Length-Dependent Calcium Inotropism in Cat Papillary Muscle

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SUMMARY We studied changes in the relationship of isometric developed force to muscle length when extracellular calcium concentration was altered in isolated cat papillary muscles. In two series of experiments at temperatures of 30°C and 32°C, and rates of 12 and 30 beats/min, 21 muscles were exposed to calcium concentrations of 1.125, 2.25, and 4.5 molar. Muscle lengths were varied between 80% and 100% of the length at which maximum developed force occurred (Lmax). Peak developed force and the time from stimulus to peak force were measured. The data indicate that force is not altered proportionately at all lengths when calcium concentration is changed. Rather, we found that a substantially greater modification of force occurs at short lengths than at long lengths. Similarly, the time to peak force increases with length at a rate which is more than 4 times greater at the lower calcium concentration than it is at the high concentration. Small but consistent shifts of Lmax also are seen. We observed that Lmax is longer when inotropic changes reduce force and shorter when force is increased. These results indicate that the inotropic effect of extracellular calcium concentration changes is dependent on muscle length.

SUGGESTIONS that inotropic and mechanical factors may be regarded as acting independently in the determination of cardiac muscle performance are found in both experimental and theoretical considerations of cardiac contraction.1 Data have been reported from isolated muscle preparations showing that the developed force-length relationship, normalized to maximum force, remains the same when the extracellular calcium concentration is changed or norepinephrine is added.2 Similar results have been obtained for a variety of isolated ventricle preparations.3

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On the other hand, indications that the inotropic effectiveness of some drugs may be altered by changes of muscle length have been available for some time.4,5 Furthermore, recent experiments on skeletal muscle have suggested that the process by which the myofilaments are activated may itself be length-dependent.6,7 This study was undertaken to elucidate whether or not muscle length and extracellular calcium concentration act independently to determine isometric force development.

METHODS

Two series of experiments were conducted to evaluate the relationship between developed force and muscle length in the presence of altered extracellular calcium concentrations. In each series right ventricular papillary muscles were rapidly excised from cats anesthetized with...
sodium pentobarbital (30 mg/kg, ip). The wall end of the muscle and the tendon were encircled and tied with 8-0 suture thread prior to removal from the heart. In the testing chamber, multifingered clamps held the tied portions of the specimen so as to maintain a cylindrical geometry and distribute the load uniformly over the circumference. The bathing solution contained (mM): Na⁺, 140; K⁺, 5.0; Ca²⁺, 2.25; Mg²⁺, 1.0; Cl⁻, 103; HCO₃⁻, 24; SO₄²⁻, 1.0; HPO₄²⁻, 1.0; acetate, 20; glucose, 10; and insulin, 2.5 U/liter. The solution was aerated with 95% O₂ and 5% CO₂. The pH was 7.4 at room temperature.

The testing apparatus consists of a linear arrangement of an electromagnetic puller, length transducer, muscle, and force transducer together with associated electronic circuitry. It operates in a feedback control mode allowing either force or length to be the controlled variable while the other is measured. Electronic signals are used to specify the time course of the controlled variable and the dynamic switching from one control mode to the other. Length is measured with a linear variable differential transformer (Hewlett-Packard 7DCDT-500). Force is determined by using a hybrid combination of a low frequency-high stability transducer (Statham UTC-2) and a high frequency-low stability transducer (Kistler-Morse DSC-3), the signals from which are combined using complementary filters. This arrangement provides low drift (less than 50 mg in 8 hours) and wide bandwidth (1 kHz). Except for the transducers and a signal generator, all of the equipment is custom-fabricated expressly for mechanical testing of tissues and has been described in greater detail elsewhere.

For data gathering, processing, and display purposes, a digital computer (Digital Equipment Corp., PDP 12) is interfaced to the apparatus. Following conversion to digital form the data have resolutions of 10 mg for force, 10 μm for muscle length at the time of stimulus, and 1 μm for length changes within the period of one stimulation cycle.

In the first series of experiments 12 muscles were tested at a temperature of 32°C using punctate stimulation (5 msec) 10-30% above threshold at 30/min. Following an initial equilibration of about 20 minutes, the muscle length at which maximum developed force occurred, was estimated. The length was then set to 0.96 L_max and the muscle allowed to equilibrate completely, as evidenced by completely stable passive and active forces. By using a signal generator to command the electronic length control feature of the apparatus, the muscle length was then varied slowly but continuously over the range 0.90–1.02 L_max. The ramp-like nature of the length change and the resulting force record are illustrated in Figure 1, which shows a single cycle. Each complete cycle occurred in 10 minutes and three consecutive cycles were used to determine the force-length relationship. Data samples were obtained from every eighth contraction.

The intent of this protocol was to produce, in a reproducible manner, an approximation to the static force-length relationship. Both slower ramps (20-minute cycle time) and full equilibration following step changes of length were used in several experiments to check this approximation. No significant differences were observed between the data produced by 10-minute ramps and those produced more slowly.

Since the results of this series were different from those reported by others, a second series of nine muscles was tested using a protocol which more nearly approximated those used by other investigators. The temperature was 30°C and field stimulation (5 msec) 10–30% above threshold was used at 12/min. Following determination of L_max and equilibration of active and passive forces, muscle length was reduced in 0.04 L_max decrements from 1.00 L_max to 0.80 L_max. At each length the muscles were allowed to equilibrate before data were collected.

In both series the data consisted of muscle length at the time of the stimulus, force at the time of the stimulus (resting force), maximum force, and time from stimulation to the occurrence of maximum force (TTP). Developed force was defined as maximum force minus resting force. Cross-sectional area was estimated from muscle weight and ranged from 0.47 to 1.2 mm² at L_max.

The calcium concentration in the bathing solution was altered to half (1.125 mM) and double (4.5 mM) its control value (2.25 mM). Some muscles underwent initial equilibration in 2.25 mM calcium, while others did so in the 1.125 mM concentration. The sequences of concentrations most used were: 2.25, 1.125, 2.25, 4.5, 2.25, 1.125, and 1.125, 2.25, 1.125, 2.25, 4.5, 2.25, 1.125.

Results

A representative example of the data of the first series of experiments is shown in Figure 2. Developed force as a function of muscle length, normalized by the maximum force at each calcium, is shown for the three calcium concentrations used. Results for all of the muscles in this series have been subjected to a secondary normalization.
FIGURE 2. Normalized developed force vs. length for a representative muscle in the first series for the three calcium concentrations used.

which expresses the forces for 1.125 and 4.5 mM calcium as a fraction of the control (2.25 mM) forces at selected lengths (Fig. 3). Because the limits of the length excursion were determined from a first estimate of $L_{\text{max}}$, the actual length range did not always turn out to be 0.90–1.02 of the correct $L_{\text{max}}$ for each muscle. The lengths selected for the secondary normalization were therefore confined to the range common to all muscles, 0.92–1.00 of the correct $L_{\text{max}}$.

Data for a representative muscle in the second series are shown in Figure 4 and those for the whole series, following a second normalization, are indicated in Figure 5. The deviation from unity seen in Figure 5 is statistically significant, $P < 0.02$. All expressions of the results from both series clearly indicate that alterations of calcium concentration do not lead to proportionate changes of developed force at all lengths. Rather, such alterations are considerable more effective at modifying force at short lengths than at long lengths.

To be sure that the length-dependent calcium effect did not result from the fact that the bathing solution contained both insulin and acetate, substances that are not present in the solutions used by many investigators, three muscles in the second series were tested with a solution modified to have no acetate or insulin. The data were indistinguishable from those obtained with the original solution, however, and are included in Figure 5.

Some of the muscles tested in each series of experiments exhibited an irreversible deterioration of developed force after equilibration with 4.5 mM calcium. That is, although a stable, augmented force-length relationship would be obtained following a change from 2.25 to 4.5 mM calcium, the forces exhibited on return to 2.25 mM calcium would be depressed from those originally found at that concentration. Evidence of a length-dependent calcium effect was apparent, however, whether the 4.5 mM calcium data were compared to the preceding or the following 2.25 mM calcium data. Only the magnitude of the force change, at a given length, was different. No such changes of performance were ever observed with alterations of calcium concentration between 1.125 mM and 2.25 mM. Notably, comparison of the force-length relationships obtained at 2.25 mM calcium before and after deterioration caused by exposure to 4.5 mM calcium exhibited the same length-dependent nonproportionality as was caused by variation in calcium concentration.
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Figure 4. Developed force-length data for a representative muscle in the second series. A: force vs. length for the three calcium concentrations used. B: force expressed as a fraction of the maximum force developed at each calcium concentration.

Figure 5. Force fraction vs. length, as in Figure 3, for all of the muscles in the second series. The heavy solid lines are the data for the same muscle that is illustrated in Figure 4, the dashed lines indicate the behavior of a central segment in the same muscle (see Discussion).

of calcium concentration. In fact, all aspects of the measured data indicated behavior following deterioration which was qualitatively and quantitatively very similar to that which accompanied a reduction of calcium concentration from 2.25 to 1.125 mM.

The continuous variation of muscle length used in the first series of experiments allowed reliable determination of $L_{\text{max}}$ for most of the muscles. Small but consistent shifts of $L_{\text{max}}$ were observed with changes of muscle condition. Compared with control (2.25 mM Ca$^{2+}$), $L_{\text{max}}$ moved toward longer lengths for lower forces and toward shorter lengths for higher forces. These shifts are summarized in Figure 6.

In both series of experiments the time from stimulus to maximum force (TTP) was measured. The manner in which these times varied with length and calcium concentration are illustrated by the example shown in Figure 7.
FIGURE 6 Muscle length at which maximum developed force occurred, $L_{\text{max}}$, for high and low calcium concentrations and calcium-induced deterioration (see text), expressed as a fraction of $L_{\text{max}}$ for the control calcium concentration. The bars indicate the mean ± 1 SD.

A particularly striking feature of these data is that the times show a greater sensitivity to length at low calcium concentration than at high. In the first series, for example, the change of TTP with length averaged 9.77, 3.95, and 2.22 msec per % $L_{\text{max}}$ at 1.125, 2.25, and 4.5 mm calcium, respectively.

In both series of experiments passive force-length relationships were invariant with changes of calcium concentration.

Discussion

The data obtained in this study demonstrate that the mechanical performance of cardiac muscle, as indicated by peak developed force, is substantially more sensitive to extracellular calcium changes at short muscle lengths than at long muscle lengths. This calcium sensitivity varies continuously with length over the range of 0.8 $L_{\text{max}}$ to 1.0 $L_{\text{max}}$. In addition, there is a consistent tendency for $L_{\text{max}}$ to shift toward longer lengths when active force is reduced and toward shorter lengths when it is increased. Though these shifts are a small fraction of muscle length (1%), they are a more substantial fraction (5%) of the length range over which active force is developed. Furthermore, the degree to which the time course of active force varies with muscle length is also altered by calcium concentration changes. Time to peak force is more sensitive to length changes at low calcium concentrations than at high.

Some investigators have reported results that are in substantial agreement with our findings. Allen et al.10 found that the effect of calcium concentration on force is length-dependent, though their measurement protocol differed significantly from that used here. Shifts of $L_{\text{max}}$ with calcium concentration changes were noted by Hoffman et al.5 The dependence of the time to peak force on muscle length has been observed by Blinks.11 Other researchers, however, have reported results that are at odds with ours.3 Sonnenblick,2 for example, found inotropic agents, including calcium, to affect the force-length relationship proportionately at all lengths. He also found no variation of $L_{\text{max}}$ with calcium or of time to maximum force with length.

One reason for concern about the validity of both the present and earlier studies is that recent investigations have cast doubt on the adequacy of the mechanical data obtained from isolated papillary muscles. Optical techniques have revealed that substantial sarcomere shortening occurs in the central region of small rat papillary muscles because of stretch in the end regions.12-13 Newly obtained data from our laboratory using infused microspheres as anatomical landmarks demonstrate that the same condition exists in the large cat papillary preparation.14 Such shortening causes the force-length relationship determined from measurements at the ends of the muscle to misrepresent that of the central region because both the length and passive force of the contracting segment at the time of peak force are overestimated. and also, possibly, because activation is reduced.15-17 However, correction of the data to account for any of these effects, using estimates based on data12-14 or assuming exponential spring behavior in the end regions, would cause the force-length curves to be shifted farther apart. and this shift would be as great or greater at short lengths as at long lengths. Thus, corrected data would exhibit the same profound length-dependent calcium inotropism as do the uncorrected data presented here.

This inference is supported by data obtained in one experiment in which microspheres 15 μm in diameter were infused into the microcirculation to provide visible indicators of internal length changes.15,16 Two microsphere markers were selected to indicate the length of a central segment in the preparation. When developed force at three different calcium concentrations is plotted vs. muscle length, the result is as shown in Figure 4 and, in
normalized form, by the heavy solid lines in Figure 5. If the force-length relationship is constructed using segment length, the normalized result is as shown by the dashed lines in Figure 5. The effect of calcium concentration changes is dependent on segment length in the same way that it is dependent on muscle length.

A number of possible mechanisms have been suggested that might account for the observed interaction of length and extracellular calcium concentration. It appears possible that length changes may (1) change the amount of force borne by an internal load;\textsuperscript{16,18} (2) alter the sensitivity of the myofilaments of calcium,\textsuperscript{19} or their structures;\textsuperscript{20} (3) change the ability of the sarcoplasmic reticulum to take up or release calcium;\textsuperscript{21,22} (4) modify the degree to which the sarcoplasmic reticulum is triggered by electrical events or calcium-induced calcium release;\textsuperscript{23-25} or (5) alter the state dependent calcium effect occurs via a relatively slow mechanism requiring several beats, such as the redistribution of calcium between the extracellular space and intracellular cellular calcium.

Although changes of extracellular calcium concentration are believed to lead directly to changes in the amount of calcium available for activation in cardiac muscle, this effect apparently takes place over a number of beats.\textsuperscript{26} In addition, it is estimated that only about 10% of the activator calcium comes from outside the cell in any one contraction.\textsuperscript{27} Since in the present study muscles were equilibrated to a new length before measurements were taken, it is not possible to determine whether the observed length-dependent calcium effect occurs via a relatively slow mechanism requiring several beats, such as the redistribution of calcium between the extracellular space and intracellular cellular stores, or whether it occurs immediately upon length change, or both. However, Allen et al.\textsuperscript{10} changed muscle length rapidly and found length-dependent calcium inotropism when they measured the first contraction following a length change. If it could be shown that this effect is fully developed immediately after a length change, the result would tend to rule out a mechanism that involves a shift of a significant amount of calcium between intracellular and extracellular sites.

Whatever the mechanism which underlies the length-dependence of calcium inotropism, the results are such as to raise doubts about the likelihood of identifying a broadly useful index of cardiac contractility. The data suggest that length may affect activation in a manner similar to that of inotropic agents. If so, it may prove to be impossible to find a performance measure that is sensitive to inotropic state but independent of muscle length.

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