Length-Induced Changes in Activation during Contraction

A Study of Mechanical Oscillations in Strontium-Mediated Contractions of Cat and Frog Heart Muscle

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SUMMARY The plateau phase of prolonged Sr-mediated contractions of preparations of cat and frog heart muscle was used to study the transient response to abrupt changes in load or length. An oscillatory response (total amplitude <5% \( L_{\text{max}} \)) was obtained. Isotonic oscillations were less damped than their isometric counterparts, implying positive feedback and thus a causal role of the perturbation in length. Oscillation frequency was 2-3 Hz at 29°C (\( Q_{10} \approx 2-3 \)); it could be increased by epinephrine or caffeine, independently of their effects on extent of shortening; it otherwise changed as a generally constant function of the length at which the oscillation occurred, whether this was altered by changes in extracellular [Sr], frequency of contraction, or load (independent of the direction and magnitude of the preceding load step). Similar oscillatory responses were induced during Sr- or Ca-mediated contractures. Cat muscles showed an additional slower component to the oscillatory response. Transient augmentation of the velocity-length relationship after abrupt reduction in load, previously described for twitch contractions under certain conditions, appears to be analogous to the first phase of the oscillatory response studied here. Our findings indicate that the oscillation is not attributable to any mechanism intrinsic to myofilament interaction, but rather that it involves length-induced changes in the level of activation, probably mediated by Ca\(^{2+}\) or Sr\(^{2+}\). We conclude that length influences the level of activation during contraction of heart muscle.

IN MAMMALIAN heart muscle, shortening velocity at any given constant load appears to be related more to the instantaneous length than to the time at which it is measured.\(^1\) \(^2\) This observation suggests that length influences mechanical activity during isotonic contraction as well as through its relationship to isometric tension development. Experiments showing that core myofibrils of skeletal muscle fibers are deactivated at short length in a manner that can be prevented by caffeine,\(^3\) and that the shape of the isometric length-active tension curve for heart muscle is related to inotropic state,\(^4\) suggest moreover that the influence of length may be mediated at least in part through changes in activation.

We have further explored this question by studying the transient response to abrupt changes in length and load imposed during contraction; we used both cat and frog preparations because of known differences between them in excitation-contraction coupling.\(^5\) \(^7\) This experimental approach has not been readily applicable to normal twitch contractions of heart muscle, but we have used strontium-mediated contractures. These have a prolonged plateau phase of steady state mechanical activity, but are analogous to normal contractions in that they respond to the usual inotropic interventions and each is associated with a single, correspondingly prolonged action potential.\(^6\) \(^9\) Abrupt changes in load during the plateau phase of these contractions induced mechanical oscillations. It will be shown that the characteristics of these oscillations are such as to imply that they reflect changes in the level of activation of the contractile apparatus. This leads to the conclusion that muscle length itself influences activation during a contraction of heart muscle.

Methods

Cat right ventricular papillary muscles and frog (Rana esculenta) ventricular strips were prepared and mounted in temperature-controlled muscle baths, containing 45 ml of bicarbonate buffer equilibrated with 95% \( O_2 \) and 5% \( CO_2 \) and maintained at 29°C, as previously described.\(^10\) \(^11\) The buffer contained (mm): NaCl, 118; KCl, 4.7; MgSO\(_4\)\(\cdot\)7\(H_2O\), 1.2; KH\(_2PO_4\), 1.1; NaHCO\(_3\), 24; CaCl\(_2\)\(\cdot\)2\(H_2O\), 2.5; and glucose, 4.5. pH was 7.4. The muscle was clipped to an underwater force transducer\(^12\) (compliance, 3 \(\mu\)m/g) and tied with a 5-cm length of thread (Tevdek 70) to an overhead electromagnetic lever system\(^13\) through which the force applied to the muscle was controlled electronically by one of two circuits which could be abruptly (in less than 5 msec) switched by a signal from the stimulator. Total compliance of the lever system was 0.4 \(\mu\)m/g and total equivalent moving mass was 225 mg, the equivalent moving mass of the lever itself being 40 mg. No additional damping\(^13\) was used. To obtain abrupt changes in length, screw stops were positioned above and below the lever and the load applied to it was altered during contraction. Displacement of the lever was recorded photoelectrically (linear range of muscle shortening = 2.2 mm) and differentiated electronically. Force, length, and velocity of shortening were recorded on a
storage oscilloscope (Tektronix 5103N with Polaroid camera C59), on a multichannel oscillograph (Devices MX4), and on analog tape (Phillips analog 7). Muscles were field-stimulated through platinum plate electrodes placed parallel to the muscle, by 5-msec rectangular pulses of voltage approximately 10% above that required to stimulate full mechanical contraction.

Muscles were equilibrated under control conditions in Ca buffer at 29°C, stimulated at a frequency of 1 per 5 seconds, and with resting length = L_{max}. For reequilibration with strontium, frequency was reduced to 1 per 10 seconds, buffer containing neither Ca nor Sr was substituted until mechanical performance declined almost to zero, and then Sr was added. Several further washes with Sr buffer (oxygenated at 29°C) were carried out during reequilibration, which took about 1 hour. Subsequently, mechanical performance remained stable for many hours. Control conditions for experiments with Sr were: temperature = 29°C; stimulus frequency = 1 per 20 seconds; Sr = 2.5 mM for cat muscles or 3.5 mM for frog muscles (unless otherwise stated). Inotropic effects were achieved by adding Sr (SrCl₂) to bring the final concentration to 5 or 7.5 mM; epinephrine (1-epinephrine bitartrate), to 5 μM; or caffeine, to 5 mM. Effects of different inotropic interventions were studied singly in separate muscles, or in random order for comparison in the same muscles. Contractures were produced by substituting buffer containing neither Ca nor Sr until mechanical performance declined almost to zero, then adding concentrated KCl solution to give a final concentration of 80 mM potassium, and 5 minutes later adding Ca (CaCl₂) or Sr.

Sr-mediated contractions and oscillatory responses remained precisely constant under constant conditions. The frequency of oscillation was measured from Polaroid photographs of oscilloscope traces and was calculated from the average of as many cycles as were measurably completed in each of any series of conditions to be compared; it always was measured from the same number of cycles in any single experiment. The extent of shortening at which an oscillatory response occurred was measured as the average length around which the measured oscillation took place. Except for experiments on the effects of load, the oscillatory response was induced by a small load step (0.1–0.2 g) to a total load near to that required to set resting length at L_{max}.

The characteristics of the muscles used were as follows (peak isometric total force (P_{0}) being measured under control conditions with Ca buffer; length, resting force, and P_{0} at L_{max}):

- Cat (n = 20): length, 5.7 ± 1.1 mm; lightly blotted wet weight, 2.1 ± 1.7 mg; mean cross-sectional area (assuming cylindrical shape and a specific gravity of 1), 0.3 ± 0.22 mm²; resting force, 0.33 ± 0.16 g; P_{0}, 2.45 ± 1.33 g.
- Frog (n = 18): length, 6.2 ± 0.9 mm; weight, 2.2 ± 0.9 mg; mean cross-sectional area, 0.3 ± 0.14 mm²; resting force, 0.38 ± 0.09 g; P_{0}, 2.04 ± 0.64 g.

These data are given as mean ± standard deviation. Student's t-test was used for statistical comparison of data except where otherwise indicated.

**Results**

**ISOTONIC OSCILLATION**

An abrupt alteration in load during the plateau phase of an isotonic Sr-mediated contraction induced a damped oscillatory response (Fig. 1). A decrease in load caused a simultaneous abrupt decrease in length, attributable to elastic recoil, followed in the activated but not in the resting muscle by a further, relatively slow increase in shortening and then a damped oscillation about the length that would be reached in a control contraction at that load. An abrupt increase in load induced a corresponding response in the opposite direction. The same oscillatory response could be elicited repeatedly in either direction during the course of a single contraction or during separate similar contractions. Total amplitude of oscillation was up to 5% of L_{max}.

In frog muscles the oscillation was more damped and at a slightly higher frequency under similar control conditions (see Methods). For cat muscle, frequency was 2.1 ± 0.2 Hz (mean ± SD, n = 14) with 2.5 mM Sr, or 2.2 ± 0.2 Hz (n = 6) with 5 mM Sr. For frog muscle, frequency was 3.1 Hz (n = 17) with 2.5 mM Sr, or 3.3 ± 0.1 Hz with 5 mM Sr.

![Figure 1 Isotonic oscillatory response to abrupt change in load during plateau phase of Sr-mediated contraction. Superimposed in each panel are (1) control contractions at constant load, (2) a contraction where load is abruptly altered from the one to the other load (as measured in the top trace), and (3) the effect of a similar alteration of load on the resting muscle (R) (bottom trace). Zero time marks the moment of stimulation (and in top left panel the simultaneous reduction to the lower load which was then maintained throughout the contraction) Note "elastic recoil" in resting and active muscle, followed by slow oscillation in active muscle.](image-url)
± 0.8 Hz (n = 6) with 3.5 mM Sr, or 3.4 ± 0.7 Hz (n = 6) with 5 mM Sr (P < 0.005 compared with frequency for cat muscle with 5 mM Sr).

Temperature. Frequency of oscillation was temperature-dependent (Fig. 2). Linear regression analysis of Arrhenius plots relating the natural logarithm of oscillation frequency to the reciprocal of absolute temperature, over the range of 23-35°C, yielded correlation coefficients equal to or greater than -0.996 for each of five cat muscles. A regression line passing through the common mean, with common slope, has equation loge Hz = -9,932/°A + 33.59. Likewise two frog muscles gave correlations equal to or greater than -0.987, and an Arrhenius pooled regression, loge Hz = -8,006/°A + 27.60.

Simplification of the temperature relationship by use of an expression in °C instead of 1/°A (nonlinearity of correspondence 0.04% at worst in this range) gives regression equations for cats: loge Hz = +0.1089°C - 2.4907; and for frogs: loge Hz = +0.0883°C - 1.4654. The temperature coefficient (Qi 0) calculated from individual values for oscillation frequency at 23 and 33°C for six cat muscles was 2.9 ± 0.3 (mean ± SD); for six frog muscles it was 2.3 ± 0.02.

The extent of shortening of the plateau phase of Sr-mediated contractions declines with increasing temperature in cat muscles, and shows a biphasic temperature response in frog muscles, declining between 20 and 29°C and increasing between 29 and 37°C.9

Load. Oscillation frequency was related to the load (or length) at which the oscillation occurred and generally increased with decreasing load/length (Fig. 3).

It always increased with decreasing load/length to approximately 30% of P o or 0.85 L max, but in some cat muscles it declined toward shorter lengths, as in the example shown in Figure 3. This relationship was independent of the direction or magnitude of the load step, not excluding the special example of "quick release" (Fig. 4). Loading conditions for preceding contractions had a very slight effect on the extent of shortening and the duration of contraction, as in the case of normal Ca-mediated contractions,14 as well as on oscillation frequency, but the present observations are independent of any such effect.

Inotropic Interventions. The frequency of the oscillatory response also was increased by positive inotropic interventions—increased [Sr], increased frequency of contraction, or the addition of epinephrine or caffeine (Figs. 5 and 6). However, oscillation frequency was not related simply to the length at which the oscillation occurred. This is demonstrated by the experiment shown in Figure 5D on cat muscle. In this case a lower temperature was used to provide conditions under which epinephrine does not alter the extent of shortening during the plateau phase. Oscillation frequency still was increased by epinephrine although length remained unchanged. It is demonstrated also by analysis of the findings at 29°C (Fig. 6). In cat muscles oscillation frequency was increased significantly more by epinephrine (5 µM) or caffeine (5 mM) than by increased [Sr] or by increased frequency of contraction, in relation to their effects on the extent of shortening during the plateau phase (i.e. the length at which the oscillation occurred). In these experiments data are taken from a control contraction and from a representative contraction after the inotropic intervention, and the geometric means are plotted (n = 5 in each case, from five cat muscles and eight frog muscles). In cat muscles, the mean angles representing the inverse tangents of the increase in oscillation frequency (%) over the increase in extent of shortening (%) were compared by Duncan's multi-
FIGURE 4  Relationship of oscillation frequency to load/length is independent of direction (A) or magnitude (B and C) of preceding load step (Sr-mediated contractions in cat muscles under control conditions).

The data in Figure 6 relating to the effects of Sr and frequency of contraction were taken at steady state, at Sr\(^{2+}\) concentrations ranging from 1.5 to 5 mM, and at contraction frequencies ranging from 1 per 10 minutes to 1 per 10 seconds, respectively, and represent the end points of an apparently linear series of intermediate points. Excluded from these series are values obtained at shorter lengths at which these values diverged from linearity, as they did in most cat muscles. The divergence was upward with higher frequencies and downward with higher Sr concentrations. Oscillation frequency of cat muscles actually declined at very short lengths both with high [Sr] and with low load (Fig. 3); the biphasic nature of the relationship is lost if the data from different muscles are normalized and pooled, but the slopes for individual muscles relating oscillation frequency to length were similar with increasing Sr concentration and with decreasing load (although the length below which oscillation frequency again began to decline was longer with changes in load than with changes in Sr concentration). In frog muscles there was no secondary decline in oscillation frequency at very short lengths and the mean slopes were similar for changes in [Sr] or load (Fig. 6). Thus in the absence of epinephrine and caffeine, oscillation frequency appears to change as a constant function of length under the influence of differences in extracellular Sr concentration, frequency of contraction, or load. Epinephrine and caffeine, however, increase oscillation frequency in a manner which is not directly dependent on length.

FIGURE 5  Effect of different inotropic interventions on frequency of oscillatory response in cat muscles: (A) increase in extracellular Sr to 5 mM, (B) 5 mM caffeine, (C) and (D) 5 μM epinephrine. Each panel shows control contraction before (lower trace in A, B, and C) and contraction after the intervention (upper traces in A, B, and C), at the same load and with the oscillatory response induced by the same load step. Control conditions in these experiments: 29°C (A, B, C) or 26°C* (D); Sr = 1 mM (A, B, C) or 2.5 mM (D); frequency = 1 per 20 seconds. Note that (1) oscillation frequency is increased by each intervention; (2) it is increased by epinephrine even when epinephrine does not alter extent of shortening of plateau phase (D); (3) there is spontaneous oscillation, especially after positive inotropic intervention; and (4) there is additional slow frequency oscillation (seen only at lower concentrations of extracellular Sr) [A], the frequency of which is increased by epinephrine (see Fig. 7) but which is abolished by caffeine.

* 26°C was chosen to provide conditions where epinephrine does not alter length during plateau.
† 1 mM Sr was chosen for these experiments to provide wide range of changes with interventions, and to illustrate low frequency component.
A similar analysis of oscillation frequency in relation to the initial velocity of shortening of the contraction showed that these too could be dissociated. The increase in oscillation frequency relative to shortening velocity in cat muscles was greater with epinephrine or caffeine than with increased [Sr] or frequency of contraction \((n = 5)\), although the differences did not reach statistical significance. In frog muscles caffeine increased oscillation frequency while decreasing initial shortening velocity; caffeine thus differed from epinephrine or increased [Sr], which increased oscillation frequency equally relative to the associated increases in shortening velocity.

**Spontaneous Early Oscillations.** When the amplitude of oscillation was large (higher contractile states as shown in Figure 5 or greater loads as in Figure 3) spontaneous oscillation occurred at the beginning of the contraction plateau after the initial rapid shortening phase. The frequency of this spontaneous oscillation was identical to that of the oscillatory response induced by an abrupt step change in load.

**Dual Oscillation.** In cat, but not frog, muscles an additional slower oscillation was seen (Fig. 7). This occurred spontaneously at 1 mM Sr but not at [Sr] \(>2.5\) mM, and was abolished by caffeine. Its frequency was \(20 \pm 0.4\%\) (mean \(\pm SE, n = 6\)) of that of the faster component. The slow oscillation frequency was increased by epinephrine relatively more than was the faster component (frequency ratio then \(= 3:1\)).

**ISOMETRIC OSCILLATION**

Figure 8 illustrates the transient changes in isometric force induced by an abrupt alteration in length. In both cat and frog muscles an isometric oscillation was observed which was more damped (and of slightly faster frequency) than the corresponding isometric oscillation. Small amplitude and highly damped oscillations of force development sometimes occurred spontaneously at the beginning of isometric contractions of cat muscle but these appeared only at high contractile states and were much less prominent than the corresponding spontaneous oscillations in length observed at the beginning of the plateau phase of isometric contractions.

**OSCILLATORY RESPONSES DURING Sr- OR Ca-MEDIATED POTASSIUM CONTRACTURES**

Abrupt changes in load induced oscillatory responses in Sr-mediated and in Ca-mediated K contractures that were...
similar to but slightly more damped than those seen during Sr-mediated contractions. The frequency of oscillation similarly was increased by an increase in [Sr] or [Ca], epinephrine, or caffeine. Oscillation amplitude declined during prolonged K contractures. This probably was due to the development of rigor because it was associated with a reduction in compliance. In cat, but not frog, muscles, dual oscillatory responses were induced (frequency ratio = 3:1).

**TRANSIENT RESPONSE TO AN ABRUPT DECREASE IN LOAD DURING ACTIVE SHORTENING**

The transient augmentation of the velocity-length relationship demonstrable for twitch contractions following an abrupt reduction in load under certain circumstances appears to be analogous to the first phase of the isotonic oscillatory response we have studied, when analyzed in terms of its velocity-length relations (Fig. 9).

**PERTURBATION-INDUCED SHORTENING OF CONTRACTION DURATION**

The duration of an isotonic Sr-mediated contraction was shortened by imposing an abrupt change in load earlier in its course. This effect did not depend on the direction of the load step, but was related to its timing. A load step very early in the plateau had no effect on the duration of contraction, but later load steps shortened it by up to 15%. Subsequent contractions were slightly depressed after a contraction whose duration (and, by implication whose action potential duration) had been shortened in this way.

**Discussion**

We have shown isotonic oscillatory responses in Sr-mediated contractions of heart muscle. Problems of internal elasticity were avoided by considering oscillations in length at constant load, and it is clear that these oscillations represent changes in mechanical activity: they are absent in resting muscle, are of relatively slow frequency (2–3 Hz at...
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30°C), and their frequency has a temperature coefficient (Q10) of 2–3; this is consistent with a chemical reaction. The oscillatory responses are not peculiar to Sr-mediated contractions but can be elicited also in Ca-mediated K contractions. The evidence suggests that they are induced directly by the change in length rather than the change in load, in that isotonic oscillations are less damped than their “isometric” counterparts (equipment and muscle compliance will allow considerable fiber shortening even during “isometric” contraction), in that oscillations occur spontaneously after initial isotonic shortening. These observations suggest the influence of positive feedback.

The oscillation appears to reflect changes in the level of activation of the contractile apparatus, mediated probably through Ca or Sr. This conclusion is based on four main observations which are considered together in the light of known mechanisms of action of epinephrine and caffeine.

1. Oscillation frequency is increased by a number of different positive inotropic interventions, but with epinephrine or caffeine this effect can be dissociated from any increase in the extent of shortening. It is difficult therefore to attribute the oscillation to any mechanism which is intrinsic to myofilament interaction, for any intervention which affected this might be expected to affect also the extent of steady state shortening. Epinephrine and caffeine do, however, influence the intracellular movements of Ca (and presumably Sr).16-22

2. Transient augmentation of the velocity-length relationship after an abrupt reduction in load, which is shown to be analogous to the first phase of the isotonic oscillatory response, is not seen in normal Ca-mediated contractions of mammalian heart muscle but occurs under three conditions: Sr-mediated contractions of cat muscle,18 Ca-mediated contractions of cat muscles exposed to caffeine,19 and contractions of frog muscles (Ca- or Sr-mediated).11 These three conditions have in common an implicit inability to sequester Ca or Sr rapidly. Isolated preparations of sarcoplasmic reticulum from skeletal muscle take up Sr less well than Ca;22 caffeine impairs the rapid binding of Ca by preparations of cardiac sarcoplasmic reticulum (Blayney et al., unpublished observations); and sarcoplasmic reticulum is less well developed in frog heart muscle than in cat.4-7

3. In cat muscles an additional, slower oscillation can be induced, the frequency of which is increased by epinephrine to a greater extent than the faster component of contraction. Since an oscillation in myoplasmic [Ca2+] or [Sr2+] implies the existence of two interacting sites for Ca2+ or Sr2+, the finding implies the participation of an additional site. It may be noted that epinephrine is thought to influence Ca transport by sarcoplasmic reticulum18 and also possibly by mitochondria,24 although it is not necessary to invoke a second epinephrine-responsive site if the one is common to both oscillatory interactions.

4. Changes in extracellular [Sr] or load result in similar changes in oscillation frequency relative to the length at which the oscillation occurs; this suggests that these two means of altering oscillation frequency might act through a common mechanism.

From these four observations, we conclude that the oscillatory response involves length-induced changes in the degree of activation of the contractile apparatus and, thus, that length influences the degree of activation during contraction of heart muscle; it probably acts by altering the level of [Ca2+] or [Sr2+] within the cell.

The transient changes in mechanical activity, which are observed in mammalian heart muscle only under abnormal conditions, presumably depend for their manifestation on quantitative differences in reaction sites at the relevant interacting sites. This does not prejudice the generality of the fundamental conclusion that length influences activation. It may, however, throw light on the identity of the sites, although this remains a matter for speculation. The sarcoplasmic reticulum is implicated by the apparent dependence of analogous transients in twitch contractions on some impairment of its function as discussed above. Mitochondria can probably be excluded (though not from the slower oscillatory component) by the observation that caffeine does not influence rapid “limited Ca loading” (Ca binding) in isolated mitochondria (Blayney et al., unpublished observations). The sarclemma possibly may be implicated by the similar effects of epinephrine and caffeine on oscillation frequency in Sr-mediated contractions of cat muscles, since both of these agents increase sarcolemmal permeability14-17,20 while apparently exerting different effects on sarcoplasmic reticulum and mitochondria.16,22,24 (Blayney et al., unpublished observations). If an associated oscillation in membrane potential could be demonstrated, as in similar oscillatory responses of turtle heart during contraction25 and in spontaneous diastolic oscillations of similar frequency in guinea pig heart,26 this would support the view that changes in Ca or Sr levels were involved; it would not necessarily indicate direct participation of the sarclemma in the oscillatory mechanism. The myofibrils, too, may participate in the oscillatory interaction as well as providing the means for its manifestation.

The findings provide no direct evidence, however, about the direction of the supposed change of Ca or Sr level which is induced by muscle shortening. The first phase of active shortening which follows a reduction of load presumably will be related primarily to sarcomere shortening because the load is then less than that which could be sustained at steady state. The initial and therefore the ultimate direction of a change in [Ca2+] or [Sr2+] associated with the subsequent oscillation will depend on whether the myofibrils are one of the oscillatory sites or, if not, on the phase lag of their response to an oscillation mediated by two or more other interacting sites. The findings neither support nor conflict with other evidence that mechanical activity declines at shorter length.4,5,7,25,26

Previously reported mechanically induced transient isometric responses have been demonstrated equally in whole muscle and in glycerinated preparations and have generally been attributed to synchronous re-formation of “cross-bridge” bonds or to configurational changes within the “cross-bridge.”19-21 These responses usually have been characterized by “stretch-activation,” but discrimination between viscoelastic and active changes is difficult. The pattern of the isotonic oscillatory response described in the present study is, however, remarkably similar to that recorded from frog skeletal fibers,22
despite its larger amplitude and much slower frequency. The amplitude (up to 5% $L_{max}$) is almost certainly too great to be accommodated within the crossbridge bond as currently envisaged. The differences in frequency appear to be part of a spectrum of differences related primarily to the type of muscle used, for the time course of isometric transients recorded from comparable glycerinated preparations (normalized approximately for differences in temperature) ranges over two orders of magnitude from that of heart muscle\(^3\) (where it is similar to that found in the present study) through mammalian skeletal muscle,\(^2\) insect fibrillar muscle,\(^2\)\(^5\) and tortoise skeletal muscle\(^8\) to amphibian skeletal muscle.\(^9\)

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References
5. Page E: Correlations between electron microscopic and physiological observations in heart muscle. J Gen Physiol 51: 211–220, 1968
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