the connectors was <0.1 mm.

Protocol for stretching and releasing the single fibers. A single-fiber preparation [4–6 mm at slack length (L₀)] was mounted horizontally in an acrylic chamber (3-ml volume) by attaching one of the connectors to a servomotor (model G-100PD, General Scanning) and the other to a force transducer (model AE-801, Aksjeselskapet). The compliances of force transducer and servomotor (including a lever arm) were 0.1 and 0.003 mm/N, respectively. The resonant frequency of the measurement system was ~5 kHz. The chamber contained a multielectrode assembly consisting of eight platinum wire electrodes, each with alternating polarity. In most experiments, cooled Ringer solution (2–3°C) was constantly circulated through the chamber at a rate of 2 ml/min with a peristaltic pump, and the temperature of the Ringer within the chamber was kept constant at 0 ± 0.1°C by a thermoelectric device (Coolinix, Yamato Kagaku). The sarcomere length of muscle fiber at rest was measured by diffraction of He-Ne laser (beam diameter, 1 mm). The resting fiber was stretched to the sarcomere length of 2.5 µm and then tetanized with a 1.1-s train of supramaximal rectangular current pulses (duration, 1 ms; frequency, 20 Hz). It was first kept isometric, and when the maximal isometric force was developed, a ramp stretch with varied velocity (amplitude, 0.02 L₀ velocity, 0.08–1.0 L₀/s), immediately followed by a ramp (isovelocity) release with a fixed velocity (amplitude, 0.02 L₀ velocity, 1.0 L₀/s), was applied with the servomotor. Because this velocity of release corresponded to ~0.3 Vmax (maximal unloaded velocity at 0°C), mechanical power generated during the release was expected to be around its maximum. Both the force and length signals were recorded with a digital oscilloscope (type 4094, Nicolet).

Subjects and apparatus for experiments with human muscles. The experiments were carried out with nine male volunteers, ages 22–29 yr. All subjects were informed about the experimental procedure to be utilized as well as the purpose of the study, and their informed consent was obtained. The testing movement was an elbow flexion. An isokinetic release after a prestretch was given during a maximal voluntary contraction by using a Kawasaki Myoret isokinetic dynamometer. The acceleration time required to attain the constant angular velocity was 50 ms at the highest velocity used in the present study (180°/s). Because the angular change during this period of time was ~9°, the amplitudes of both stretch and release were set at 40°, and data obtained for both ends by 10° within the whole excursion of 40° were discarded (Data analysis). Particular attention was paid to avoid any possible injury associated with the generation of large force during stretch, and measurements were stopped when a subject exhibited any sign of pain. The study was approved by the Ethics Committee for Human Experiments, University of Tokyo.

Protocol for stretching and releasing the human muscles. Subjects were familiarized with the testing procedure on several occasions before the experiment. They each sat on a chair, with their backs and arms upright and with their right forearms firmly attached to the lever of the Myoret. A pivot of the lever was correctly aligned with the rotation axis of the elbow joint, and the requisite axial alignment of joint and dynamometer axes was maintained during the movement. First, we examined the torque–elbow joint angle relations for each subject and determined the optimal angle for isometric torque generation (Aₒ) at an angular resolution of 10°. Then we determined the range of motion for each subject as Aₒ ± 20°, where the elbow angle was expressed as included angle, i.e., 180° at full extension. Elbow flexions were performed at the maximal effort, with or without short-range (40°) pre-stretch of varied velocities (30, 60, 90, 120, 150, 180°/s) followed by an isovelocity (90°/s) shortening. In the movement without prestretch (control), the maximal isometric contraction was performed at the largest elbow angle (Aₒ + 20°); after the torque reached a plateau, the isovelocity release was given. In the movement with prestretch, the stretch followed by a release was applied immediately after the rise of voluntary torque ended, so as to minimize the effect of fatigue. The subjects were allowed to rest for 5 min between each measurement. Major muscles involved in the elbow flexion are biceps brachii, brachialis, and brachioradialis, and their relative contributions have been shown to be 34, 47, and 19%, respectively (20). For the biceps brachii, an anatomic study of cadavers has shown that the change in elbow angle by 120° induces the length change of biceps brachii by ~0.7 L₀ (21), so that the distance of stretch and release used in the present study is estimated to be ~0.2 L₀, which is 10 times as large as that for frog single fibers. However, the velocities of stretch and release are estimated to be 0.18–1.1 and 0.54 L₀/s, respectively; both are comparable with those for frog single fibers.

Recording of electromyographic (EMG) activities. During force measurements, EMG signals were recorded simultaneously from the biceps brachii and from the lateral head of triceps brachii, an antagonist elbow extensor. Bipolar surface electrodes (5-mm diameter) were placed over the bellies of muscles, with a constant interelectrode distance of 30 mm. The EMG signals were amplified and fed into full-wave rectifier through both low (time constant, 0.03 s) and high (1 kHz) cut filters.

Data analysis. Collection, analysis, and storage of all kinds of signals were performed on a Fujitsu FM/V personal computer. Torque, angle, and EMG data were collected throughout the range of motion. The positive and negative work done by the muscle was determined for each contraction by integrating force with respect to length. The EMG signals were analyzed for the middle part of the range of motion, spanning 20°, and the data from both ends of the movement were discarded, because of the presence of artifacts associated with the acceleration and deceleration of the lever (Subjects and apparatus for experiments with human muscles). Integrated EMG (iEMG) was used to evaluate the degree of motor-unit recruitment (5, 6). Analysis of statistical significance was based on Student's paired t-test.

RESULTS

Enhancement of mechanical work in frog single muscle fibers. Figure 1 shows typical length and force records when a single fiber was tetanized isometrically, stretched by 0.02 L₀ with varied velocities (0.08–1.0 L₀/s), and then immediately released at a constant velocity (1.0 L₀/s). In unstretched control, it was first tetanized isometrically at the same fiber length as that attained after the stretch and then released in the same manner. The fiber responded to the stretch with the generation of extremely large force. In all the fibers examined, the force generated before release increased consistently with the velocity of stretch.
The work produced by the fiber during the release and the work done on the fiber externally during the stretch were measured from the force-length diagrams (Fig. 2) as positive and negative work, respectively. The values of both positive and negative work were normalized to the positive work produced during the release without prestretch and were plotted against the velocity of stretch for three muscle fibers examined (Fig. 3).

Enhancement of mechanical work in human muscles. When the isovelocity stretch-release protocol similar to that for frog single fibers was applied to contractions of human elbow flexors, responses qualitatively similar to those of frog single fibers were observed. Figure 5 shows representative length and force records. The muscles responded to the stretch with the generation of...
large eccentric force, and during the subsequent release, they retained a slightly higher level of force (Fig. 5B) than that during the release without stretch (unstretched control, Fig. 5A).

As in the case with the frog single fibers, both positive and negative work production was measured from the force-length relations (Fig. 6), normalized to the positive work produced in the unstretched control, and plotted against the velocity of stretch (Fig. 7). Although both the amount of negative work and the degree of the enhancement of positive work were relatively small, their dependencies on the velocity of stretch were substantially similar to those in frog single fibers. The positive work was significantly ($P < 0.05$) larger than that in the unstretched control at stretch velocities of 60, 90, 120, and 150°/s and was maximal at 90°/s (0.54 L_o/s for biceps brachii).

When the positive work was plotted against the eccentric force attained before release, it showed a sharp peak at $-1.25 P_o$, where $P_o$ is the isometric force generated in contractions without stretch (Fig. 8). However, this optimal eccentric force for work production was much smaller than that in the frog single fibers ($-1.8 P_o$).

Effects of prestretch on EMG activity. To see whether any change in the motor-unit recruitment occurs and plays a role in the enhancement of work production after the stretch, we analyzed EMG signals from both elbow flexors (biceps brachii) and extensors (triceps brachii). The iEMGs were obtained during both the stretch and release and were normalized to those during the release in unstretched control. Figure 9, A and B, shows the relationship between the velocity of stretch and the iEMG from biceps brachii and triceps brachii, respectively. In the biceps brachii, the iEMG during both the stretch and release tended to be larger.

Fig. 5. Records of elbow joint angle (expressed as 180° at full extension) and force in contractions of human elbow flexors. A: control. Maximal voluntary, isometric contraction was performed at largest angle in experimental range of motion (optimal angle for force generation +20°) (left), then release by 40° at constant velocity (90°/s) was given (right). B: contraction was started at smallest angle in range of motion (optimal angle for force generation 20°) (left), and then ramp stretch (velocity, 90°/s in this record) immediately followed by a release, both by 40°, was given during contraction (right).

Fig. 6. Force-length diagrams for stretch-release cycles of human elbow flexors obtained from records shown in Fig. 5. A, control; B, release at 90°/s subsequent to stretch at 90°/s.
than that during the release in unstretched control (Fig. 9A). However, the difference was not significant ($P > 0.05$) at stretch velocities of 30, 90, 120, and 150°/s. Notably, the mean iEMG was minimal at the stretch velocities of 90–120°/s, at which the maximal enhancement of work production was seen (Fig. 7). In triceps brachii, the iEMG during the stretch and release was consistently unchanged from that during the release in unstretched control, with an exception of a slight increase ($P < 0.05$) during the release subsequent to the stretch at 30°/s (Fig. 9B). These results suggest that the change in the motor unit-recruitment pattern is not primarily related to the enhancement of work production observed in the present experimental condition.

**DISCUSSION**

The present study showed that, in both frog single fibers and human elbow flexors, the mechanical work production during the shortening preceded by a stretch is a function of stretch velocity and that an optimal velocity of stretch exists for the enhancement of work production. Because the force attained before release increased consistently with the velocity of stretch, there also existed an optimal eccentric force (Figs. 4 and 8) for the enhancement of work production.

However, one must use caution when comparing the results on human muscles with those on frog single fibers, because the distance of stretch differed considerably between experiments with these two kinds of muscle. In frog single fibers, the distance of stretch was limited to within 2% of $L_o$, because stretches with longer distance tended to have an injurious effect and often caused irreversible damage in the fibers. Such a short length change is likely within a range of single to a few cross-bridge strokes, so that the obtained results would manifest events occurring in single to several cross-bridge cycles. On the other hand, the distance of stretch in human muscles was subjected to severe mechanical constraints imposed on the isokinetic dynamometer. Because of the large mass of the lever system and that of the forearm, the acceleration and deceleration took time (50 ms), so that the stretch had to be much larger in distance (~0.2 $L_o$) than in frog single fibers. Therefore, the results in human muscles would represent more steady-state events and thus may exhibit some quantitative differences from those on frog single fibers.

Among other differences, we should refer to the difference between the optimal force against stretch for the subsequent work production in human muscles
(−1.3 \text{ P}_0) and that in frog single fibers (−1.8 \text{ P}_0). The recent study of Ishii et al. (19) with an in vitro motility assay system, in which velocities of steady-state sliding between isolated myosin filaments and actin filaments were directly measured, has shown that the load exceeding \text{P}_0 caused the sliding movement directed opposite to the polarity of actin filament (corresponding to forced lengthening in muscle). In this system, thick filaments were detached from actin filaments when the force exceeded −1.4 \text{ P}_0 during the steady-state sliding. This finding is apparently consistent with the present results on human muscles and suggests that the markedly large force exerted by frog single fibers represents transient, unsteady force developed by individual cross bridges at the instant when they are forcibly detached.

A part of the negative work absorbed by frog single fibers and human muscles during stretch must be stored by the SEC in the form of elastic energy. Because this elastic energy increases with force on the SEC, it would be maximal at the maximal velocity of stretch attained within the range used in the present experiment. Therefore, the reutilization of elastic energy stored in the SEC cannot fully account for the present, biphasic dependence of the work production on the velocity of stretch.

The work made by the contractile element (CE) after stretch is, however, not as simply evaluated in the isovelocity release experiments. The large force exerted by the CE during the forced lengthening stretches the SEC to a greater extent than in isometric contractions. Although this process gives the SEC larger elastic energy, it reduces the distance of shortening of the CE within a limited range of release. Even if the force generated by the CE during the shortening were enhanced by the previous stretch (see below), the diminished distance of shortening would have an effect of reducing the work produced by the CE, depending on both the degree of force enhancement and the decrease in the distance of shortening. Such a mechanism may provide one possible reason why the total amount of work (work by SEC plus that by CE) was reduced when either stretch velocity or force exceeded a certain level (15).

The other possible mechanism for the present finding is related directly to the contractile properties of the CE. Since Abbot and Aubert (1) demonstrated, for the first time, the stretch-induced improvement of the ability of a frog muscle to generate tension, this phenomenon has been studied extensively with single-fiber preparations. When isometrically contracting single fibers are stretched from lengths at the descending limb of their length-tension relations and then kept at stretched lengths, they generate extremely large tension during the stretch, which is then followed by an exponential decay toward the new steady-state level after the end of stretch. This level of tension is higher than that determined on the length-tension relation (13). The peak tension attained during stretch increases with the velocity of stretch, whereas the level of the after-tension maintenance increases with decreasing velocity of stretch (26), suggesting that the enhancement of tension-generating capability is related to some complicated modification in actin-myosin interaction.

Measurements of stiffness of contracting single fibers (30) have shown that the stiffness after the end of stretch is not larger than without stretch, despite the maintenance of higher tension. This suggests that the enhancement of tension-generating capability is not caused by an increase in the number of interacting actin-myosin cross bridges. On the other hand, an X-ray diffraction study by Sugi et al. (27) has shown the increase in the irregularity of the hexagonal myofilament lattice on stretch, which may bring about an elevation of the electrostatic potential and cause an additive effect on the generation of active tension.

Such an enhancement of tension-generating capability greatly influences the dynamic properties of muscle. When isometrically contracting frog single fibers are stretched and then released isotonically, they exhibit larger shortening velocity than is attained under the same load without stretch, resulting in the upward shift of the force-velocity relations (8, 13, 28).

Notably, the effect of SEC is completely excluded in this type of experiment, since the steady-state shortening velocity is determined after the end of shortening (elastic recoil) of the SEC. Also, isovelocity stretch-release experiments (9) have shown that the force at given shortening velocity increased after the stretch, resulting in the increase in mechanical work produced for the same distance of shortening.

In human muscles, many additional factors still exist that influence muscle contractile properties in situ. Nervous control, including the stretch reflex, is the most likely to be considered, because stretching the muscle may immediately cause an additional recruitment of motor unit and also may cause an inhibitory effect when the force exceeds a certain level. However, in the present study, measurement of electrical activity showed no significant change in iEMG during the shortening after the stretch of 90–150°/s, where the largest enhancement of work production was observed (Figs. 7 and 9). This suggests that the neural modification plays a minor role in the present experimental condition. This result is consistent with the report by Gulch et al. (18) that no appreciable change in iEMG occurred, despite an enhancement of tension, when isometrically contracting elbow flexors were stretched and then held at stretched state. On the other hand, knee extensors have been shown to exhibit much-reduced EMG activities during forced lengthening compared with the EMG activities during isometric contraction (31).

Recent studies of motor control of eccentric contractions have shown that the neural commands controlling eccentric contractions differ considerably from those controlling concentric contractions (14). Among other differences, motoneurons with higher recruitment threshold (motor units for fast-twitch fibers) are
predominantly recruited in submaximal eccentric contractions, as opposed to the “size principle” operating in the concentric contractions (23). In addition, the neural commands associated with the eccentric contractions may influence the activity of motoneurons for synergistic muscles (24, 25). Such mechanisms can also enhance the mechanical output during the subsequent concentric contractions. In the present study, however, stretches and subsequent releases were done after the isometric force reached the level of maximal voluntary contraction, so that the eccentric contractions presumably caused no detectable change in the iEMG from the agonist muscle during the following concentric contractions. However, the possibility of increased activity in synergistic muscles needs further examination.

The present results for the elbow flexor muscles may not be applied directly to the whole body movements that are composed of coordinated contractions of numerous muscle groups with a variety of structural and contractile properties. For instance, the role played by the SEC may be more important in muscles with longer tendons, such as plantar flexors. Indeed, storage and reutilization of elastic energy by Achilles tendon have been shown to be involved in hopping movements in humans and other mammals (2, 17). The use of the stretch-shortening cycle of the SEC would make this particular type movement more energy efficient, because the length changes of the muscle fibers would be so small that they would not themselves produce large mechanical work. In addition, this elastic energy may be used in more complex movements involving the planter flexion, not only for saving energy but also for adding it to the mechanical work produced by other muscles and consequently gaining higher performance. However, experiments on drop-jump movements of humans (plyometric actions) have shown the presence of an optimal drop distance (and thus the optimal amount of negative work) to perform the highest jump (22), implying that the mechanism shown in the present study can also operate in much more complex movements. In relation to sports, the presence of such an optimal eccentric force would be of particular importance, because this suggests that an eccentric counter-movement preceding a concentric action should be taken at an appropriate velocity and deceleration rate to gain the highest performance.

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