Muscle stiffness measured under conditions simulating natural sound production

Lynn E. Dobrunz, Dominic G. Pelletier, and Thomas A. McMahon
Division of Applied Sciences, Harvard University, Cambridge, Massachusetts 02138 USA

ABSTRACT Isolated whole frog gastrocnemius muscles were electrically stimulated to peak twitch tension while held isometrically in a bath at 4°C. A quartz hydrophone detected vibrations of the muscle by measuring the pressure fluctuations caused by muscle movement. A small steel collar was slipped over the belly of the muscle. Transient forces including plucks and steady sinusoidal driving were applied to the collar by causing currents to flow in a coil held near the collar. The instantaneous resonant frequencies measured by the pluck and driving techniques were the same at various times during a twitch contraction cycle. The strain produced by the plucking technique in the outermost fibers was $<1.6 \times 10^{-4}$%, a strain three orders of magnitude less than that required to drop the tension to zero in quick-length-change experiments. Because the pressure transients recorded by the hydrophone during plucks and naturally occurring sounds were of comparable amplitude, strains in the muscle due to naturally occurring sounds must also be of the order 10^{-3}%. A simple model assuming that the muscle is an elastic bar under tension was used to calculate the instantaneous elastic modulus E as a function of time during a twitch, given the tension and resonant frequency. The result for E_{max}, the peak value of E during a twitch, was typically 2.8×10^6 N/m^2. The methods used here for measuring muscle stiffness are unusual in that the apparatus used for measuring stiffness is separate from the apparatus controlling and measuring force and length.

INTRODUCTION

When skeletal muscles contract, they give rise to a sound which may be heard using a stethoscope over the skin. Muscles isolated from the body of an animal also give rise to pressure fluctuations in the bathing medium. The pressure fluctuations are produced as an isolated muscle vibrates laterally and rings as a consequence of even a single force development (Barry, 1987; Frangioni et al., 1987). Recently interest has focused on the possibility of using muscle sounds to measure some feature of the mechanical status of a muscle, with an eye to the potential for using muscle sounds as a diagnostic tool (Barry and Cole, 1990).

This paper reports on experiments in which lateral vibrations of the same mode shape found in spontaneous sound generation were induced in whole frog muscles to measure the resonant frequency as a function of time during a twitch. The measurements were conducted at low temperatures to ensure that the isometric force event was slow compared with the decay time of the pressure fluctuations after a lateral pluck. Only very small strains are induced in the muscle fibers as a consequence of its lateral vibrations, making it unlikely that the elements giving rise to the stiffness are themselves disturbed or altered by the measurement. The paper concludes with a curve showing the stiffness as a function of time calculated on the basis of a simple model.

Address correspondence to Thomas A. McMahon, Division of Applied Sciences, Pierce Hall 325, Harvard University, Cambridge, MA 02138.

METHODS

Apparatus

Frog gastrocnemius muscles were clamped vertically in an isometric muscle holder made of two stainless steel posts and beams. The resonant frequency of the muscle holder was 428 Hz, measured from the ringing response obtained by lightly tapping the distal muscle clamp from below when a taut rubber band simulating a muscle was in place between the clamps. Because the frequency of the muscle sounds recorded in the experiments was never >150 Hz, the muscle holder was adequately stiff. A quartz force transducer (model 9203; Kistler Instrument Corp., Amherst, NY) mounted on a sliding stage held the isolated gastrocnemius muscle via a stainless-steel clamp designed to accept the knee. A lower clamp gripped the distal tendon. The range (0–500 N), sensitivity, and natural frequency (7.7 kHz) of the force transducer with the proximal muscle clamp attached were adequate for the measurements.

Sound pressure in the bath was measured using a quartz crystal hydrophone (model LC-10; Celesco Transducer Products, Inc., Canoga Park, CA). The transducer was suspended in the bath with its tip pointing at the muscle belly, usually 2 mm from the surface of the muscle. The sensitivity and frequency response of the hydrophone and its amplifier were calibrated in the bath by comparing the hydrophone output to the output of a calibrated catheter-tip pressure transducer (model PC-380; Millar and Frey Screw Machine Products, South Plainfield, NJ) when both were in the far field of a loudspeaker driven by

0006-3495/90/08/557/09 $2.00

a sinusoidal source at frequencies from 50 to 500 Hz. Further details regarding the apparatus may be found in Frangioni et al. (1987), including results of experiments proving that the muscle was the source of the sounds and that the bath and apparatus influenced neither the frequency nor the amplitude of the pressure measurements.

In many of the experiments, a short segment of an 8.6-mm outside diameter steel tube was cut to form a collar for the muscle. The collar (mass = 0.354 g) was slipped over the muscle belly, where friction held it in place throughout the experiments. A 250-turn steel-core coil sealed in room temperature vulcanizing silicone rubber was immersed in the bath 5.0 mm from the muscle belly. The purpose of the coil and the collar was to provide a lateral driving force to the muscle, as will be explained.

Protocols

The gastrocnemius muscles were removed from both legs of 30 frogs (*Rana pipiens*), preserving the sciatic nerve intact along the entire length of the femur. The bath contained a Ringer's solution (100 mM NaCl, 2.5 mM KCl, 1.17 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2 \cdot 6H_2O$, 5.0 mM glucose, buffered to pH 7.9 using Hepes buffer) maintained at 4°C, monitored by a Hg thermometer. In some experiments, the bath was at room temperature, 20–24°C. The cut end of the sciatic nerve was stimulated at 5 V, a voltage well above that found to produce a twitch of maximum amplitude. The stimulator (model 6BP Pulsar; Frederick Haer) was operated under software control. Force and pressure records were sampled (12 bits) at 1.0 kHz (DT-2801-A A/D board; Data Translation, Marlboro, MA) and recorded on a hard disk within a microcomputer (model 286 Desk Pro; Compaq Computer, Houston, TX).

In 14 muscles, tension and pressure were recorded in single isolated twitch stimulations. The muscle was held at a range of lengths from L_0 to 1.08 L_0, where L_0 is the length for maximum developed tension (total peak twitch tension minus passive resting tension). The muscle length was measured as the distance from one end of the muscle belly to the other, excluding the proximal and distal tendons.

In five unstimulated muscles, the damped natural frequency was measured as a function of length and tension for muscles held at a range of lengths and forced to vibrate laterally by releasing a steady force (pluck technique) or by applying a constant-amplitude sinusoidal current through the coil (driving technique). In three muscles, the influence of collars of various mass was assessed by plucking the passive muscle and recording the damped sinusoidal pressure transient that ensued.

In the remaining experiments, muscles were given single twitch stimuli and the tension and pressure were recorded as the muscle was held as close as possible to L_{max}, the length where the sound was of greatest amplitude. In five muscles, the sound pressure was measured as the hydrophone was moved around the azimuth of the muscle belly, at a fixed distance from the proximal muscle clamp, with and without a collar present, during twitch stimulations. In nine muscles, both the pluck and driving techniques were used to determine the time course of the damped natural frequency (hereafter called the resonant frequency) during a twitch.

RESULTS

In Fig. 1, curves are shown for the passive tension (P), the maximum twitch tension (T), and the developed twitch tension ($D = T - P$) for a frog gastrocnemius at 4°C. As shown by the points, the spontaneously occurring muscle

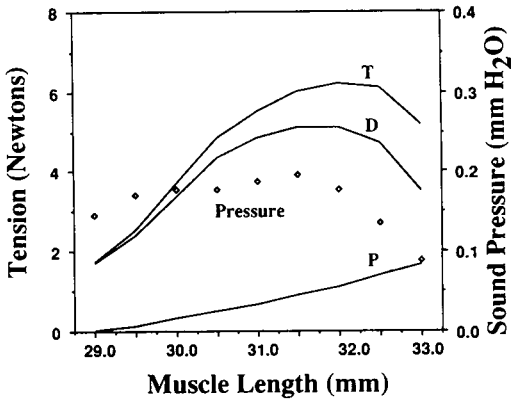

FIGURE 1 Tension and sound pressure amplitude for frog gastrocnemius muscle at 4°C. T = tension at the peak of the twitch; P = passive (unstimulated) tension; D = developed tension, $D = T - P$. The length at which the sound pressure amplitude is maximum, L_{max}, is just below the length at which the developed tension is greatest.

sound pressure recorded by the hydrophone as the tension rises has an amplitude which is a function of muscle length. The length at which the sound pressure amplitude is greatest will be called L_{max}. In most of the muscles studied, L_{max} was ~97% of L_0, the length at which the developed tension was greatest. In the particular muscle shown in Fig. 1, L_{max} was ~0.99 L_0.

Our first task was to assess the degree to which the steel collar influenced the pattern and frequency of muscle vibrations. In Fig. 2, the amplitude of the first half-cycle of the spontaneously occurring sound pressure waveform is shown as a function of azimuth angle for measurements

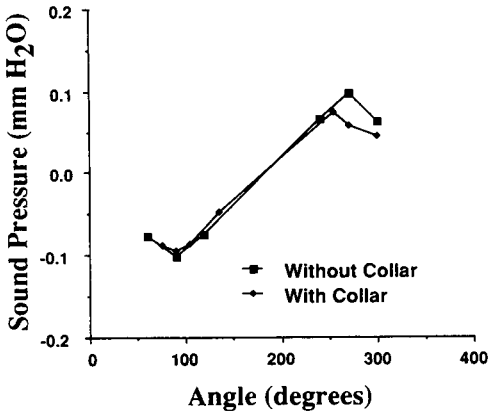

FIGURE 2 Amplitude of the first half-cycle of sound pressure vs. azimuth angle at 4°C. The hydrophone was 2 mm from the surface of the muscle. The distance from the proximal muscle clamp remained fixed as the hydrophone was rotated around the muscle belly. Pressure records show that the mode of lateral vibration was similar with and without the steel collar.

made at the midpoint of the muscle belly in muscles held at L_{max} and given a twitch stimulation (not plucked or driven). The mass of the muscle in this experiment was 0.725 g and the mass of the collar was 0.354 g. Amplitudes measured with and without the collar are very similar. Measurements of the frequency of resonant vibration recorded in a passive (unstimulated) muscle after a pluck showed that the collar reduced the resonant frequency at any particular tension and length, but the evidence from Fig. 2 supports the conclusion that the presence of the collar did not make important changes in the mode shape. With and without the collar, the mode of spontaneously occurring vibrations during twitches is a lateral oscillation with the greatest amplitude in the central region of the muscle.

As shown in Fig. 3, the resonant frequency of a passive muscle with a collar was found to be the same whether the pluck or the driving method was used. In the pluck method, the resonant frequency was obtained by fitting an exponentially decaying damped sine wave to the pressure signal recorded by the hydrophone after the release of a lateral force applied to the collar. In the driving method, a sinusoidal lateral driving force was applied to the collar, and the resonant frequency was found by varying the driving frequency until the pressure oscillations recorded by the hydrophone reached their greatest amplitude.

In Fig. 4, tension and sound pressure are presented as functions of time for a pluck released at various times after a twitch stimulation. The strength of the steady lateral force applied through the collar was adjusted until its release gave rise to a sound event only modestly greater in amplitude than the spontaneously occurring sound near the beginning of each record as the tension begins to rise. Doubling the strength of the pluck and therefore the amplitude of the resulting sound did not affect the frequency of the sound. Results from the experiment using the sinusoidal driving technique are given in Fig. 5. In Fig. 5, *a–c*, the steady driving frequencies during subsequent twitches were 45, 65, and 75 Hz, respectively. The large-amplitude episodes on the pressure record show that the muscle was forced into resonant lateral vibrations twice during each twitch cycle, once when the muscle tension was rising and again as it was falling. In Fig. 5 *a*, the driving frequency (45 Hz) is half the resonant frequency during the peak of the tension trace, and the 45-Hz forcing is exciting not only a 45-Hz vibration but also a small 90-Hz vibration in the muscle at that time. In Fig. 5 *d*, the frequency of the forcing signal (90 Hz) applied to the collar matches the maximum resonant frequency of the muscle so that a resonant response occurs only once during the twitch cycle.

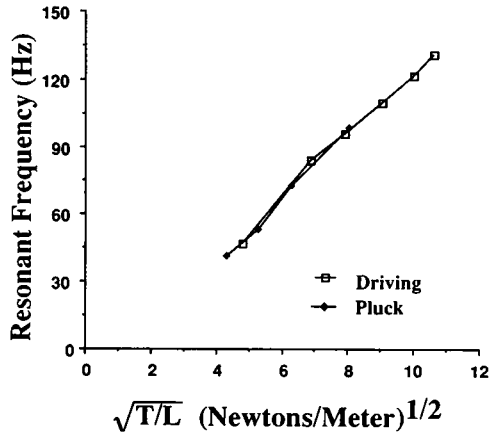

FIGURE 3 Resonant frequency of a passive (unstimulated) muscle at 20°C. The resonant frequency obtained using the pluck and driving methods was the same at a particular length and tension.

The resonant frequencies obtained by the pluck and driving methods during a series of identical twitches are compared in Fig. 6. The resonant frequency obtained at any given time during the cycle of force development is practically the same whether the pluck or driving technique is used. The tension curve appearing in Fig. 6 was recorded during one of the twitches of the driving experiments; other tension records in this series were almost identical.

A simple model of the muscle considered as an elastic bar under tension is presented in Appendix A. When the resonant frequency measured by the plucking technique is used for f, the result for the relative stiffness E/E_{max} as a function of time during a twitch is shown in Fig. 7. Here E_{max} is the (calculated) maximum stiffness measured during the twitch.

DISCUSSION

Are the pluck and driving techniques adequate to measure resonant frequency?

The purpose of the experiments was to measure the time course of the changing resonant frequency of a muscle during a twitch. In making these measurements, we wished to excite a mode of vibration similar to, although not necessarily identical to, the one excited by the spontaneously occurring vibrations accompanying muscle sounds. Are the techniques employing the collar appropriate and adequate for the task?

It is certainly true that the collar influenced the frequency of vibration. This was to be expected because the mass of the collar (0.354 g) was a large fraction of the total mass including the collar and the muscle (1.079 g for the muscle of Fig. 2). Even so, the results shown in Fig. 2 imply that the half-wavelength standing wave pattern of

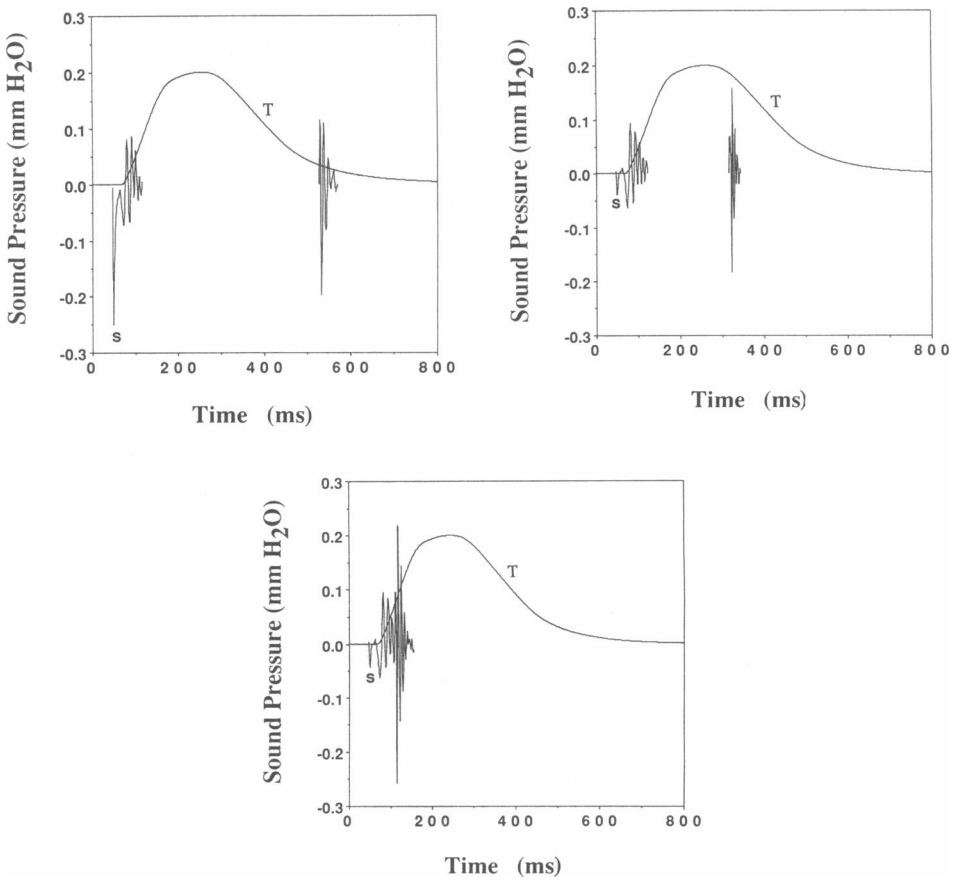

FIGURE 4 Plucks: tension (T) and sound pressure vs. time for twitches at 4.3° C. The muscle was plucked once per twitch. The events marked S on the pressure record are artefacts due to the nerve stimulus. The vibration on the pressure record coincident with the rise in tension is the spontaneously occurring muscle sound. The tension peak is 0.62 N above the baseline (passive) tension. Muscle mass = 0.98 g, length = 32.0 mm.

lateral vibration is not very different with and without the collar, so that forcing vibrations using the collar results in deformations and strain patterns within the muscle that should be similar to those accompanying muscle sounds. Therefore, plucking a muscle at various times during a twitch (Fig. 4) gives essentially the same information as would be available if the natural sound could be made to occur at arbitrarily-specified times. In fact, there is a slight advantage to weighting the muscle at the center using the collar. After the first half-cycle of vibration, we found that the pressure transient after a pluck could be fitted to an exponentially decaying sinusoid to a greater precision than could a spontaneously excited muscle sound. The spontaneous sound probably is excited by nonuniform development of contractile force throughout the muscle. Evidently, the use of the collar to develop a plucking force is more often able to excite a single mode of decaying vibration than is a spontaneous sound.

An essential feature of our technique was the cold temperature. We found that the time constant for decay of the pressure transient after a pluck was not influenced by the temperature of the bath, but the time required for the twitch tension to rise and fall was ~8 times longer (650 ms compared with 80 ms) at 4°C than it was at 20°C. At 4°C, the time constant for the pressure transient after a pluck was 26 ms whereas the time constant for decay of the twitch tension was ~220 ms. Thus the rate of decay of the pressure signal is typically 8 times faster than the rate of change of tension, providing support for the assumption that the tension varies slowly enough to make it reasonable to fit a constant-frequency sinusoid to the first few cycles of the pressure signal elicited by a pluck. For example, in Fig. 4 *b* the pressure transient after a pluck lasts for ~22 ms. Fig. 6 shows that the frequency is changing at ~140 Hz/s at this time. Hence, the change in frequency during the pressure transient is 3.08 Hz, or ~3.4%, which is small enough to allow the oscillating pressure transient to be described by a single frequency.

Note that at 20°C, where the time constants for pressure decay and tension decline can be almost the

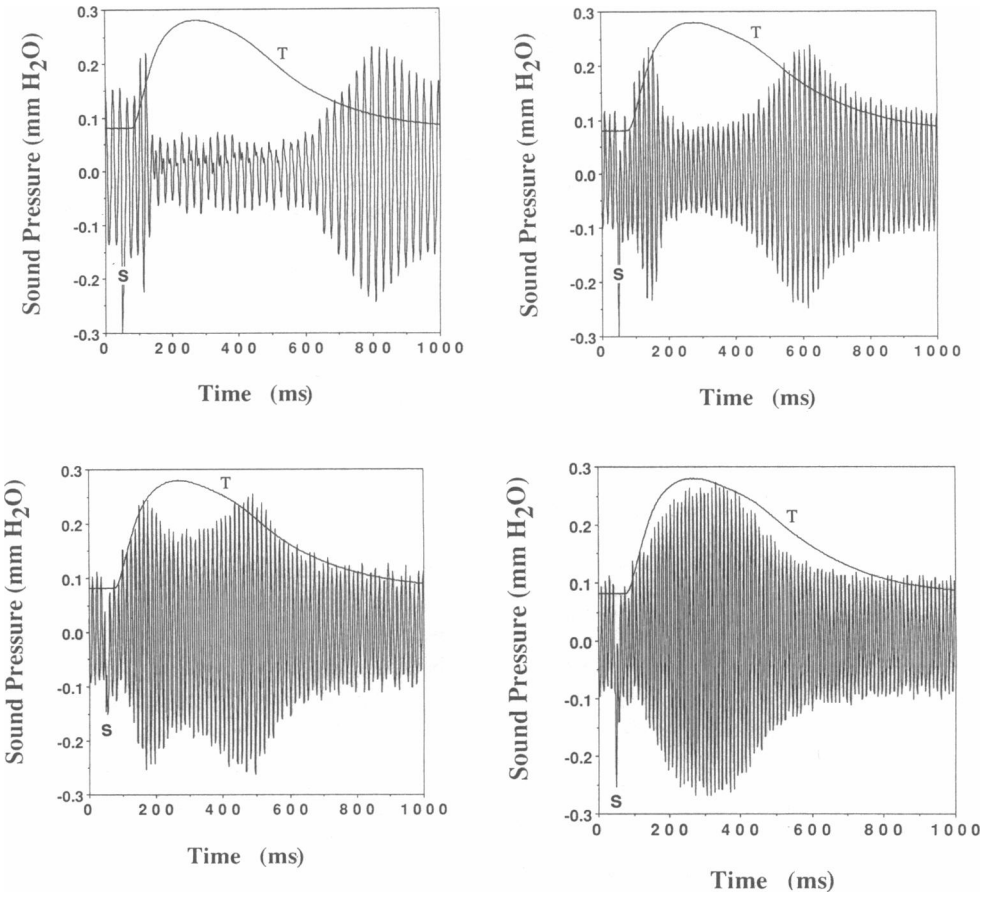

FIGURE 5 Driving: tension (T) and sound pressure vs. time for twitches at 4.3°C. The steady sinusoidal driving frequency was (*a*) 45 Hz, (*b*) 65 Hz, (*c*) 75 Hz, and (*d*) 90 Hz. Artefacts due to stimulation of the nerve are marked S. The tension peak is 1.46 N above the baseline (passive) tension. Muscle mass = 0.80 g, length = 31.5 mm.

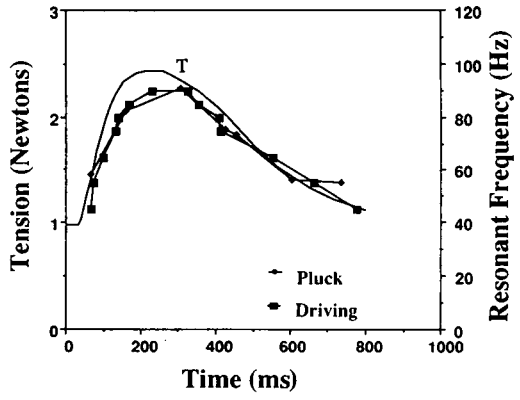

FIGURE 6 Resonant frequency and tension vs. time for twitches at 4.3° C. The instantaneous resonant frequencies obtained using the pluck and driving methods were essentially identical. Muscle mass = 0.73 g, length = 30.5 mm.

same, the assumption that the pressure transient decays before its frequency changes probably is not valid. Barry and Cole (1990) made measurements comparable to ours on muscles at temperatures of 20°C and higher, and obtained instantaneous frequency spectra using time-frequency transformations including the Wigner Transform and the Exponential Distribution. These techniques allowed them to calculate the peak instantaneous frequency even when the frequency was changing rapidly.

Can the resonant frequency be used to calculate *E* during a twitch?

Fast changes in length (completed in 0.2 ms) of <0.5% of the initial length have been shown to cause little or no change in the rapid elasticity of single fibers of frog anterior tibialis muscles at 0°C (Ford et al., 1974), presumably because such small, rapid stretches and releases are not sufficient to disrupt attached cross-bridges. Measurements of stiffness in muscle fibers using

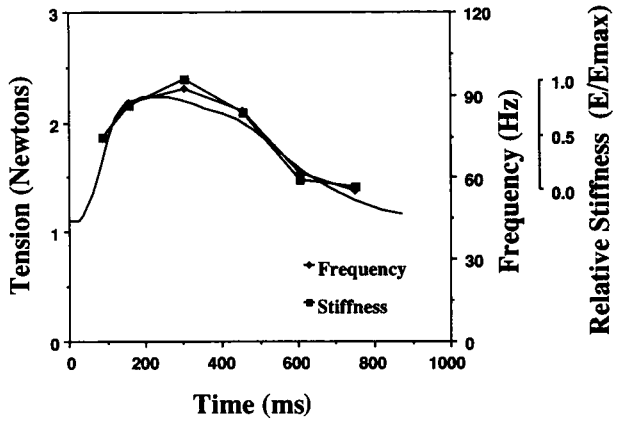

FIGURE 7 Calculated relative stiffness E/E_{max} for twitches at 4.3°C. Muscle mass = 0.80 g, length = 31.5 mm. The resonant frequencies obtained from plucking experiments were used, along with the tension, to calculate the instantaneous E (Appendix A). The calculation takes account of the added mass due to the surrounding saline solution, the mass of the collar, the spindle shape of the muscle, and the fact that the mass of the collar was lumped at the midpoint of the muscle.

small-amplitude vibrational length changes (Cecchi et al., 1982, 1986) generally corroborate the assertion that only strains <0.5% can be applied to a fiber for the purpose of measuring stiffness without risking a change in the stiffness caused by the imposed strain. In Appendix B, a short calculation is given estimating the peak strain of fibers within the peripheral bundles of the muscles in our experiments. The calculations concluding Appendix B show that for the experiments of Figs. 7 and 8 at the peak stiffness, the strain in the outer muscle fibers was $\epsilon = 1.6 \times 10^{-6}$. Assuming that the length of a half-sarcomere is 1.0 μm, this strain gives rise to a sliding displacement between thick and thin filaments of 1.6×10^{-3} nm per

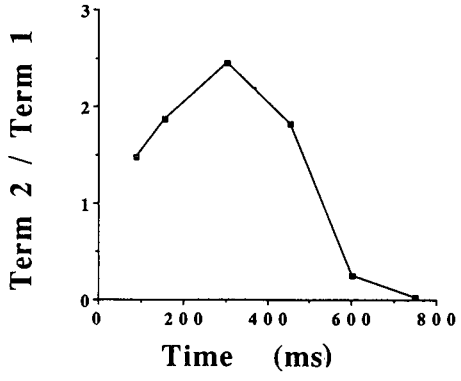

FIGURE 8 Ratio of term 2 (stiffness term) to term 1 (tension term) in Eq. A-12. When this ratio is large, stiffness dominates as the restoring force in the resonant vibrations. The muscle is the same one as in Fig. 7 at 4.3°C.

half sarcomere. Because the displacement required to drop the tension to zero in rapid-length-change experiments on single muscle fibers is of the order 4 or 5 nm per half sarcomere (Ford et al., 1974), the strain imposed by our technique is <0.04% of the strain that would be expected to buckle an average attached cross-bridge.

Stiffness has been found to decline at frequencies below 4 kHz in studies employing sinusoidal length changes of small amplitude in single frog muscle fibers at 4°C (Cecchi et al., 1986). Although the strains imposed in our experiments would not be expected to cause filament shearing motions of such a magnitude that cross-bridge detachment and reattachment would be made necessary, the frequencies used in our experiments (<150 Hz) were too low to be confident that cross-bridges had inadequate time to cycle or to change from one attached state to another (the $T_1 - T_2$ transition) during a vibration period. Even so, our calculations show an approximately constant stiffness-to-force ratio throughout the twitch. The ratio of stiffness to force has been found to be constant also in single-fiber experiments employing a length change thought to be more rapid than cross-bridge cycling or $T_1 - T_2$ transitions (Ford et al., 1981).

In an earlier study, we concluded that the restoring force sustaining the lateral vibrations that give rise to muscle sound are due to bending stiffness as well as tension in an active muscle but are due almost exclusively to tension in a passive muscle (Frangioni et al., 1987). These conclusions have been corroborated and extended in the present work. In Fig. 3, the resonant frequency for a passive muscle maintained at different values of tension T and length L is a nearly-linear function of $(T/L)^{1/2}$, as would be expected for a string or rubber band having no bending stiffness that was gripped at each end and stretched to various lengths. In Eq. A-12, two terms are evident in the expression for the square of frequency, one dependent on T (term 1) and the other on elastic modulus E (term 2). When the ratio of term 2 to term 1 is small, tension dominates as the restoring force sustaining the vibration; when the ratio of term 2 to term 1 is large, elastic stiffness dominates. As shown in Fig. 8, the ratio of term 2 to term 1 rises to a peak value near 2.5 at the time when the resonant frequency is highest, then falls to values <0.1 when the twitch force declines. Thus either a spontaneously-excited sound or a vibration plucked or driven by the collar is sustained primarily by bending forces throughout most of the time when the tension is elevated during a twitch.

A central conclusion of this paper is given in Fig. 7, where the relative stiffness E/E_{max} is found to rise and fall after a time course nearly the same as that of the tension during a twitch. This finding is consistent with results obtained by Schoenberg and Wells (1984) using their

transmission-time technique, and other studies using length steps or vibrations, including those by Bressler and Clinch (1974) and Cecchi et al. (1982). Hatta et al. (1988) found similar results using measurements of the wave speed of ultrasonic waves (3–7 MHz) to calculate the stiffness of whole frog skeletal muscle during isometric twitches and tetanii.

The peak value E_{max} reached (during a twitch) by the elastic modulus of an equivalent homogeneous rod of the same length, mass, density, and resonant frequency as the muscle is 2.8×10^6 N/m^2 (Appendix A). This number may be compared with the value 2.4×10^7 N/m^2 obtained by Hatta et al. (1988) using ultrasonic waves in a tetanized semitendinosus muscle. Our result for E is smaller, and several reasons come to mind for the difference. Our measurements were carried out for twitch stimulation, whereas theirs were for tetanus, and other measurements of theirs showed that the peak stiffness reached in a twitch was little more than half that reached in a tetanus. The strain imposed upon individual sarcomeres in the Hatta et al. study was $\sim 2.0 \times 10^{-4}$% (see Appendix B), a level similar to the strains imposed during our experiments. A major difference between our methods and theirs concerned the frequency of the imposed sinusoidal local length changes. In our experiments, typically done at 100 Hz, a stretch (half of a full cycle) takes 5 ms, which is about twice the time constant for $T_1 - T_2$ state transitions (Ford et al. 1977). Thus the frequency in our experiments was far too low to presume that $T_1 - T_2$ state transitions did not take place during the stretch, although that presumption would be valid for the 3–7 MHz methods of Hatta et al. The fact that state transitions would be expected during the time sarcomeres were changing length in our experiments probably accounts for a large part of the (factor of 10) difference between our stiffness estimate and that of Hatta et al.

Because the amplitudes of the oscillating pressure transients after our plucks were adjusted to be only modestly greater than those accompanying naturally occurring muscle sounds (Fig. 4), we conclude that the estimates of muscle fiber strain given in Appendix B also may be taken to form an upper bound on the fiber strains accompanying naturally occurring sounds.

The techniques reported here are unconventional in that they separate the apparatus used to probe muscle stiffness (the collar and its driver) from the apparatus regulating and measuring force and length. We have already pointed out one advantage of such methods: they give rise to particularly small imposed length disturbances within the fibers. Unlike the ultrasound transmission technique employed by Hatta et al. (1988), our methods did not require mechanical contact of fixed sound transducer heads with the central region of the muscle, so the investigator would be free to perform simultaneous measurements of force-velocity and stiffness properties. Another advantage of our techniques is that a very fast servo lever and a force transducer with high frequency response are not needed to measure stiffness, since neither participates in the stiffness measurement. In fact, it may be possible to measure the resonant frequency of muscles in intact animals by exciting the muscle into resonant lateral vibrations through a force applied to the skin surface. Provided the tension and length of the muscle could also be measured in some way, the instantaneous elastic modulus of the muscle could be estimated using the methods discussed here.

APPENDIX A

Calculating the elastic modulus E, given the frequency and tension

In this appendix we use Rayleigh's method to derive a relation between the natural frequency of vibration, the longitudinal tension, and the elastic modulus of a cylindrical bar under constant tension. We then solve for the elastic modulus. The following variables will be used:

A = cross-sectional area of the bar
E = elastic modulus of the bar
I = second moment of the area of the cross-section = $\pi R^4/4$
L = length of the bar
R = radius of the bar
T = longitudinal tension
t = time
x = longitudinal distance from the end
y = transverse displacement
ρ = mass density of the bar (muscle)
ρ_0 = mass density of the surrounding fluid (water)
ρ_s = mass density of steel
ω = natural frequency of vibration.

Mode shape

We shall assume that the bar vibrates in a sinusoidal half-wavelength mode given by

$$y(x, t) = y_0 \sin(\pi x/L) \sin \omega t.$$

Potential energy

The potential energy of the bar is greatest at $\omega t = n\pi/2$, where n is odd. The two components of the potential energy are: (*a*) that due to longitudinal tension; and (*b*) that due to bending. The contribution due to tension will be evaluated first.

$$V_T = (-T/2) \int_{x=0}^{x=L} y(d^2y/dx^2)\, dx \qquad \text{(A1)}$$

(Lamb, 1931, p. 60). Substituting for y and d^2y/dx^2,

$$V_T = (\pi^2 T/2L^2) \int_{x=0}^{x=L} y_0^2 \sin^2(\pi x/L)\, dx = \pi^2 y_0^2 T/4L. \qquad \text{(A2)}$$

The contribution due to bending is

$$V_B = (EI/2) \int_{x=0}^{x=L} (d^2y/dx^2)^2 \, dx \quad \text{(A3)}$$

(Lamb, 1931, p. 125). Thus,

$$V_B = (\pi^4 y_0^2 EI/2L^4) \int_{x=0}^{x=L} \sin^2 (\pi x/L) \, dx = \pi^4 y_0^2 EI/4L^3. \quad \text{(A4)}$$

The total potential energy V of the bar when it is stationary at its maximum displacement is

$$V = V_T + V_B = (\pi^4 y_0^2/4L^3)(TL^2/\pi^2 + EI). \quad \text{(A5)}$$

Kinetic energy

The total kinetic energy is made up of three parts: (*a*) the kinetic energy of the moving bar; (*b*) the kinetic energy of the collar mass, M, at the center of the bar, and (*c*) the kinetic energy of the fluid motions in the bath surrounding the bar and the collar mass. The kinetic energy is greatest at $\omega t = 0, \pi, 2\pi$, etc. The kinetic energy due to motion of the bar (muscle) is

$$KE_{muscle} = (\rho A/2) \int_{x=0}^{x=L} (dy/dt)^2 \, dx \quad \text{(A6)}$$

(Lamb, 1931, p. 125). Evaluating the integral gives

$$KE_{muscle} = (\rho y_0^2 A\omega^2/2) \int_{x=0}^{x=L} \sin^2 (\pi x/L) \, dx = \rho\omega^2 y_0^2 AL/4. \quad \text{(A7)}$$

The kinetic energy of the collar mass, M, at $x = L/2$ is

$$KE_{collar} = M(dy/dt)^2/2 = M\omega^2 y_0^2/2. \quad \text{(A8)}$$

Lamb (1932, p. 77) shows that when a bar in a fluid is moving at right angles to its axis, the kinetic energy due to fluid moving around the bar has an apparent mass equal to the fluid displaced by the bar and a velocity equal to that of the bar. Hence,

$$KE_{added\ mass} = (\rho_0 AL/4)\omega^2 y_0^2 + (\rho_0/\rho_s) M\omega^2 y_0^2/2. \quad \text{(A9)}$$

Rayleigh's method

In Rayleigh's method for determining the resonant frequency of a system with no dissipation or damping, the maximum potential energy (when $dy/dt = 0$) is equated to the maximum kinetic energy (when $y = 0$). Equating the expression for $KE_{total} = KE_{muscle} + KE_{collar} + KE_{added\ mass}$ to the expression for V and solving for E, using $\omega = 2\pi f$, gives

$$E = (16 f^2 L^3/\pi^2 I)[(\rho + \rho_0) AL/4 + (1 + \rho_0/\rho_s) M/2] - TL^2/I\pi^2. \quad \text{(A10)}$$

We assume that the muscle is a cylinder of radius R, so that $I = \pi R^4/4$. The muscle mass $m = \rho AL$. Taking the density of the muscle ρ to be approximately the density of water ρ_0 (Wilkie, 1954), and taking the ratio $\rho_0/\rho_s = 0.126$ (Ashby and Jones, 1980), a result is obtained for the elastic modulus in terms of L, R, f, m, M, and T:

$$E = (32L^3/\pi^3 R^4)[f^2(m + 1.126M) - T/8L. \quad \text{(A11)}$$

The relative importance of bending stiffness and longitudinal tension as restoring forces can be appreciated by solving the above for f^2.

$$f^2 = \underbrace{T/8L(m + 1.126M)}_{\text{term 1}} + \underbrace{\pi^3 R^4 E/32L^3(m + 1.126M)}_{\text{term 2}}. \quad \text{(A12)}$$

For a spindle-shaped muscle such as the gastrocnemius muscles used in this study, the total mass of the muscle is not distributed uniformly over the length. Instead, the central regions are more massive than the end regions. Comparing Eqs. A7 and A8, it is apparent that if the distributed mass of a vibrating bar were concentrated at its center point, the effective mass would be doubled (assuming that the mode shape and amplitude were unchanged). Hence, in the limit as all the muscle mass is concentrated at its center, the m in Eqs. A11 and A12 should be replaced by $2m$. Because the spindle shape of gastrocnemius muscles neither concentrates all the mass at the center nor distributes it uniformly, as an approximation we took into account the shape of the muscle by replacing m by $1.5m$ when we used Eqs. A11 and A12 to perform the calculations for Figs. 7 and 8. The variation in cross-sectional area would also have an effect on the potential energy, and if taken into account would lead to a decrease in the estimate for E_{max}. For parallel-fibered muscles without a bulge in the middle, Eqs. A11 and A12 may be used for calculations as they stand.

For the experimental results presented in Fig. 7, $L = 0.0315$ m, $R = 0.0028$ m, $m = 0.0008$ kg, and $M = 0.000365$ kg. The maximum value of E is obtained when $T = 2.14$ N and $f = 92.36$ Hz, giving $E_{max} = 2.8 \times 10^6$ N/m^2.

APPENDIX B

Estimating the peak strain in peripheral muscle fibers.

Assume the muscle plus tendon can be represented as an equivalent homogeneous bar under longitudinal tension subject to a lateral load applied to the center. For a simply supported beam with longitudinal tension T loaded in bending by a single load P at the center, the deflection in the center δ is given by

$$\delta = \delta_0/(1 + \alpha), \quad \text{(B1)}$$

(Timoshenko, 1956), where α is the ratio of the longitudinal force to the critical (buckling) value of the axial load:

$$\alpha = TL^2/EI\pi^2 \quad \text{(B2)}$$

and δ_0 is the deflection produced by the lateral load P only:

$$\delta_0 = PL^3/48EI. \quad \text{(B3)}$$

For a bar with a circular cross-section and radius R,

$$I = \pi R^4/4. \quad \text{(B4)}$$

The strain in the outermost fibers is

$$\epsilon = R(d^2y/dx^2). \quad \text{(B5)}$$

Assuming the mode shape to be specified by

$$y(x) = \delta \sin (\pi x/L) \quad \text{(B6)}$$

and differentiating gives

$$dy/dx = (\pi\delta/L)\cos(\pi x/L) \tag{B7}$$

and

$$d^2y/dx^2 = -(\pi^2\delta/L^2)\sin(\pi x/L). \tag{B8}$$

At $x = L/2$,

$$d^2y/dx^2 = -\pi^2\delta/L^2. \tag{B9}$$

Therefore the strain in the outer muscle fibers at the midpoint of the muscle length is a tensile strain of magnitude

$$\epsilon = R\pi^2\delta/L^2. \tag{B10}$$

Using Eqs. B1, B2, B3, B4, and B10, and substituting the values $L = 0.0315$ m, $R = 0.0028$ m, $E = 2.8 \times 10^6$ N/m^2, $P = 3.09 \times 10^{-5}$ N, and $T = 2.14$ N (from the experiment of Figs. 7 and 8 at the maximum twitch tension) gives a peak tensile strain in the outer muscle fibers of $\epsilon = 1.6 \times 10^{-4}$%. The value for P used in these calculations was obtained in a calibration experiment in which a constant current of the magnitude used in the pluck experiments was caused to flow in the coil. The steel collar was tied in the center of a cotton thread which replaced the muscle in the muscle holder. The coil was positioned the same distance from the collar as it was in the muscle experiments. A photograph was taken and later analyzed to give the angle of the thread (21°) with respect to the center line. The angle and the measured (steady) tension in the string were used to calculate the lateral force P. Because the same current amplitude was used in the plucking and driving experiments, the calibration discussed above measured the maximum of the force pulling the muscle laterally using the driving technique as well as the magnitude of the lateral force just before the release in the pluck technique.

The technique for stiffness evaluation in whole frog muscle employed by Hatta et al. (1988) was based on measuring the wave speed of 3–7 MHz ultrasonic waves in muscle. These authors estimated that the local length increase caused by the passage of an ultrasonic wave was ~0.1 nm. They found that the wavelength at 7 MHz was ~0.2 mm. Therefore the segment length within which sarcomeres are stretched is 0.1 mm, and the average strain of a sarcomere in that segment is (0.1 nm)/(0.1 mm) = 10^{-6} = 10^{-4}%. This figure estimates the average strain; the peak strain in the middle of the segment would be expected to be twice as great, 2.0×10^{-4}%. Thus the ultrasound technique employed by Hatta et al. and the lateral forcing technique used in the present study provide about the same level of strain to individual sarcomeres.

The authors are grateful to Dr. Daniel T. Barry and Dr. David Morgan for helpful discussions.

This work was supported by Harvard University and by grant number 86-10-9 to Dr. McMahon from the Sloan Foundation, and by a National Science Foundation Award for Creativity in Engineering to Ms. Dobrunz.

Received for publication 20 October 1989 and in final form 20 April 1990.

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