Interfilament Spacing Is Preserved during Sarcomere Length Isometric Contractions in Rat Cardiac Trabeculae

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ABSTRACT It is generally assumed that the myofilament lattice in intact (i.e., nonskinned) striated muscle obeys constant volume. However, whether such is the case during the myocardial contraction is unknown. Accordingly, we measured interfilament spacing by x-ray diffraction in ultra-thin isolated rat right ventricular trabeculae during a short 10 ms shuttered exposure either just before electrical stimulation (diastole), or at the peak of the contraction (systole); sarcomere length (SL) was held constant throughout the contraction using an iterative feedback control system. SL was thus varied in a series of SL-clamped contractions; the relationship between SL and interfilament spacing was not different between diastole and systole within 1%; this was true also over a wide range of inotropic states induced by varied $[Ca^{2+}]_o$. We conclude that the cardiac myofilament lattice maintains constant volume, and thus constant interfilament spacing, during contraction.

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A unifying theory that has gained acceptance proposes that the impact of sarcomere length (SL) on myofilament Ca^{2+} sensitivity is due to changes in the spacing between the thick and thin filaments (1,2). Because myofibrils are believed to maintain close to constant volume (3), elongation of the sarcomere is expected to lead to a reduction of the distance between the thick and thin filaments. A closer approximation of the myosin heads to actin may be expected to increase the probability of strong cross-bridge formation at a given concentration of activating calcium. Several experiments have provided support for this theory. Osmotic compression of the myofilament lattice by high molecular weight molecules that cannot enter the myofilament lattice structure, such as dextran, induces an increase in myofilament Ca^{2+} sensitivity concomitant with a reduction of muscle diameter (1–3). Since it has been shown that a reduction in muscle diameter occurs in parallel with a reduction in myofilament lattice spacing in skeletal muscle, the increase in myofilament Ca^{2+} sensitivity after dextran treatment without a change in SL is consistent with the interfilament spacing theory. Fuchs et al., who showed that myofilament Ca^{2+} sensitivity could be rendered length independent when muscle diameter was kept constant by applying an appropriate amount of dextran at each SL (1), provided more direct support for this theory. A similar result was obtained by McDonald et al., who showed in single permeabilized cardiac myocytes that application of dextran at a short SL was sufficient to increase myofilament Ca^{2+} sensitivity to match that recorded at the high SL, despite the fact that SL had not changed (2). On the other hand, we have recently suggested that interfilament spacing alone cannot be the only determinant of myofilament Ca^{2+} sensitivity (4,5). These conclusions, however, are based entirely on measurements of either muscle diameter or interfilament spacing in relaxed, noncontracting muscle preparations. It is possible that the myofilament lattice rearranges during contraction, the very condition that exists when myofilament Ca^{2+} sensitivity is assessed (3). Early experiments in skinned striated muscle suggested that upon activation there is an expansion of the myofilament lattice, which is likely due to the attachment of the myosin heads to the thin filament. A similar conclusion was also derived by Cecchi et al. (6), using a segment length clamp protocol in intact amphibian skeletal muscle. As in skeletal muscle, interfilament spacing in both intact and skinned myocardium is a function of SL (4), and large changes in SL usually accompany active contraction in isolated mammalian myocardium (7). Thus, whether systolic myofilament lattice spacing deviates from the diastolic lattice spacing in mammalian myocardium independent of changes in SL is currently unknown. Accordingly, to answer this question, we implemented an iterative feedback technique to control SL throughout the contraction in isolated rat cardiac trabeculae while interfilament spacing was simultaneously assessed by x-ray diffraction, either during diastole or at the peak of systole and over a wide range of SL and inotropic states.

All experiments were performed according to University of Illinois, Chicago, institutional guidelines concerning the care and use of experimental animals. Male rats (LBNF-1; 250-350 g) received intraperitoneal injections of 50 mg/kg sodium pentobarbital and 1.5 ml heparin (5). Under deep anesthesia, the heart was excised and perfused retrograde with a modified Krebs-Henseleit solution also containing 0.2 mM $CaCl_2$ and 20 mM 2,3-butanedione monoxime to inhibit spontaneous contractions (25°C; pH 7.4) (5). Right ventricular trabeculae were dissected, mounted in the experimental

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apparatus, stimulated at 1 Hz, and left to equilibrate for ~30 min at $[Ca^{2+}] = 1.2$ mM and diastolic SL = 2.1 μm. Next, three separate experimental runs were conducted at $[Ca^{2+}] =$ 0.3, 0.7 and 1.2 mM. In each run, SL was clamped by iterative feedback every 10th contraction at SL = 1.9, 2.0, 2.1, or 2.2 μm as previously described in detail (8). Briefly, SL clamp is achieved in the central segment of the muscle preparation close to the stationary attachment site of the muscle (i.e., the side attached to the force transducer) by appropriately stretching or releasing the muscle at the other side (connected to a high-speed motor). This approach allows for a SL clamp with minimal motion artifact at the site of measurement (8); an iterative feedback algorithm derives the muscle length profile that is required to achieve SL clamp. Briefly, the actual SL attained during the contraction (as measured by laser diffraction) is compared to the desired SL waveform so as to calculate a muscle length waveform via standard proportional integrative-differential feedback, which is to be applied during the next controlled contraction; this process is repeated over several contractions—usually 5–8—until the algorithm converges onto the desired SL waveform, after which several contractions can be recorded in succession under SL control (we averaged five contractions in this study). We carefully aligned a focused, shuttered x-ray beam (250 × 250 μm; $\lambda = 0.103$ nm; 10 ms) perpendicular to the laser beam and at the same area of the muscle where SL was controlled; this arrangement allows for the simultaneous measurement of myofilament lattice spacing and SL. Details of the x-ray apparatus have been described previously (5).

Fig. 1 *A* illustrates representative recordings obtained from a rat cardiac trabecula in a series of contractions with SL control from SL = 1.9 μm to 2.2 μm. In between the SL clamped contractions, diastolic SL was kept constant at SL = 2.1 μm such that each controlled contraction commenced at a comparable contractile state. For each SL controlled contraction, SL was stretched or released to the desired SL just before electrical stimulation and then held constant at that SL. Consistent with our previous studies (8), twitch force under SL clamp was both enhanced and prolonged as compared to SL auxotonic contractions (data not shown); furthermore, peak twitch force increased with increasing SL. The x-ray beam shutter was opened either during the peak of the contraction or just before stimulation in separate contractions under SL clamp as illustrated in panel *C*. The x-ray diffraction pattern was captured on a cooled charge-coupled device detector; diffraction patterns thus obtained either in diastole or peak systole allowed for accurate determination of interfilament spacing based on the d1,0 x-ray reflections. Fig. 1, panel *D*, illustrates the broadening as well as the shift in the ratio of the 1,0–1,1 intensity in going from diastole to systole, consistent with previous reports (3,6,9). It should be noted, however, that quantitative analysis of those aspects of the data would have required much longer x-ray exposures, and this was not the purpose of our experiments.

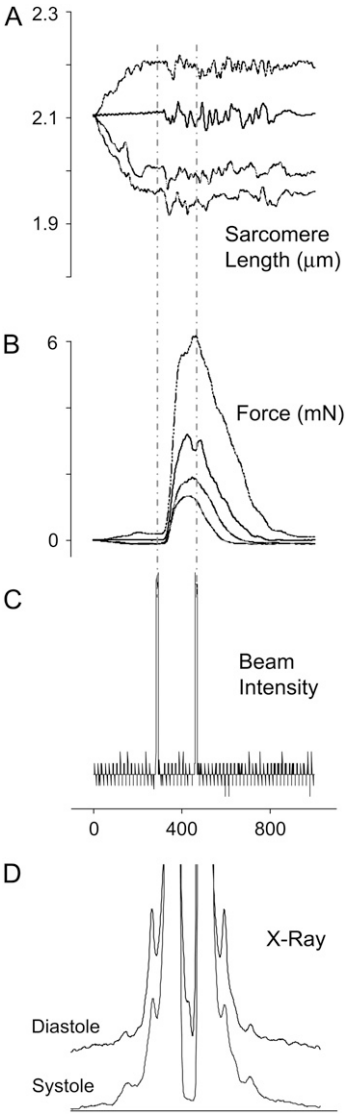

FIGURE 1 (*A* and *B*) Sarcomere length and force recorded during iterative feedback length control in an isolated rat cardiac trabecula. (*C*) Times at which the x-ray beam shutter was opened (10 ms) either during diastole or at the peak of systole. (*D*) The one-dimensional projection of the equatorial x-ray diffraction pattern recorded at SL = 2.2 μm and at the times indicated in *C*.

Fig. 2 summarizes the average data obtained in all experimental series at three levels of contractile state as varied by alterations in extracellular $[Ca^{2+}]$. In the top panel, the interfilament spacing normalized to the spacing obtained in diastole at SL = 2.1 μm is plotted as a function of SL. The solid symbols indicate the diastolic lattice spacing, whereas the open symbols indicate the lattice spacing at peak systole; the dashed line in this panel indicates normalized interfilament spacing as predicted from constant myofilament lattice volume behavior. Two observations are apparent from these data. First, interfilament spacing is inversely proportional to SL with little deviation between diastole and the peak of

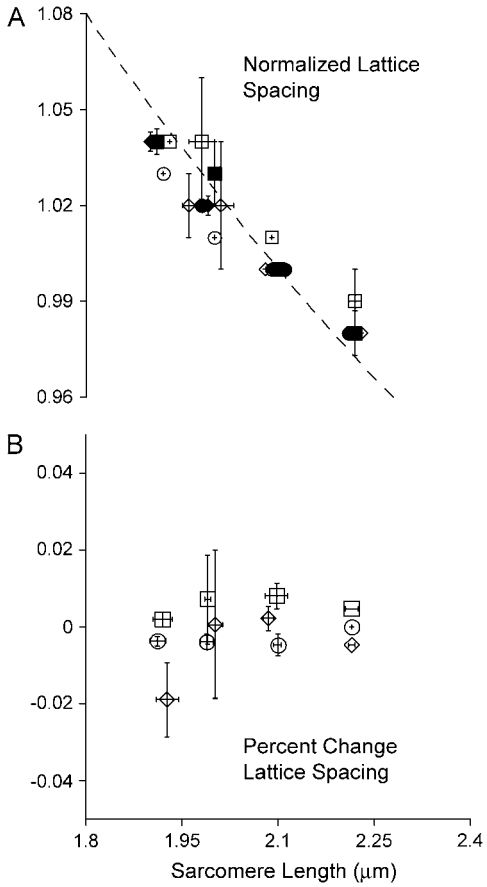

FIGURE 2 (*A*) Diastolic (*solid symbols*) and systolic (*open symbols*) interfilament spacing normalized to the 2.10 μm diastolic spacing as function of sarcomere length (SL) measured at varied contractile state ($[Ca^{2+}]_o$ = 0.3 mM (○), $[Ca^{2+}]_o$ = 0.7 mM (□), and $[Ca^{2+}]_o$ = 1.2 mM (◇)). The dashed line indicates interfilament spacing as predicted by constant myofilament lattice volume. (*B*) Peak systolic interfilament spacing relative to diastolic interfilament spacing. Data presented as mean ± SE; *n* = 3.

systole. Second, the data are closely clustered to the dashed line, indicating that mammalian myocardium adheres close to constant myofilament lattice volume regardless of either SL or activation state. The notion that interfilament spacing is not affected by the transition from diastole to systole is further confirmed by the data shown in Fig. 2 *B*. Here, peak systolic interfilament spacing relative to the diastolic spacing is plotted as function of SL. As is clear from these data, there is $<1\%$ change in interfilament spacing during SL isometric cardiac muscle contractions, regardless of inotropic state or SL. Thus, our data support the use of relaxed interfilament spacing to predict the behavior of the cardiac sarcomere throughout the contraction. It should be noted that in our study, we did not assess dynamic changes in interfilament spacing in activated skinned myocardium, nor did we investigate whether our results also hold true for muscle diameter, a parameter closely correlated with interfilament spacing.

In conclusion, in our study in isolated rat myocardium, we found that active SL isometric muscle contraction occurs without a physiologically significant change in interfilament spacing. Our data support the notion of a constant volume of the sarcomere lattice during the course of an active twitching contraction.

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