Dynamic Stiffness Measured in Central Segment of Excised Rabbit Papillary Muscles During Barium Contracture

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The dynamic mechanical behavior of excised rabbit papillary muscles that had been tonically activated by replacing bathing Ca\(^{2+}\) with Ba\(^{2+}\) was studied. Steady activation was used to visualize the dynamic behavior of cardiac myofilaments more clearly than is possible during twitches, which are complicated by the kinetics of excitation-contraction coupling. To avoid artifacts due to damaged ends of the muscle, the length of a central segment, which was defined by 2 tungsten pins inserted through the muscle, was measured. To test the mechanical behavior of the contractured muscles (at 24°C), the central segment length was sinusoidally oscillated (amplitude 1%) at 15 different frequencies (0.05–30 Hz). The dynamic stiffness of the central segment was calculated from the ratio of force response amplitude to length perturbation amplitude. At low frequencies (below 0.4 Hz), stiffness was approximately constant and reflected the force-length relation. However, in a localized range near 1 Hz, there was a distinct drop in the magnitude of dynamic stiffness to approximately half its low-frequency baseline. This range may reflect the dynamics of attachment and detachment of force generators. The frequency of minimum stiffness was consistent among all muscles (1.3 ± 0.3 Hz). Moreover, no significant change in this frequency was found over the examined range of lengths (90–100% of the segment length that produced maximal developed force) and activation levels (Ba\(^{2+}\) concentration 0.3–1.0 mM). From 2 to 8 Hz, dynamic stiffness appeared to reflect force-velocity properties, but at higher frequencies, another elastic property emerged. At 30 Hz, stiffness was proportional to force, with an apparent series elasticity less than 1.8%. Even though the muscles had only moderate longitudinal inhomogeneity, quantitatively significant (35%) errors would have been introduced had the study relied on total muscle length instead of central segment length. *Circulation Research* 1987;60:756–769

It is easier to analyze the mechanical properties of a steadily contracting muscle that has a constant level of activation than to deal with a twitching muscle in which activation varies periodically with time. Unlike skeletal muscle, however, heart muscle cannot normally be tetanically contracted by repeated stimulation. This feature of heart muscle makes analyses of its mechanical properties rather difficult. Steiger et al.10 and Saeki et al.11 have studied heart muscle in a state of very stable contracture produced by replacing bathing Ca\(^{2+}\) with Ba\(^{2+}\). Since Ba\(^{2+}\) appears to activate muscle in the same way as Ca\(^{2+}\), the results from such contractures can be extrapolated to a normal twitch if one assumes that contracture is a still picture of the twitch process at a corresponding degree of activation. Moreover, the findings from contracture will be more directly related to the mechanics of myocardial filaments than results from twitches in which the kinetics of cyclic excitation-contraction coupling complicate interpretation of the data.

Another difficulty in studying myocardial mechanics is the artifactual series compliance at the damaged ends of excised papillary muscles. Thus, several investigations have shown that the central, presumably healthy portion of papillary muscles shortened during a twitch even though the ends of the excised muscle were held isometric.12–14 The amount of the shortening can be as much as 10–15% of the length of the resting central portion of the excised muscle.

In the present study, the mechanical properties of papillary muscles during Ba\(^{2+}\) contracture were investigated, but artifacts from the damaged ends were avoided by measuring the length of a central segment of the muscle. This segment was defined by 2 pins placed transversely through the muscle.9 Small sinusoidal oscillations of muscle length over a wide frequency range were used as the perturbation signal, and the dynamic stiffness of the central segment was calculated from the force and segment length responses. The results indicate that the frequency-dependent variation of both the magnitude of dynamic stiffness and its phase shift showed significantly larger increases when measured by the length of the central, healthy segment rather than the whole muscle length.

Materials and Methods

Preparation

New Zealand white rabbits weighing 3–4 kg were anesthetized with an intravenous injection of pentobar-
bital sodium (40 mg/kg), and the hearts were excised rapidly. The aorta was cannulated above the aortic valves, and the heart was perfused with Tyrode's solution (37°C) equilibrated with a 98% O2-2% CO2 gas mixture. The perfusate contained (in mM) NaCl 130, KCl 4, NaHCO3 10, MgCl2 1, CaCl2 2.5; NaH2PO4 0.43, glucose 5.56, pH 7.2-7.4. During the perfusion, a papillary muscle from the right ventricle was dissected and then mounted horizontally in a tissue bath perfused with the same oxygenated solution as above. The tendinous end of the muscle was tied by 6-0 black silk to a stainless steel wire that was connected to the force transducer. The nontendinous end was fixed by a clamp that was connected to a linear motor used to change muscle length. The papillary muscle was stimulated 30 times per minute with electrical pulses of 3-millisecond duration and intensity of 20-70% above the threshold through a pair of parallel platinum wire electrodes positioned on both sides of the muscle. The temperature of the perfusate was gradually decreased from 37°C to 24°C over about 30 minutes after mounting the muscle in the bath. Higher temperature may facilitate the healing-over process10 and has been recommended before.11 To reduce oxygen consumption, muscle length was low (below 90% Lmax) during this time.

After 2 hours of equilibration at 24°C, 2 tungsten pins were inserted vertically into the papillary muscle by a micromanipulator. The pins were separated by 2-3 mm and were used to define the central segment between them (Figure 1). During pin insertion, electrical stimulation was stopped to minimize damage to the papillary muscle, and the muscle was stretched to a length where there was significant passive force (100-98% Lmax, defined below). The tungsten pins were 5 mm in length and 50 μm in diameter. The tip was electrolytically sharpened to a diameter less than 1 μm. The weight of the pin insertion appeared negligible because the developed steady-state force before (4.37 ± 1.00 g/mm², mean ± SD) and after pin insertions (4.29 ± 0.91 g/mm²) did not show any significant difference (p > 0.1 by paired t test). The distance between the pins was measured both above and below the muscle by the optical method described later.

The top surface of the tissue bath (10 mm x 40 mm x 12 mm) was then covered by a glass during the experiment to prevent the exchange of gases in the perfusate with room air. The perfusate entered the bath via 2 holes at the base and overflowed through 2 gaps in the end walls of the chamber through which the 2 rods were passed that connected to the force transducer at one end and the linear motor at the other.

After 1 more hour of equilibration, Lmax, which is defined as the muscle length at which developed twitch force was maximal, was determined by gradually stretching the papillary muscle over 5 minutes. Under this condition, Lmax, defined as the length of the central segment between the pins at its maximal shortening in the twitch, was also measured. Muscle diameter at Lmax was measured optically during the resting phase at the same length. The mean Lmax and the standard deviations from 13 muscles were 4.75 ± 0.92 mm, and the corresponding diameter values were 0.84 ± 0.12 mm (cross-sectional area 0.57 ± 0.16 mm²). The mean value of Lmax was 2.45 ± 0.33 mm (42.7 ± 3.1% of Lmax).

The criteria for accepting a given muscle preparation in this study were as follows: 1) diameter less than 1 mm, 2) maximal developed force at Lmax more than 4 g/mm², 3) resting force less than 17% of total force at Lmax, and 4) limited transverse inhomogeneity of fiber shortening represented by movements of the 2 pins (described in the next section). The muscles studied in the early phase of the series, for which the difference between the top and bottom pin distances was not examined, were excluded if inspection by video monitoring showed large relative rotation of either pin (indicating large transverse inhomogeneity).

Measurement Apparatus

A linear-variable differential transformer (LVDT) was used to continuously monitor the total muscle length. Muscle length was controlled by a Ling V-203 linear motor (Ling Dynamic Systems, Ltd., Royston, England) and a Crown DC-300A driver amplifier (Crown International, Inc., Elkhart, Ind.). Force was measured by a Kulite BG-25 strain gauge transducer (Kulite Semiconductors, Ridgefield, N.J.). The compliance of the system was less than 0.6 μm/g.

The segment length between 2 pins was obtained by measuring the distance between the optical images of 2 pins projected onto a Reticon 1024 photodiode array (EG&G Reticon, Sunnyvale, Calif.) with optical magnification of about 5 times. Overall, the system can resolve a change of 2 μm in segment length. Two pin distances, which were imaged on 2 arrays through a beam-splitter, were measured simultaneously (Figure 1). One pin distance was a projection of an imaginary segment of line drawn between the pins parallel to and approximately 0.5 mm above the top edge of the muscle; the other was a projection of a line segment 0.5 mm below the bottom edge (Figure 1). The central muscle segment length (Lc) was the average of these two distances. This averaged value was considered to represent the length of muscle segment coursing axially through the center of the papillary muscle.

In 7 muscles, a signal equal to half the difference between the pin distances measured above and below the muscle (ΔLc) was recorded. This signal was considered to represent the amount of transverse inhomogeneity of fiber shortening within the muscle (Figure 2). Transverse inhomogeneity will cause the 2 pins not to move in parallel because length changes in the upper half of the muscle will differ from those in the lower half. During sinusoidal oscillations in Ba2+ contracture, ΔLc averaged 31 ± 13% SD of ΔLc for the 7 muscles where ΔLc was measured. This degree of transverse inhomogeneity corresponds to one edge of the muscle oscillating with an amplitude 14% larger than along the axis and the opposite edge oscillating with 14% less amplitude than the axis. Thus, the aver-
FIGURE 1. Schematic representation of system to measure segment length between 2 tungsten pins. Lenses situated between lamp and beam splitter have been omitted for clarity. Pin distances above and below the muscle are measured separately by 2 photodiode arrays. L_t, average of 2 measured segment lengths; L_m, muscle length defined as length from the clamp to silk tie; ΔL_s(avg), change in the averaged segment length; ΔL_s(diff), half the difference between the changes in top and bottom pin distances.

age range of displacements (0.86–1.14%) was not so nonuniform. When ΔL_s(diff) exceeded 50% of ΔL_s(avg) during oscillations in contracture, the muscles were excluded from the study. However, during twitches, the transverse inhomogeneity was greater; ΔL_s(diff) averaged 57% of ΔL_s(avg). Although data from twitches were not analyzed, muscles with excessive twitch inhomogeneity (ΔL_s(diff) > 100% ΔL_s(avg)) were still excluded.

The electrical recording of both central segment length and overall muscle length was calibrated by optical measurements of these dimensions under a trinocular microscope (American Optical A0580, American Optical Corporation, N.Y.) with a calibration scale. The muscle preparation in the tissue bath was also continuously visualized by a video camera, recording, and display system (RCA TC2000, RCA Corporation, Lancaster, Pa.; Sony V02610, Sony Corporation, New York, N.Y.).

It is important to estimate the magnitude of the force required to accelerate the mass of the pins compared with the force oscillations generated within the muscle. The largest acceleration was estimated by considering the clamped end (which has the largest motion relative to the force transducer) of the longest muscle (8 mm) for the largest amplitude of oscillation (±1%), which is 2% peak-to-peak) at the highest frequency (30 Hz). For this case, calculating the maximum acceleration yielded a value equal to 30% of the acceleration due to gravity. Thus, the force required to accelerate a pin can be no more than 30% of the weight of the pin, i.e., 30% of 0.2 mg. This force is less than 1% of the smallest force oscillation ever observed (which approximated ±10 mg for the thinnest muscle tested at rest), and it is an even smaller fraction of the force oscillation at 30 Hz during contracture. Thus, any inertial effect due to the mass of the pins should be negligible.

Protocols

After the equilibration period in normal Tyrode's solution, the dynamic stiffness of resting muscle was studied by stopping stimulation for a short period and sinusoidally changing muscle length at 8 different frequencies ranging from 0.05 to 30 Hz (Figure 3). The amplitude of muscle length perturbation was adjusted so that the central segment length would change approximately 1% of L_m(max) at any of the different frequencies of perturbation. Stimulation was restarted as soon as the last frequency was measured. This protocol

![FIGURE 2. Records of central segment length, whole muscle length, and force during a twitch contraction with the ends of the muscle held fixed. F, total force produced by the papillary muscle; L_m(max) and L_s(max), muscle and central segment length at which developed twitch force is maximal. Other abbreviations are the same as in Figure 1. The central segment shortened about 4% L_s(max). Cross-sectional area at L_m(max) = 0.78 mm².](image-url)
FIGURE 3. Frequency responses of a muscle to small length perturbations in resting state (A), in Ba^2+ contracture (B), and in rigor state (C) at 95% Lmmax. Cross-sectional area at Lmmax, 0.50 mm². Relevant changes in muscle length and force, respectively, are indicated by numerical figures at the top and bottom, and the frequencies at which length was perturbed. Abbreviations are the same as Figures 1 and 2.
was repeated for 3 central segment lengths, i.e., 100, 95, and 90% $L_{max}$. Before inducing Ba$^{2+}$ contracture, the muscle was incubated in Ca$^{2+}$-free Tyrode's solution for 10–20 minutes until force development almost disappeared. Whereas a calcium-free environment may damage the plasma membrane of myocardial cells under some conditions, the subphysiologic temperature (24° C) used appears to protect the myocardium. BaCl$_2$ was then added to the Ca$^{2+}$-free Tyrode’s solution, and contracture developed over the next 5–10 minutes.

After contracture force had stabilized, the dynamic stiffness of 7 muscles was measured at 3 different lengths (100, 95, and 90% $L_{max}$) with the perfusate Ba$^{2+}$ concentration always at 0.5 mM. In another 4 muscles, the effects of Ba$^{2+}$ concentration (0.3, 0.5, and 1.0 mM) were examined with the central segment length always at 95% $L_{max}$. Experimental measurements in a given muscle were completed within 1 hour (average duration 41.5 ± 11.5 minutes, mean ± SD) after the induction of Ba$^{2+}$ contracture.

Under each condition of contracture, the muscle was perturbed by sinusoidal oscillations applied to its clamped end. Fortunately, for the small amplitude applied here, the oscillation of central segment length remained sinusoidal in form at all frequencies, as verified in Figure 4. Thus, sinusoidal oscillations could be produced indirectly in the central segment without having to control its length directly, e.g., via a feedback servo system. However, as frequency changed, the amplitude of oscillations applied to the whole muscle had to be varied to maintain the same amplitude of oscillation in the central segment. In summary, the sinusoidal oscillations induced in the central segment had an amplitude of approximately 1% $L_{max}$ (ranging from 0.7–1.2 $L_{max}$) and were at 15 different frequencies ranging from 0.05 to 30 Hz (Figures 3 and 4).

Small length step perturbations were also applied to some muscles in the resting state, Ba$^{2+}$ contracture, and rigor, and the force transient responses were obtained.

When the stiffness measurement during Ba$^{2+}$ contracture was completed, the muscle was perfused with glucose-free Ba$^{2+}$-Tyrode’s solution bubbled with 98% N$_2$-2% CO$_2$ for 1½ hours in order to produce a rigor state. Papillary muscles in rigor, with the segment length fixed at 95% $L_{max}$, were perturbed with sinusoidal length changes at 15 different frequencies in the same way as during Ba$^{2+}$ contracture (Figure 3).

**Data Analysis**

The muscle length ($L_m$) was defined as the length from the clamp to the silk tie and the central segment length ($L_{cseg}$) as the average of the distances between 2 pins measured above and below the muscle (Figure 1). The modulus of dynamic stiffness of muscle ($K_m$) and of central segment ($K_{cseg}$) were defined as follows:

$$K_m = \frac{\Delta F}{\Delta L_m}$$  

$$K_{cseg} = \frac{\Delta F}{\Delta L_{cseg}}$$

where $\Delta F$ is the amplitude of force oscillations, $\Delta L_m$ is the amplitude of length oscillation of the whole muscle, and $\Delta L_{cseg}$ is the amplitude of the length oscillation of the central segment. Each dynamic stiffness value was then normalized to the value that would be obtained if each unit (whole muscle or central segment) had a length of 10 mm and a cross-sectional area of 1 mm$^2$.

The time difference between the peaks of the oscillations in the force and central segment length was expressed as a phase shift ($\phi$). The phase shift ($\phi_m$) between the force and whole muscle length was similarly defined. A positive value of the phase shift means that the force wave leads the length wave. Time differ-

![Figure 4](http://circres.ahajournals.org/fig4/)

**Figure 4.** Frequency responses of muscle in Ba$^{2+}$ contracture at 0.4, 1, 1.2, 5, and 15 Hz. The amplitude of segment-length oscillations at 1 Hz was slightly larger than at other frequencies. Also note distortion of force waveform near 1 Hz: Force rose more slowly than its rate of decline at 1 Hz, but this pattern reversed at 1.2 Hz. Force calibration at left is full scale of 0.2 g and that at right is applied only for trace at 15 Hz. $L_1$, 95% $L_{max}$; $L_m$, 99.1% $L_{max}$; steady contracture force, 3.65 g/mm$^2$; $L_{cseg}$, 2.87 mm; $L_{max}$, 6.60 mm; cross-sectional area at $L_{max}$, 0.58 mm$^2$.
ence were corrected for the 1.3-millisecond delay in the muscle length signal and the 8.3-millisecond delay in the segment length signal produced by filters in the electronic instruments.

Since there were statistically significant differences between the dynamic stiffness of the central segment and whole muscle (see "Results"), the dynamic stiffness of the ends of the muscle was estimated. The end region was defined as the combination of both ends of the muscle outside the pins. Since the central segment and end region were arranged in series, the complex compliance of the whole muscle (1/Km) is equal to the sum of the compliances of the central segment and the end region (1/Kc and 1/Ke, respectively):

\[ 1/K_m = 1/K_c + 1/K_e. \]

In this equation, each K represents a complex value of stiffness. In terms of magnitudes (K) and phases (φ):

\[ -\cos \phi_e = (1 - \cos \phi_m - \cos \phi_c) = A \]
\[ K_c \]
\[ -\sin \phi_e = (1 - \sin \phi_m - \sin \phi_c) = B \]
\[ K_m \]
\[ K_e \]

From these intermediate results, the magnitude and phase shift (Kφ) of stiffness in the end region were calculated:

\[ K_e = 1/\sqrt{A^2 + B^2}, \quad \phi_e = \tan^{-1}(B/A) \]

When Km and Ke had nearly the same phase shift (<20° difference), Ke could be approximated without reference to phase shifts:

\[ K_e = K_m - K_n \]

Note that unnormalized values of Kc and Ke used to calculate Kφ, which was then normalized as described above.

The calculation of dynamic stiffness for the end region should be viewed as an approximation, and the potential limitations in the assumptions underlying this calculation should be borne in mind. First of all, everything outside the central segment was lumped into a single end region. Unlike the central segment, which several studies have shown to be relatively homogeneous, the end region is heterogeneous. It certainly contains a significant fraction of healthy tissue since there is no clearly discernible boundary between damaged and healthy tissue, but the proportion and organization of healthy vs. damaged tissue could vary greatly between preparations. There is also considerable geometric nonuniformity produced by the clamp and the natural tapering of the muscle at its tendinous end.

Secondly, the calculation assumes only the central segment and end region in series and no other structures in parallel. This would require that the endothelial membrane, connective tissue matrix, and other vertical planes of tissue (parallel to the one measured with the pins) all deform longitudinally in the same way as the column of myocardial cells between the pins. There was no evidence that these assumptions were grossly violated. On the other hand, with no sensitive way to test whether the assumptions were correct, an influence from parallel structures cannot be ruled out.

Because of the similar shape of the stiffness modulus-frequency curves for the central segment and whole muscle, three stiffness moduli (Kcm, Kc, Kc) were selected for statistical comparisons. Kcm was the average of modulus values at 0.1 and 0.2 Hz. Kc was the minimum value of stiffness, which usually occurred near 1 Hz. Kc was the average stiffness at 20 and 30 Hz. To compare the frequency-dependent phase shift, the values of phase shift at 0.4 Hz (φc) and 4 Hz (φc) were used.

Analysis of variance, Duncan's multiple comparison method, and t test were used for statistical analysis of the results. A p value of 0.05 was chosen as the threshold of statistical significance. Since the stiffness modulus and phase shift of the end region are functions of (and thereby dependent on) those of the central segment and the whole muscle, only the variables of the central segment were compared statistically with those of the whole muscle using analysis of variance. For the data from contracture, the logarithmic values of stiffness modulus in the analysis of variance were used because a wide range of stiffness values needed to be compared. All values are reported as mean ± SD in this paper.

Results

Twitch Contractions

Thirteen papillary muscles met the criteria for acceptance and were analyzed in this study. Developed force during twitches averaged 4.91 ± 0.68 g/mm² at Lmax, while the resting force was 0.65 ± 0.047 g/mm² (11.9 ± 2.9% of the total force). With the muscle held isometric at Lmax, the central segment shortened 4.4 ± 1.5% (n = 11) of its resting length. At 82% of Lmax, the muscles still developed about half the force of Lmax. An example of isometrically twitching muscle, showing a shortening of the central segment, is presented in Figure 2. The amount of central segment shortening depended on the muscle length; there was more shortening at lengths below Lmax. Shortening was maximum (6.9 ± 1.1%) at a length of 90% Lmax, the same length at which Huntsman et al observed maximum shortening. The 2 pins did not move completely in parallel. There was always a slight transverse inhomogeneity in muscle fiber movement as indicated by finite magnitudes of Ldiff (Figure 2). The amount of Ldiff varied with muscle length even in the same specimen.

Properties of Muscle in Barium Contracture

With a Ba²⁺ concentration of 0.5 mM, the average total contracture force of 9 muscles at Lmax was 3.59 ± 0.84 g/mm², which was 63.9% of the total peak force of isometric twitch contractions (5.56 ± 0.63 g/mm², n = 9). As contracture developed after adding
Ba\textsuperscript{2+}, the central segment shortened 6.9 ± 1.5% below its previous length when there was no bathing Ca\textsuperscript{2+} and little developed tension.

As length was oscillated at approximately 1% amplitude, the force oscillation generated by the muscle showed a dramatic change in amplitude with the frequency of length perturbation (Figures 3B and 4). At lower frequencies, the amplitude of force was small, and it became smaller as the frequency increased. It reached a minimum value at about 1 Hz and thereafter rapidly increased with frequency until 20–30 Hz, where force no longer increased so markedly with frequency. The waveform of force output was almost sinusoidal except at about 1 Hz, at which frequency the force waveform was slightly distorted (Figure 4). The mean force level during length oscillation did not change at lower frequencies, but it dropped at high frequencies (10–30 Hz, Figure 3B). At 30 Hz, mean force was 10.9 ± 4.3% less than the steady force level with no oscillations.

The frequency spectrum of dynamic stiffness, (ΔF/ΔL), showed a prominent frequency dependence (Figure 5), reflecting the dramatic changes in the amount of force output with frequency. At low frequencies (below 0.4 Hz), the stiffness modulus was nearly constant. The stiffness modulus rapidly decreased above 0.4 Hz and reached a minimum value at 1–1.5 Hz. The frequency at which minimum stiffness occurred was labelled $f^\text{\text{*}}$. From that point, stiffness increased first rapidly, then gradually to 30 Hz.

The phase shift between force and segment length oscillations also demonstrated a marked frequency dependence (Figure 5). Phase shift was either slightly positive (force change occurred before length change) or negative (force lagged behind length) below $f^\text{\text{*}}$. Above that frequency, phase shift abruptly became much more positive over a narrow range of frequencies between 1 and 2 Hz. Over the range 2–4 Hz, in which the stiffness modulus increased markedly, the phase shift reached a maximal value (>70°) and thereafter gradually decreased toward zero.

The strong frequency dependence of stiffness modulus and phase shift was a characteristic feature of muscles in Ba\textsuperscript{2+} contracture and was not seen either in resting muscle or in rigor muscle (see below).

Comparison of Dynamic Stiffness Between Central Segment and Whole Muscle During Barium Contracture

Whereas the frequency spectra showed qualitatively similar patterns among the central segment, the whole muscle, and the end region, there were significant quantitative differences among them. There were differences in length oscillation between the central segment and whole muscle. For example, at low frequency, a 1% oscillation of muscle length produced a larger (1.36%) amplitude of oscillation in central segment length. Moreover, the amplitude of central segment length oscillations often increased even further (range of percent increase 0–25%) near 1 or 1.5 Hz despite a constant amplitude of whole muscle oscillation (Figure 4). At high frequency, the amplitude of muscle length oscillation had to be increased to maintain the same amplitude of central segment oscillation as at low frequency. Also, at high frequency, the mean length of the central segment increased slightly (e.g., by 0.35 ± 0.17% of $L_{	ext{max}}^\text{S}$ at 30 Hz). The amount of transverse inhomogeneity (ΔL$_{\text{trans}}$) also varied slightly with frequency; it tended to become slightly larger at 1–1.5 Hz (Figure 3B).

Figure 5 presents representative stiffness and phase shift spectra as measured for the central segment and whole muscle and as estimated for a lumped end region. Below 10 Hz, the modulus of central segment stiffness was always less than that of the whole muscle, suggesting that the lumped end region would have an even greater stiffness. The maximal amount of positive phase shift of the central segment exceeded that of the whole muscle, suggesting an even smaller phase shift for the end region. These were common findings in all 13 specimens.

Statistical analysis of dynamic stiffness for all muscles (at 0.5 mM Ba\textsuperscript{2+} and 95% $L_{\text{max}}^\text{S}$) is shown in Table 1. The stiffness modulus at low frequencies ($K_{\text{low}}$) and the minimum stiffness ($K_{\text{min}}$) of the central segment
Table 1. Stiffness Modulus, Ratio $K_{low}/K_{max}$, and Phase Shift of Muscles in Ba$^{2+}$ Contracture

<table>
<thead>
<tr>
<th>Variables</th>
<th>Central segment</th>
<th>Whole muscle</th>
<th>End region</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{low}$ (g/mm)</td>
<td>1.95 ± 0.58*</td>
<td>2.65 ± 0.60</td>
<td>4.04 ± 1.60</td>
</tr>
<tr>
<td>$K_{max}$ (g/mm)</td>
<td>1.09 ± 0.44*</td>
<td>1.58 ± 0.49</td>
<td>2.85 ± 1.61</td>
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<tr>
<td>$K_{ns}$ (g/mm)</td>
<td>13.62 ± 4.11</td>
<td>13.18 ± 3.24</td>
<td>13.68 ± 4.33</td>
</tr>
<tr>
<td>$K_{ns}/K_{low}$</td>
<td>0.560 ± 0.148</td>
<td>0.594 ± 0.122</td>
<td>0.677 ± 0.127</td>
</tr>
<tr>
<td>$f_{min}$ (Hz)</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>$\phi_4$ (degrees)</td>
<td>-0.1 ± 4.8</td>
<td>-0.1 ± 4.8</td>
<td>0.3 ± 4.3</td>
</tr>
<tr>
<td>$\phi_f$ (degrees)</td>
<td>82.5 ± 9.4*</td>
<td>72.7 ± 7.7</td>
<td>57.8 ± 14.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 13). *Implies a significant difference (p < 0.05) between central segment and whole muscle. $K_{low}$, stiffness modulus at low frequencies (0.1 and 0.2 Hz); $K_{min}$, minimum modulus of stiffness; $K_{ns}$, stiffness modulus at high frequencies (20 and 30 Hz); $f_{min}$, frequency at minimum modulus of stiffness; $\phi_4$, phase shift at 0.4 Hz; $\phi_f$, phase shift at 4 Hz. Positive value of phase shift means that force wave leads length wave.

were significantly smaller (p < 0.01) than those of the whole muscle. However, there was no significant difference at high frequency ($K_{ns}$) between the segment and the whole muscle. Also, $f_{min}$ for the central segment and the whole muscle were not statistically different. The phase shift at 4 Hz ($\phi_f$) of the central segment was significantly larger than that of the whole muscle ($p < 0.025$), but at 0.4 Hz ($\phi_4$), there was no difference. The relative change of stiffness from low to high frequencies, as expressed by the ratio $K_{ns}/K_{low}$, was significantly higher for the central segment than for the whole muscle ($p < 0.01$). These tendencies were maintained at other muscle lengths (90 and 100% $L_{max}$) and at different Ba$^{2+}$ concentrations (0.3 and 1.0 mM).

In summary, the central segment showed more prominent frequency dependent changes in stiffness modulus and phase shift than the whole muscle. In other words, the stiffness of the end region of contracted muscle must have varied less with frequency and attenuated the frequency dependence of whole muscle stiffness compared with the central portion.

Effect of Barium Concentration

Total force of muscles (n = 4) in Ba$^{2+}$ contracture at 95% $L_{max}$ with Ba$^{2+}$ concentrations of 1, 0.5, and 0.3 mM were 3.37 ± 0.55, 2.35 ± 0.50, and 0.92 ± 0.54 g/mm$^2$, respectively.

The frequency spectra of central segment stiffness and phase shift were examined for the three different concentrations of barium. In a typical muscle, the stiffness modulus curve shifted upward roughly uniformly with an increase in Ba$^{2+}$ concentration (see Figures 6 and 7). Therefore, $K_{low}$, $K_{min}$, and $K_{ns}$ all significantly increased with increase in Ba$^{2+}$ concentration (as summarized in Figure 6A), but the ratios $K_{ns}/K_{low}$ and $K_{ns}/K_{max}$ showed no significant difference. The spectra of phase shifts for different Ba$^{2+}$ concentrations were almost superimposable so that $\phi_4$ and $\phi_f$ for three Ba$^{2+}$ concentrations were not statistically different.

The $f_{min}$s for different Ba$^{2+}$ concentrations were the same (mean value 1.4 Hz). Thus, changes in Ba$^{2+}$ concentration affected only the level of stiffness moduli but did not change the shape of the stiffness or phase shift spectra.

In summary, the central segment showed more prominent frequency dependent changes in stiffness modulus and phase shift than the whole muscle. In other words, the stiffness of the end region of contracted muscle must have varied less with frequency and attenuated the frequency dependence of whole muscle stiffness compared with the central portion.

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Total force of muscles (n = 4) in Ba$^{2+}$ contracture at 95% $L_{max}$ with Ba$^{2+}$ concentrations of 1, 0.5, and 0.3 mM were 3.37 ± 0.55, 2.35 ± 0.50, and 0.92 ± 0.54 g/mm$^2$, respectively.

The frequency spectra of central segment stiffness and phase shift were examined for the three different concentrations of barium. In a typical muscle, the stiffness modulus curve shifted upward roughly uniformly with an increase in Ba$^{2+}$ concentration (see Figures 6 and 7). Therefore, $K_{low}$, $K_{min}$, and $K_{ns}$ all significantly increased with increase in Ba$^{2+}$ concentration (as summarized in Figure 6A), but the ratios $K_{ns}/K_{low}$ and $K_{ns}/K_{max}$ showed no significant difference. The spectra of phase shifts for different Ba$^{2+}$ concentrations were almost superimposable so that $\phi_4$ and $\phi_f$ for three Ba$^{2+}$ concentrations were not statistically different.

FIGURE 6. Summary of mean values for modulus of dynamic stiffness from all preparations. Stiffness at low, minimum (middle), and high frequencies for muscle in Ba$^{2+}$ contracture, resting state, and rigor. (A) Effect of Ba$^{2+}$ concentration on the stiffness moduli, muscles at 95% $L_{max}$. (B) Effect of central segment length on the stiffness moduli, muscles in contracture at 0.5 mm Ba$^{2+}$. (C) Muscles in rigor and at rest (Solid circles, stiffness modulus of rigor muscles at 95% $L_{max}$; open symbols, stiffness modulus of resting muscles at different central segment lengths). Bars show standard deviations.
Figure 7. Typical example of the effect of Ba\textsuperscript{2+} concentration on dynamic stiffness curves of the central segment. Solid square, 1 mM; solid circle, 0.5 mM; solid triangle, 0.3 mM; open circle, resting muscle; steady contracture forces, 3.43, 3.05, and 0.65 g/mm\textsuperscript{2} at 1, 0.5, and 0.3 mM Ba\textsuperscript{2+}; resting force, 0.05 g/mm\textsuperscript{2}; L\textsubscript{m}, 95\% L\textsubscript{m\textsuperscript{max}}; L\textsubscript{m\textsuperscript{max}}, 4.60 mm; cross-sectional area at L\textsubscript{m\textsuperscript{max}}, 0.65 mm\textsuperscript{2}.

Effect of Muscle Length on Muscle Stiffness in Barium Contracture

Total forces of muscles in 0.5 mM Ba\textsuperscript{2+} contracture (n = 7) at L\textsubscript{m\textsuperscript{max}}, 95\% L\textsubscript{m\textsuperscript{max}}, and 90\% L\textsubscript{m\textsuperscript{max}} were 3.79 ± 0.85, 3.14 ± 0.78, and 2.55 ± 0.70 g/mm\textsuperscript{2}, respectively.

Figure 8 shows a typical example of the effect of muscle length on the dynamic stiffness of the central segment, and these trends are summarized in Figure 6B. Consistent with the increase of mean force at longer lengths, K\textsubscript{y} became significantly greater with each increase in length. At low frequency, on the other hand, there was no significant difference in stiffness between 90 and 95\% L\textsubscript{m\textsuperscript{max}}. The significantly greater K\textsubscript{y} at L\textsubscript{m\textsuperscript{max}} perhaps reflects an underlying curvilinear force-length relation. The relative decrease of stiffness at f\textsubscript{min} (K\textsubscript{min}, K\textsubscript{y\textsuperscript{min}}) was the same at all lengths. While \phi\textsubscript{y\textsuperscript{max}} did not show any significant difference with length, in the frequency range of large phase shifts, \phi\textsubscript{y\textsuperscript{max}} (76.7 ± 9.9°) was significantly smaller than those of 95\% L\textsubscript{m\textsuperscript{max}} (87.6 ± 9.4°) and 90\% L\textsubscript{m\textsuperscript{max}} (84.8 ± 15.2°).

High-Frequency Stiffness vs. Force

The increase in high-frequency stiffness with changes in Ba\textsuperscript{2+} concentration or muscle length suggested that K\textsubscript{y} might be governed by the level of force in either condition, much as it is in skeletal muscle. Therefore, Figure 9 plots stiffness at 30 Hz vs. the mean level of force during oscillation at 30 Hz. Comparing the effect of changing muscle length (open symbols) with the effect of changing Ba\textsuperscript{2+} concentration (solid symbols) showed no statistical difference between the coefficients of linear regression in the two cases. For the combined data set, the correlation between stiffness and tension (force per cross-section) was K\textsubscript{30Hz} = 5.5F + 0.6 (r = 0.86).

To interpret this correlation, the size of a sudden shortening step that would be required to bring force instantaneously to zero was computed. Assuming a linear relation between force and length during sudden shortening, from the definition of stiffness, the equation: stiffness = (original force − 0)/(shortening step) solves to shortening step = original force/stiffness. Figure 9 shows that force and stiffness maintain approximately the same ratio at all points. Consequently, the amount of sudden shortening required to reduce force to zero would be approximately the same for all
conditions regardless of segment length or activation. The required size of such a sudden shortening step was estimated as follows: if x is the mean tension g/mm², stiffness would be 5.5 x g/mm. Since this stiffness pertains to a normalized "standard" muscle of 1 mm² area, the tension corresponds to a total force of x grams. Thus, the shortening step = x grams/5.5 x g/mm² = 0.18 mm. Relative to the normalized length of the "standard" muscle (10 mm), this is a change of 1.8%.

Resting Muscle Properties

Resting forces (n = 8) at Lₚₘₐₓ, 95% Lₚₘₐₓ, and 90% Lₚₘₐₓ were 0.26 ± 0.09, 0.14 ± 0.08, and 0.10 ± 0.09 g/mm², respectively.

The stiffness spectra of resting muscles were almost rectilinear (Figures 7 and 8). Stiffness increased only slightly as the frequency of length oscillations became higher, and phase shift was fairly constant over all the frequencies. As Figure 6C summarizes, Kₚₐₜ of Lₚₘₐₓ was significantly greater than at 95% Lₚₘₐₓ, but there was no significant difference between 95% Lₚₘₐₓ and 90% Lₚₘₐₓ. No significant differences could be shown with respect to φ₀ₐₜ or φₐₜ. The two nonlinear responses seen during contracture, i.e., distortion of force waveform at around 1 Hz and a decrease in mean force level during rapid oscillations, were not detected in resting muscle.

There was no significant difference between the stiffness of the central segment and that of the whole muscle over the entire range of frequencies examined.

Dynamic Stiffness of Muscle in Rigor

The total force of muscles (n = 4) in 1 mM Ba²⁺ contracture at 95% Lₚₘₐₓ before incubation in hypoxic Ba²⁺ Tyrode's solution was 3.35 ± 0.52 g/mm². The total force rose slightly immediately after the onset of perfusion with the hypoxic solution and then decreased significantly over the subsequent 10–20 minutes. Total force gradually increased again over the following 30–60 minutes and finally reached a new steady level of 1.94 ± 0.59 g/mm².

The amplitude of force oscillations did not show much difference over all frequencies. Therefore, the stiffness modulus curve was almost flat (Figure 6C). On average, Kₚₐₜ of rigor was much larger than that of contracture (22.5 vs. 12.3 g/mm) despite the lower force level. The phase shift value was slightly positive (6–7°) and did not show substantial change over the frequency range. Thus, muscle in rigor showed little dependence of stiffness and phase on frequency, just as in the resting state. Also similar to resting muscle, neither nonsinusoidal distortion of force waveforms nor a decrease in mean force during rapid oscillations was observed in rigor (Figure 3).

In contrast to muscles in Ba²⁺ contracture and at rest, the central segment of muscle in rigor was always 50% stiffer than the whole muscle at all frequencies.

Step Perturbation of Muscle Length

Figure 10 illustrates changes in the central segment length and force that follow a step change in muscle length by about 1% of Lₚₘₐₓ. Such length steps were applied to resting muscles, muscles in Ba²⁺ contracture, and muscles in rigor. The force transient responses were similar to those reported earlier.¹¹⁷ Muscles in Ba²⁺ contracture showed a quite different force transient response from that seen in resting and rigor muscles. This transient consisted of an instantaneous force change, a rapid force recovery, and a complex approach to the final steady level that was composed of a delayed force response and damped oscillations. The trace of central segment length was steplike in the resting condition and rigor state but not in Ba²⁺ contracture. After a rapid initial change that overshot the eventual equilibrium segment length, the central segment length showed several damped oscillations that seemed to be associated with the force oscillations.

Discussion

Barium as an Activator

Ba²⁺ contracture was chosen as a means to activate heart muscle steadily for a period long enough to measure several frequency spectra of stiffness under varying conditions. Barium ion is believed to produce a steady force because it is not sequestered as avidly as calcium by sarcoplasmic reticulum after entering the cardiac cell through the same channel as calcium ion.¹⁹ The mechanism by which barium activates the contractile proteins seems to be similar to that of calcium since barium has an affinity to troponin C and can induce force in glycereinated cardiac muscle. Besides, transient force responses for stepwise length perturbations of cardiac muscle in Ba²⁺ contracture¹⁷ and Ba²⁺-activated glycereinated cardiac muscle are similar to...
those of Ca2+-based activated glycerinated cardiac muscles.

Ba2+ produces a very stable level of force for more than 1 hour. However, 40–60 minutes after the onset of Ba2+ contracture, changes were observed in the spectrum of dynamic stiffness that indicated a potential deterioration in the physiologic state of the muscle. The main changes were as follows: 1) stiffness at lower frequencies increased, 2) the dip of the stiffness modulus curve around 1 Hz became shallower and finally disappeared, and 3) the maximum value of positive phase shift became smaller. All contracture data were obtained before these changes occurred. They are probably caused by formation of rigor complexes at some of the contractile reactive sites. The rigor complexes may be indirectly induced by a long exposure to Ba2+ in the cytoplasm. For this reason, all the data reported here were obtained no more than 1 hour after inducing Ba2+ contracture.

Properties of Muscle in Barium Contracture

Dynamic stiffness during Ba2+ contracture should reflect the mechanical behavior of cardiac myofilaments. Contracture offers a direct view of myofilament mechanics without the intervening screens of excitation-contraction coupling or the activating and relaxing processes. Different portions of the dynamic stiffness spectrum appear to be related to different mechanical properties of the myofilaments. For this discussion, the spectrum is divided into 4 regions: 1) low frequency stiffness, which relates to the incremental slope of the force-length relation; 2) the region of the dip in stiffness, which seems to reflect active cycling of force generators; 3) the rise in stiffness with frequency over the range above \( f_{on} \) that is expected from the force-velocity property, and 4) the very high frequency range, over which the effective series elasticity of the muscle absorbs length oscillations and stiffness tends toward a plateau.

At low frequencies (0.05–0.2 Hz), force and length oscillated in synchrony (phase shift almost zero), and stiffness did not vary with frequency to any significant degree. Both of these phenomena are characteristic of purely elastic behavior, for which force is only governed by the length of the sarcomeres. Thus, the muscle appears to be oscillating along its force-length relation. The data from different lengths suggest that such a relation between total force and length in Ba2+ contracture would curve away from the length axis as force and length increased because the slope, i.e., stiffness, was greater at 100% \( L_{max} \) than at lower lengths.

If elasticity, i.e., the force-length relation, were the only property governing muscle behavior, then stiffness would not change as frequency increased, and phase shift would remain near zero. Indeed, that was nearly the case in our data both at rest and during rigor. Active muscles, on the other hand, exhibited large changes in dynamic stiffness with frequency. The apparent viscoelasticity of muscle is the source of much of the change in stiffness. However, the dip in stiffness near 1 Hz and the rapidity of phase changes at and slightly above the dip frequency are not consistent with standard viscoelastic behavior. In our opinion, the dip in stiffness is related to active cycling of cross-bridges within the myofilaments. It is a characteristic that has been found in all active muscles for which dynamic stiffness has been measured.

The frequency at which the dip in stiffness occurs may be related to the rate of contractile reactions within the myofilaments. An important result from this study was the constancy of this frequency despite...
changes in length or the degree of activation. This finding suggests that the dip frequency may be a characteristic of the individual force-generating units regardless of how many units are operating at one time or the degree of overlap between the thick and thin filaments. When the basic rate of such contractile units is altered (via changes in temperature or the isozymic structure of myosin), it has been shown\(^2\) that the dip frequency shifts as expected. Although a frequency near 1 Hz may seem surprisingly slow to be characteristic of cross-bridge rates, it is near the range measured for other slow muscles. For example, Kawai\(^24\) found the dip frequency for a slow skeletal muscle (soleus) from the rabbit to be even lower (0.5 Hz) at only a slightly lower temperature (20°C).

At frequencies between 2 and 8 Hz, force oscillations occurred ahead of length oscillations by approximately one quarter of a cycle. With this phase shift, the moment of peak force would coincide with the moment of peak velocity of lengthening, and the moment of minimum force would coincide with the moment of peak velocity of shortening. This region of the dynamic stiffness spectrum, therefore, seems to reflect the force-velocity relation of muscle. Further support for this inference is provided by the increase in amplitude of force oscillation (and thus stiffness) over this frequency range. This correlated with the increase in the maximum velocity of lengthening or shortening expected for sinusoidal length oscillations of constant amplitude (such as were used in this study) as frequency increased.

How does the ability of Ba\(^{2+}\)-contractured muscle to shorten rapidly compare with shortening ability in other active states? At 10 Hz, a 1% peak-to-peak length oscillation generates a peak velocity of shortening of approximately one-third \(L_{\max}\) per second. This velocity would be expected to reduce force below 60% of its isometric value for either twitching\(^2\) or tonically active rabbit myocardium at 24°C.\(^26\) Yet, in our studies, the minimum force during oscillation never fell below 60% of its isometric value. Evidently, the Ba\(^{2+}\)-contracted muscles had the ability to shorten at least as fast as normally activated muscle. Nevertheless, a strong influence of velocity on force is evident in the stiffness spectrum.

Above 10 Hz, stiffness failed to rise as quickly with frequency as it did from 2 to 8 Hz, and the phase difference between force and length oscillations diminished (see Figure 5). These phenomena are evidence for an elastic element that absorbs an increasing portion of the applied length oscillation as frequency increases above 10 Hz. Note that this elasticity is a property of the central segment itself and cannot be due to the end regions because in our study segment length was measured directly. It is likely that the main source of this elasticity resides within cross-bridges just as has been shown for skeletal muscle.\(^27\) Consistent with this suggestion is our observation that high-frequency (30 Hz) stiffness was nearly proportional to mean force under all conditions of length or activation in Ba\(^{2+}\) contracture. The scatter about the regression line in Figure 9 may be largely due to measurement errors, but a small intrinsic variation with conditions cannot be ruled out. For instance, in rigor, there was more stiffness (relative to the level of force) than in contracture.

Our estimate of the amount of sudden elastic recoil that would make force zero (1.8% of \(L_{\max}\)) was very close to the estimate (1.6%) from a study using sarcoplasmic length measurements.\(^28\) However, these values are still almost double the recoil that is observed in skeletal muscles (less than 1% of \(L_{\max}\)). There is also one report of an equally small recoil in cardiac muscle.\(^29\) Because stiffness often failed to reach a final plateau by 30 Hz, the stiffness of the element responding to sudden changes was almost certainly underestimated, and thus its recoil was overestimated. The actual value is probably closer to that for skeletal muscle, although whether it is identical is uncertain from our results.

Several other phenomena were noted that appear to relate to the active processes of muscle contraction but are not expressed by the dynamic stiffness spectrum. For example, there was mild distortion of the force waveform near the dip frequency. Also, at high frequencies of perturbation, the mean force level during oscillation decreased but then recovered quickly when oscillation was stopped. These two phenomena were unique features of activated muscles in Ba\(^{2+}\) contracture; they were absent in the resting and rigor states. The decrease in mean force level at high frequencies of perturbation is likely to be related to the uncoupling effect\(^30\) caused by rapid stretches or releases.

**Comparison With Earlier Studies Based on Frequency Response Method**

Qualitatively, the stiffness and phase shift spectra measured in the central segment are similar to the results reported by earlier investigators,\(^1\) who measured only total muscle length. However, a quantitative comparison suggests distortions in the earlier data. For instance, high-frequency stiffness was only 4 times larger than at low frequency (at 96% \(L_{\max}\)),\(^2\) compared with 7 times larger in the present segment data (at 95% \(L_{\max}\)). Similarly, the phase shift spectra in that study did not show as abrupt or large a rise (between 1 and 2 Hz) as in the present segment data. Thus, it appears that the damaged ends induced distortions in the dynamic stiffness curves obtained in the previous studies that measured only total muscle length. These distortions seem to be quantitatively similar to the difference noted in our study between whole muscle and central segment stiffness.

Another difference between previous results\(^2\) and this study concerns the magnitude of stiffness. Their resting stiffness was an order of magnitude greater than was found in the present study (10 g/mm at \(L_{\max}\), vs. 0.8 g/mm at \(L_{\max}\)). One probable reason for this difference is the higher resting force in their report. Another reason may be a difference in the definition of the 100% reference length; our study used the central segment length at peak force (which is shorter than its resting...
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end region to lapse more into rigor and thus made it contracture may have induced the already damaged change in stiffness. Finally, it is possible that Ba\textsuperscript{2+} was stiffer.

pin signals on the oscilloscopes and the image of muscles that were clamped at both ends.\textsuperscript{6-9} However, the incremental stiffness in relation to the oscillation amplitude over the range 0.5-2.0% L\textsubscript{Jmu}

Surprisingly, however, the incremental stiffness in response to small length oscillations during Ba\textsuperscript{2+} contracture was significantly greater in the end region than the central segment for frequencies less than 10 Hz.

Perhaps, the stretch of the end region placed it at a higher concentration of Ba\textsuperscript{2+} and partial rigor.

Several previous studies have also applied steplike changes in muscle length.\textsuperscript{1,17} However, when such a perturbation was applied in our investigation, a sharp steplike change in the length of the central segment could not be obtained. Segment length always overshot its eventual equilibrium length and then oscillated in a damped manner. The potential influence of such an artifact on studies measuring transient changes in force, length, or velocity following abrupt steps needs to be evaluated.

**Longitudinal and Transverse Inhomogeneity**

In our muscle preparations, the tendinous end of the muscle was tied with thin silk, and the nontendinous end was held by a clamp. We recognized, as did Julian and Sollins,\textsuperscript{3} that central segment shortening during twitches resulted predominantly from stretching of the nontendinous end of the muscle as observed from the 2 pin signals on the oscilloscopes and the image of muscle and pins on the microscope-video-TV monitoring system. This observation suggests that in our preparations, damage in the tendinous end was minimal and the small amount of lengthening of this portion during isometric contraction was caused mainly by the high stress due to the small cross-section of this end. That the clamped end of the preparation was predominantly stretched during twitch contraction could be one factor to explain why the shortening of the central segment of our preparation was only about half of that measured in muscles that were clamped at both ends.\textsuperscript{6-9} However, Julian and Sollins\textsuperscript{3} reported a fairly large amount of shortening (10-12%) of central sarcomeres despite using a method of mounting similar to ours.

Similar to the stretch of the ends during twitches, the lumped end region in our study also stretched as contracture force was developing. This observation might be interpreted as suggesting that the ends would be more compliant than the undamaged central segment. Surprisingly, however, the incremental stiffness in response to small length oscillations during Ba\textsuperscript{2+} contracture was significantly greater in the end region than the central segment for frequencies less than 10 Hz. Perhaps, the stretch of the end region placed it at a length where its incremental force-length relation was steeper than in the central segment. Additionally, some of the shortening of the central segment may have been due to a greater ability of the healthy center to decrease its unstressed length with no requisite change in stiffness. Finally, it is possible that Ba\textsuperscript{2+} contracture may have induced the already damaged end region to lapse more into rigor and thus made it stiffer.

In the damaged end regions, more force seems to be borne by passive means (either rigor bonds or resting properties) rather than by actively cycling cross-bridges. Two observations support this hypothesis: 1) Compared to the center, the stiffness of the end region varied less with frequency (Figure 5). Recall that for whole muscles in both rigor and resting states, stiffness changed only slightly over the entire frequency range. 2) Less uncoupling due to rapid displacements seemed to occur in the end regions. Recall that during high-frequency perturbations the mean length of the central segment had to increase to balance the mean force borne by the end regions, which must not have lost as much contractile ability during the oscillations.

Transverse inhomogeneity, i.e., variation in length changes at different sites across the diameter of the muscle, was also observed in the central segment. Most of the transverse inhomogeneity may be related to misalignment of the mounted muscle and inappropriate pin insertion. It is difficult to mount a muscle so perfectly that all parts of its cross-section receive the same stretch that they would in vivo. Fortunately, the transverse inhomogeneity during sinusoidal perturbation of muscles at rest, in Ba\textsuperscript{2+} contracture, and in rigor was much less than that during twitches.

It is not felt that the amount of transverse inhomogeneity that we observed would affect the stiffness data. For example, the amount of transverse inhomogeneity did not vary much with frequency despite the order of magnitude changes in dynamic stiffness. Furthermore, if most of the transverse inhomogeneity were due to transverse differences in mounting at the ends rather than inhomogeneity in myocardial properties across the muscle, then there would only be transverse differences in the average length and the amplitude of oscillation of fibers in the central segment. In other studies with the same technique,\textsuperscript{21} changes in oscillation amplitude over the range 0.5-2.0% L\textsubscript{max} did not greatly affect the dynamic stiffness.

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