The Force-Velocity Relation and Stepwise Shortening in Cardiac Muscle

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SUMMARY. A series of experiments was carried out to determine the effects of load variation on the character of stepwise shortening. We imposed afterloaded isotonic contractions on rat ventricular trabeculae, and measured the effect of load on pause duration, i.e., on the duration of the periods during which there was no sarcomere shortening. Sarcomere lengths were measured by optical diffraction. Increases of load brought about increases of pause duration; the relation was linear. The relation did not appear to depend on time during contraction, but did depend on sarcomere length: for a given load, pauses were longer at shorter sarcomere lengths. In a supplementary protocol in which we measured the dynamics of the central segment of the muscle during muscle isometric contraction, we found that the velocity of sarcomere shortening during the shortening step was approximately independent of load. These results provide a framework for interpretation of muscle force-velocity relations: diminished velocity at high loads may be the result of increased pause durations. (Circ Res 51: 37-42, 1982)

ALTHOUGH stepwise shortening was first reported several years ago, the phenomenon has proved controversial. In the original experiments (Pollack et al., 1977) made with optical diffraction, it was shown that the shortening pattern in cardiac and skeletal muscle was discontinuous. Periods of rapid shortening, or steps, generally alternated with periods during which there was little or no shortening, or pauses. The shortening waveform often, although not always, resembled a staircase.

Since publication of that paper, several investigators have proposed that the phenomenon was an artifact attendant with the use of optical diffraction (Morgan, 1978; Rüdel and Zite-Ferenczy, 1979). In response to this criticism, several alternative methods were pursued. Analysis of high-speed cinemicrographs of the striation pattern (Delay et al., 1981) indicated clear stepwise shortening; since the image comprises information from several diffractions, an artifactual pause arising out of Bragg effects in any one order would not be expected to produce an artifactual pause in analysis of the image. Similarly, when the striation pattern obtained with incoherent light microscopy was projected onto a photodiode array, and the signal processed with a phase-locked loop, the resulting sarcomere length output was again stepwise (Jacobson et al., 1981). Finally, we have observed that shortening muscles generate discrete sound bursts, again indicating synchronous behavior (Brozovich and Pollack, 1981).

The present study represents an initial attempt to explore the physiological properties of the phenomenon. We attempted to measure the influence of load and sarcomere length on the stepwise shortening waveform. In particular, we wished to determine whether the reduced muscle shortening velocity at high load, i.e., the force-velocity relation, was the result of increased pause duration, reduced step velocity, or some combination of these.

Methods

Specimens

Thin trabeculae isolated from the right, and occasionally the left, ventricles of rats were used for these experiments. The hearts of rats, anesthetized with ether, were removed and rapidly transferred to a dissection chamber where they were perfused through the aortic stump with a physiological salt solution (see below) at room temperature. This served to clear blood from the coronary vasculature and to allow ample time for selection and dissection of specimens.

Satisfactory specimens were usually 2-3 mm long, 30-200 μm wide, and 25-150 μm thick. Often, they were ribbon shaped, the small dimension oriented along the optical axis.

Specimens were mounted under stereomicroscopic observation in a small (1.5 ml) chamber designed to give laminar flow of the bathing solution, thereby minimizing turbulent mechanical noise. The chamber was made of teflon with a glass floor to allow entry of laser light. The specimen was mounted between miniature stainless steel, spring-loaded clamps, one of which was attached to a moving coil, and the other to a grass stem and thence to a tension transducer. Details of the coil and tension transducer are found elsewhere (Delay et al., 1979).

The bathing solution had the following composition (in mM): Na+, 145; Cl-, 102; HCO3-; 24; acetate, 20.5; K+, 5.0; Ca++, 1.8; Mg++, 1.0; SO4•-; 1.0. Insulin was added (10 U/liter) along with 1.8 g/liter dextrose. The solution was gassed with 95% O2 and 5% CO2 and maintained at 26 ± 1°C. Preparations were stimulated at 12/min through a pair of platinum electrodes running along the full length of the specimen.

Stabilization, usually requiring 1½ hours, resulted in a general improvement of muscle performance—diminished resting tension, increased active tension, more complete
activation of the specimen (determined microscopically), and diminished stimulus threshold. The hallmark of the recovery process was the diminution in the intensity and extensiveness of the quasi-random sarcomeric "dithering" occurring between contractions. We suspect the dithering was caused by the damage inflicted by dissecting and clamping the specimen, and spreading from the clamped regions toward the central region of the muscle (Krueger and Pollack, 1975).

In rare instances, dithering diminished naturally to an imperceptible level; in such preparations, fine details of the sarcomere shortening patterns were highly reproducible from contraction to contraction. In other specimens, some dithering remained in evidence even after the period of stabilization. There the reproducibility was not as good, presumably because the initial condition was inconsistent. Where dithering remained substantial enough to eliminate reproducibility, we added lidocaine (Astra) in progressively increasing quantities (up to 50 mg/liter) as required. Lidocaine diminished dithering, presumably by virtue of its antiarrhythmic effect. Microscopically, one could also observe a salutary effect on synchrony: preparations with substantial dithering often exhibited sizeable, well-striated regions which failed to respond even to massive stimulation. After lidocaine, such regions generally regained their responsiveness. If not, the preparation was discarded.

Measurements of tension and sarcomere length (by light diffraction) were carried out as described in previous papers (Pollack et al., 1979; Iwazumi and Pollack, 1979). Sarcomere length resolution was approximately 2 nm (Iwazumi and Pollack, 1979). The diameter of the illuminated region was adjusted to be approximately equal to that of the specimen. The laser light was circularly polarized. Sarcomere length was computed from one of the two first orders. Cross-sectional area was computed from dimensions measured stereomicroscopically from above the specimen and, with the aid of a mirror, from the side.

**Protocols**

Two major protocols were employed, one using afterloaded isotonic contractions, and the other using isometric contractions. The afterloaded isotonic protocol was used to study the effect of load on pause duration. Because of the low tensions involved in some runs, we found it convenient to effect these contractions by servo-control of muscle length rather than tension. By applying a suitably chosen exponential shortening pattern, the tension could be kept constant (within ±5% at intermediate and high loads) for a substantial length of time during contraction. Measurements of sarcomere length during constant tension were recorded on Polaroid film. Such measurements were restricted to regions in which the specimen had uniform dimensions. We usually made measurements in several contiguous regions along the specimen 50–100 μm apart.

An isometric protocol was used to complement the afterloaded isotonic series. Though the specimen is held isometrically, the sarcomeres in the central region shorten at the expense of stretch of the end regions adjacent to the clamp.

**Criteria for Acceptance of Data**

Data from a given record (or portions thereof) were admitted into the study only if the waveshape varied imperceptibly over a minimum of three successive contractions, usually five. Whereas many records showed absolute pauses, i.e., intervals during which the sarcomere length change was indistinguishable from zero, some records showed slight progressive increases or decreases of sarcomere length, and still others showed noise-like deviations from zero velocity. Thus, we adopted the following criteria for acceptance of a particular pause: (1) the onset and the termination must have been abrupt; i.e., there must have been a discontinuity in the slope; (2) during the putative pause, all points on the waveform must have fallen between two lines with slope ±1 nm/msec emanating from the point of origin of the pause, except (3) for short pauses, less than or equal to 5 msec, larger noise-like deviations of up to 5 nm were tolerated, provided the slopes of the shortening steps before and after the pause were equal (within 10%), thus demonstrating a clear "gap" in the shortening record. For these short pauses, criterion (2) was not imposed.

**Effect of Beam Size**

Since we used specimens of varying cross-section, and since we restricted the beam diameter to be roughly equal to the width of the fiber, the volume of tissue sampled varied widely among experiments. Figure 1 shows that pause durations were generally longer in the smaller specimens. Because of the wide variation in pause duration among specimens, we found it expedient to normalize the results obtained in each specimen relative to a specific set of conditions (e.g., load, time, sarcomere length) for that specimen. The results were then analyzed among different specimens.

**Results**

Representative tracings of sarcomere length changes in an afterloaded isotonic contraction are shown in Figure 2. From data such as these we examined the effect of load variation on pause duration.

**Pause Durations**

Figure 3 shows that pause durations increased linearly with load. Only low-to-moderate loads were used (these fibers develop up to 10 g/mm² at L_max).
Higher loads were more difficult to obtain with the afterloaded isotonic protocol, since most of the shortening takes place prior to the time the high load is reached.

Figure 3 includes data taken at various times after stimulation and at various instantaneous sarcomere lengths. It is possible that the load dependence arises in part out of consistent differences of sarcomere length or time between runs at low and high load. For example, the onset of shortening at high load is later than that at low load, so the data are consequently biased toward later times after stimulation. We therefore felt it prudent to test for both time dependence and sarcomere length dependence of pause duration.

Time dependence was checked by employing a supplementary experimental protocol. The objective was to allow pauses at a given load and sarcomere length to occur at different times after stimulation. To achieve this, we allowed each of three specimens to contract against zero load from two initial lengths; thus, particular sarcomere lengths were reached at different times. The results, shown in Figure 4, indicate that the effect of time, or history, is relatively small.

The influence of sarcomere length is shown in Figure 5. Figure 5A shows that pauses are substantially longer at shorter sarcomere lengths; the relation between pause duration and load, however, remains approximately linear. Figure 5B shows the same data, plotted with sarcomere length on the abscissa to better illustrate the substantial sarcomere length dependence of pause duration.

For reasons discussed in Methods, it should be noted that relative, not absolute, pause durations were plotted in Figure 5, A and B. These graphs were obtained in the following manner: For each specimen, the mean pause duration for an unloaded contraction (actual load 0-0.08 g/mm²) at sarcomere length 2.05 μm (range 2.00-2.09) was considered as 100%. All data from a given experiment were normalized to this value. Comparable data from all specimens then were pooled and the values shown on Figure 5 were obtained. To provide some measure of absolute scale it should be noted that the highest point in Figure 5B corresponds to a pause duration of 28 msec.

It appears that the pauses grow longer at higher loads and at shorter sarcomere lengths; however, they are not functionally dependent upon time after stimulation.
Step Shortening Velocity

Initial attempts to quantify the effects of load and sarcomere length on step velocity gave no conclusive results. The data showed considerable scatter, particularly with the afterloaded isotonic protocol. To test the possibility that some functional dependence might emerge from selected data, we examined those records which were most staircase-like. Here we used records obtained with the isometric protocol, so that higher loads could be included. The criterion for selection was that there were at least three successive pauses in which the sarcomere length decrements between pauses differed by less than 10%. The rationale was that such records were likeliest to be representative of events at the molecular level, but this is evidently an assumption. Figure 6 shows the result of such analysis. Though the velocities at the lowest loads are slightly elevated, the overall result is consistent with the possibility that the load dependence is small or zero.

Discussion

The results indicate that the characteristics of step-wise shortening are influenced by instantaneous load and by sarcomere length.

Pauses

The pause duration varied directly with the load against which the muscle was contracting. At zero load, the pause durations were lowest, falling into the range of about 2–9 msec at 26°C, depending on sarcomere length. They increased linearly with load up to about 2 g/mm². Although we did not study higher loads systematically, records taken in muscle isometric contractions often indicated protracted pauses as the peak of contraction approached, pauses extending as long as 50 msec. Although the interpretation of such records is open to question, as load was not held constant and the activation level may have begun to decrease, such records tend to indicate that...
the increase of pause duration with increased load is likely to extend beyond loads of 2 g/mm².

The load dependence of pause duration may justify some preliminary speculation as to the molecular mechanism underlying the pause. If the pause is associated with the progress of some process, such as a chemical reaction, predisposing the specimen for some preliminary speculation as to the molecular mechanism underlying the pause. It might be anticipated that the larger the load (and hence the larger the required energy expenditure for a step of fixed size), the longer the time required to complete the process. That this was found to be true gives some weight to its consideration as a preliminary working hypothesis.

We also found that the pause durations increased with decreasing sarcomere length. On a macroscopic level, it is of interest that muscle shortening velocity also decreases at shorter sarcomere length both at intermediate loads (Martyn et al., 1980) and at zero load (Pollack and Krueger, 1976; Martyn et al., 1980). It would appear, then, that these reduced muscle velocities stem at least in part from increased pause durations. The mechanism underlying the increased pauses at shorter sarcomere lengths remains to be elucidated. Elevated restoring forces may play a role.

A simplifying result is the observation that the sarcomere length and load dependence of pause duration appear relatively unmodulated by time after stimulation. Figure 4 shows that this is true when the load is zero. For the higher loads, however, the conclusion is only tentative. The fact that the data points on Figure 5 were obtained at different times and yet do not impair the linear relation suggest that time after stimulation, or previous contractile history, is a relatively unimportant variable. This may not be true late in contraction, where the diminished activation level could well influence pause durations.

The effect of beam size (Fig. 1) on measured pauses is significant in two respects. First, it confirms the expectation that the measured pattern of stepwise shortening is somewhat smoothed relative to the fundamental response at the molecular level, presumably as a result of the capturing of more than one region of synchrony by the laser beam. Second, though there is considerable scatter in the data, extrapolation to a beam size of zero, thereby giving the fundamental, local response, gives pause durations that are increased only modestly over those obtained using beams of 30–50 μm wide. Thus, the absolute values of pause duration obtained with restricted beam widths may have quantitative relevance at the molecular level.

Shortening Steps

The effect of load on step shortening velocity was somewhat less definitive, because of the scatter. The scatter may result from a less than "ideal" degree of synchrony.

To be specific, consider the time course of the degree of synchrony during contraction. Let us assume that the first step of shortening occurs relatively synchronously throughout a sizeable region. If pauses depend on load and sarcomere length, as described above, then any regional variation in these would bring about similar regional variation in pause duration, resulting in progressively increasing phase differences among local signals, and hence a progressive diminution of synchrony. Measurements made later in contraction might therefore be expected to be more "contaminated" by the effects of asynchrony than those made early in contraction.

Consequently, we examined the effects of load on step velocity from records which we assumed to reflect a high degree of synchrony throughout contraction. Mostly, these were records obtained from preparations with particularly clear striations, and a sharp diffraction pattern whose first order did not diminish in intensity or broaden substantially during contraction. The objective criterion for selection of such records was that the records show a succession of three or more pauses in which the sarcomere length decrements between pauses varied less than 10%. The results (Fig. 6) showed little or no dependence of step velocity on load over the full range of loads. The lack of dependence of step velocity on load is sometimes strikingly apparent in experiments on skeletal muscle (c.f. Pollack et al., 1977, Fig. 2b).

Implications

These results have direct implications as to the genesis of the load-velocity relation. The variation of step velocity with load is relatively small. However, the pause durations increase directly with load. The reduced muscle velocities at high loads may therefore stem from the increased pause durations. In other words, at least within the range of loads studied, the force-velocity relation may be a straightforward reflection of the relative pause duration at different loads.

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