Effect of Initial Sarcomere Length on Sarcomere Kinetics and Force Development in Single Frog Atrial Cardiac Cells

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SUMMARY We studied sarcomere performance in single isolated intact frog atrial cells using techniques that allow direct measurement of sarcomere length and force. The purpose of this investigation was to determine whether length-dependent alterations in contractile activation occur in the single isolated cardiac cell. This was accomplished by determining the effect of initial sarcomere length on the time course of sarcomere shortening and force development during auxotonic twitch contractions. The results presented in this paper demonstrate that the velocity of sarcomere shortening, the rate of force development, and the magnitude of force development during auxotonic twitch contractions all increase as initial sarcomere length increases over the range of about 2 μm to greater than 3 μm. These results indicate that the level of contractile activation increases as initial sarcomere length increases. Also, results are presented that indicate that the rate of increase of contractile activation during a twitch contraction also increases as initial sarcomere length increases. These length-dependent effects on contractile activation in conjunction with the slow time course of contractile activation cause the force-velocity-length relationship to be time-dependent: i.e., the velocity of sarcomere shortening at a given sarcomere length and load depends on the time during the contraction when the sarcomere reaches that length. The results suggest that length-dependent alterations in contractile activation may play a major role in the improved contractile performance that accompanies an increase in initial sarcomere length in cardiac muscle. Circ Res 49: 767-774, 1981

IT is well established that the contractile performance of striated muscle is affected by two parameters: the sarcomere length and the level of contractile activation. Recent evidence indicates that these parameters are not independent regulators of contractile performance, since contractile activation is regulated to some extent by sarcomere length. Indeed, it has been suggested (see Jewell, 1977) that length-dependent alterations in contractile activation may play a major role in the improved contractile performance which accompanies increased ventricular filling (i.e., Starling's Law of the Heart).

The purpose of the present investigation was to determine whether length-dependent alterations in contractile activation occur in single frog atrial cells. This was accomplished by determining (1) the effect of initial sarcomere length on the time course of sarcomere shortening and force development during auxotonic twitch contractions and (2) the peak force-sarcomere length relationship of auxotonic twitch contractions. Such an investigation is of interest for several reasons: First, it now is recognized that direct measurement of sarcomere length is necessary for the evaluation of cardiac sarcomere performance. This conclusion results from the fact that the time course and extent of sarcomere shortening in intact cardiac tissue may bear very little relationship to the time course of overall muscle shortening. It is known, for instance, that sarcomeres in the central region of the muscle shorten and stretch the damaged regions of the tissue near the clamps holding the ends of the preparation during so-called isometric contractions in frog (Nassar et al., 1974) and rat (Krueger and Pollack, 1975) cardiac preparations. Thus, damaged end regions cause uncertainty in the evaluation of sarcomere performance based on tissue length measurements. However, such uncertainties do not exist when sarcomere lengths within the preparation are measured directly. Second, at present few data are available in which the effect of initial sarcomere length on the kinetics of sarcomere shortening has been directly determined. Nassar et al. (1974) used the laser diffraction technique to assess directly the performance of a large group of sarcomeres in the central region of small thin bundles of frog atrial tissue during so-called isometric contractions and found that the velocity of sarcomere shortening appeared to be independent of initial sarcomere length and force. Since force development increased as initial sarcomere length increased from 2.2 μm to over 3.0 μm, the data suggest that the contractile activation increases as sarcomere length increases. However, the interpretation of these data suffers from uncertainty about the distribution of the total force among the large number of cells that exist in...
parallel in the tissue (see Manning et al., 1977). Third, although elegant experiments have been performed on single-skinned cardiac cells from a variety of animals (rat, dog, and frog) which demonstrate that length-dependent activation occurs in partially activated cardiac sarcomeres (see Fabiato and Fabiato, 1976, 1978), it is difficult to extrapolate from these data to predict quantitatively what effect length-dependent activation would have on sarcomere performance in intact tissue. For example, Edman and Nilsson (1971) have found that the magnitude and duration of the so-called active state in both rabbit papillary muscle and frog skeletal muscle increases with increased initial length. However, Brutsaert and Sonnenblick (1969) found that the phase-plane velocity-length relation in cat papillary muscle was the same for a considerable portion of the contraction, regardless of the length from which shortening began. That is, during a significant portion of a twitch contraction, the velocity of tissue shortening at any given muscle length and any given total load did not depend on the initial length of the muscle or the time during the contraction when the muscle shortened to that length. A time-independent force-velocity-length relationship could occur if time and length-dependent increases in contractile activation are offset by decreases in contractile activation which occur during active shortening of muscle (see Edman and Kiessling, 1971).

The single isolated cardiac cells used in the present investigation offer some distinct advantages over the intact tissue. First, the sarcomeres within the cell can be observed directly, using conventional light microscope techniques. Second, the single cell is only 1–2 myofibrils wide and the sarcomere performance appears to be uniform across the width of the cell. It follows, therefore, that the sarcomeres under observation must be load-bearing, and that the total force is distributed equally between the parallel myofibrils. Third, the single cardiac cell provides an opportunity to assess directly the force-sarcomere velocity-sarcomere length interactions in the simplest intact cardiac preparation.

**Methods**

The techniques of cell preparation, the preparation and calibration of cantilevered glass force beams, the method of attachment of a single cell to the glass beams, and the method of recording and determining sarcomere lengths and force development during a twitch contraction have been reported previously (see Tarr and Trank, 1976; Tarr et al., 1979, 1981a, 1981b). Briefly, one end of a single cell is attached to a glass beam of known compliance; the displacement of this beam as the cell shortens and develops force during a twitch contraction is proportional to the force developed by the cell. If the other end of the cell is attached to another glass beam which is essentially noncompliant, then during a twitch contraction the cell will shorten and develop force in an auxotonic fashion (see Figs. 1, 2, and 4). The extent of shortening varies as the compliance of the force beam. In this type of experiment, we secured the cell to the glass beams by performing end-to-end rotation of the cell about the beams in such a manner as to wrap each end of the cell around the beam by one to two turns. An alternative experiment is to attach only one end of the cell to a glass beam (the force beam) and draw the other end of the cell into a fluid-filled pipette with controlled suction. In this case the cell shortens during a twitch contraction in a lightly loaded and relatively isotonic fashion, since the end of the cell in the suction pipette is relatively free to move (see Fig. 3). In this type of experiment, the end of the cell attached to the glass beam is not wrapped around the beam. Conventional bright field light microscope techniques are used to view the cell, the sarcomere pattern within the cell, and the position of the calibrated compliant force beam. Synchronized stroboscopic illumination is used to "freeze" the motion of the sarcomeres and the force beam at time intervals of 16.67 msec during the contraction. The data are recorded on a closed circuit TV-video tape system and analyzed using the stop frame capability of the video tape recorder in combination with a double TV cursor (see Tarr et al., 1979). By such an analysis, the time course of sarcomere length changes and force development which occur during a twitch contraction can be analyzed (Tarr et al., 1981a, 1981b). The resolution limit of the light microscope (0.5 μm in our system) sets the accuracy with which sarcomere length and force can be measured. For sarcomere length determinations, the length occupied by a small group of sarcomeres (usually 6–10) is determined and an average sarcomere length is calculated. The accuracy of this average sarcomere length determination is 0.5 μm divided by the number of sarcomeres within the unit of length measured; for 10 sarcomeres, the accuracy is ±0.05 μm. The accuracy of the force measurement is 0.5 μm divided by the compliance of the force beam; for a force beam having a compliance of 0.2 μm/nN, the force is measured with an accuracy of ±2.5 nN. The cells used in the present investigation were bathed in Ringer's solution having the following composition: NaCl = 111 mM, KCl = 5.4 mM, CaCl₂ = 1.8 mM, Tris = 10 mM, glucose = 4 mM, and HCl as required to adjust the pH to 7.3. All experiments were done at room temperature (~25°C).

**Results**

Figure 1 presents the time course of sarcomere shortening and force development during four auxotonic contractions in a single cell beginning from different initial sarcomere lengths. In these contractions the cell was attached to a relatively compliant force beam, and the amount of sarcomere shorten-
LENGTH-DEPENDENT ACTIVATION IN SINGLE HEART CELLS/Tarr et al.

Figure 1 Time course of sarcomere shortening and force development (total force) during auxotonic twitch contractions beginning from different initial sarcomere lengths. The performance of the same group of eight sarcomeres was analyzed in all four contractions. Force beam compliance was 0.26 \( \mu \)m/nN.

Figuring was considerable (20-40% of the initial sarcomere length.) These data demonstrate that the velocity of sarcomere shortening, the rate of force development, and the magnitude of force development all increase as initial sarcomere length increases. The data also demonstrate that the velocity of sarcomere shortening as the sarcomere passes through given sarcomere lengths (e.g., 2.46 \( \mu \)m; see arrows) depends on the initial sarcomere length from which the contraction begins, even though the force (see arrows) supported by the sarcomere as it passes through a given sarcomere length (e.g., 2.46 \( \mu \)m; see arrows) increases as initial sarcomere length increases. For example, the contraction beginning from a sarcomere length of 3.25 \( \mu \)m has a sarcomere velocity of 4.1 \( \mu \)m/sec and a force of 116 nN when the sarcomere passes through a length of 2.46 \( \mu \)m. In contrast, the contraction beginning from a sarcomere length of 2.78 \( \mu \)m has a sarcomere velocity of 3.5 \( \mu \)m/sec and a force of 43 nN when the sarcomere passes through a length of 2.46 \( \mu \)m. Thus, the increase in sarcomere length appears to increase the level of contractile activation such that the sarcomere at a given sarcomere length could shorten at an increased velocity even in the face of an increased load. Such a finding suggests that the force-sarcomere velocity-sarcomere length relationship at any given sarcomere length during an auxotonic contraction depends on the sarcomere length from which the contraction begins.

Another example of enhanced contractile activation associated with increased sarcomere length is demonstrated in Figure 2. The data presented in this figure were obtained from a different cell than those presented in Figure 1. Again, an increased velocity of sarcomere shortening, an increased rate of force development, and an increased magnitude of force development were associated with an increase in initial sarcomere length. Also, in this example there is a considerable range of sarcomere lengths (~2.45 \( \mu \)m to 2.05 \( \mu \)m) during which the sarcomere shortening in the contraction beginning from the long sarcomere length has a higher velocity, even in the face of an increased force, than does the sarcomere shortening in the contraction beginning from the short sarcomere length. In fact, in both contractions the sarcomere shortened to the same sarcomere length (~2.0 \( \mu \)m) and reached this length at the same time although the peak force (an isometric force) developed by the sarcomere was significantly different in the two contractions. Thus, it appears that the isometric force which can be developed by the sarcomere at a given sarcomere length and at a given time during an auxotonic contraction can be influenced by the initial sarcomere length from which the contraction begins.

The data presented in Figures 1 and 2 demonstrate that the initial sarcomere length influences the velocity of sarcomere shortening during an auxotonic contraction. To determine if the variability in force which normally occurs during an auxotonic contraction could have affected the result, a somewhat different experiment was performed in which the sarcomeres were allowed to shorten under relatively isotonic conditions at loads approximating the preloads required to set the initial sarcomere length. This was accomplished by attaching one end of the cell to the calibrated force beam and drawing the other end of the cell into the opening of a suction pipette under controlled vacuum (see Tarr et al., 1981a). The initial sarcomere length was
The performance of the same group of 10 sarcomeres was analyzed in both contractions. Force beam compliance was 0.21 μm/nN. The results of such an experiment are shown in Figure 3. An increase in initial sarcomere length was associated with an increased preload, but the long sarcomere still shortened at this preload at the same or perhaps a slightly increased velocity compared to the contraction initiated at the short sarcomere length with a decreased preload. Also, in the sarcomere length range of about 2.8 to 1.9 μm, the contraction initiated from the long sarcomere length (e.g., 3.45 μm) passed through any given sarcomere length (e.g., 2.4 μm) with similar velocity but with greater force than the contraction initiated from the short sarcomere length (e.g., 2.8 μm). Thus, these data again support the conclusion that the force-sarcomere velocity-sarcomere length interrelation depends on the sarcomere length from which the contraction begins.

The data presented in Figures 1-2 demonstrate that an increase in initial sarcomere length over a length range of 2 μm to greater than 3 μm resulted
in an increase in both the rate and magnitude of force development during auxotonic twitch contractions in which the single cell was allowed to contract against a relatively compliant force beam. Such a result would be expected if the sarcomere were operating on the ascending limb of an isometric force-sarcomere length relationship which was time-dependent (see Discussion). To determine whether the force-generating capability of the sarcomere increased as sarcomere length increased above sarcomere lengths of 2.2 μm, we performed experiments in which the cell was allowed to contract auxotonic against a relatively stiff force beam in order to reduce the overall change in sarcomere length which normally occurs during an auxotonic contraction. The results of one such experiment are given in Figure 4. It is apparent that the peak total force (filled circles) developed by the cell during a relatively "isometric" contraction (the amount of sarcomere shortening averaged 3.4% of the initial sarcomere length) increased as sarcomere length increased over the sarcomere length range of about 2.5 μm to about 2.7 μm.

**Discussion**

The data presented in this paper demonstrate that increases in initial sarcomere length over a length range of about 2 μm to greater than 3 μm resulted in an increase in both the rate of force development and magnitude of force development during auxotonic twitch contractions in the single cardiac cells. These findings are very similar to those found by Nassar et al. (1974) and Manring et al., (1977) during so-called isometric contractions in very thin bundles of frog atrial muscle in which sarcomere dynamics were measured by means of the laser diffraction technique. Even under so-called isometric conditions, these investigators found that the sarcomeres contracted auxotonically and shortened to a considerable extent (up to 27%). In contrast to our results, these investigators found that the velocity of sarcomere shortening was essentially the same for contractions initiated from long sarcomere lengths (e.g., 3 μm) and short sarcomere lengths (e.g., 2.2 μm). Our results indicate that an increased velocity of sarcomere shortening generally was associated with an increase in initial sarcomere length. Nevertheless, both our results (Figs. 1 and 3) and those of Nassar et al. (1974) demonstrate that the long sarcomere (e.g., 2.8 μm) is capable of supporting a greater load during a twitch contraction than the short sarcomere (e.g., 2.2 μm), and it is also capable of moving this load with the same (Nassar et al., 1974) or greater velocity (our result) than the short sarcomere. As suggested by Manring et al. (1977), such a result would be possible if the force-generating capability (i.e., isometric force) of the sarcomere increased over the sarcomere length range of 2-3 μm. Such a length-dependent increase in isometric force has been reported for both partially activated skinned cardiac (Fabiato and Fabiato, 1978) and skinned skeletal muscle cells (Endo, 1973). Also, it is well established that intact skeletal muscle operates during twitch contractions on the ascending limb of an isometric force (P0)-sarcomere length relationship up to sarcomere lengths on the order of 2.8 to 3 μm (Rack and Westbury, 1969; Close, 1972). Our data (see Fig. 4) indicate that the intact single cardiac cell also operates during a twitch contraction on the ascending limb of a P0-sarcomere length relationship up to sarcomere lengths on the order of 2.8 μm.

As previously discussed, our data, as well as those obtained by other investigators, suggest that an increase in sarcomere length over the length range of about 2 μm to perhaps in excess of 3 μm results
in an increase in contractile activation such that the long sarcomere is capable of moving a given load at higher velocity than the short sarcomere. However, our finding that the velocity of sarcomere shortening at any given sarcomere length depends on the initial sarcomere length prior to the onset of shortening is not consistent with the ideas put forth by Brutsaert and Sonnenblick (1969), Brutsaert and Henderson (1973), and Henderson and Brutsaert (1974) that cardiac muscle has a force-velocity-length relationship during an isotonic twitch contraction that depends only on tissue length and is independent of time or the prior history of the contraction. Our results in the single frog cardiac cell indicate that the increased contractile activation which accompanies an increase in initial sarcomere length allows the sarcomere to shorten at increased velocity at any given sarcomere length and any given load as the cell shortens auxotonically. In this case, the velocity of sarcomere shortening at any given sarcomere length and load would depend on the prior history of the contraction, and the force-velocity-length relationship would become time-dependent in such a contraction. Such a time-dependent force-sarcomere velocity-sarcomere length relationship can easily be explained by a time-dependent $P_0$-sarcomere length relationship which increases as sarcomere length increases (see below). A time-independent force-velocity-length relationship as found by Brutsaert and co-workers could occur, as suggested by Brutsaert (1974), if the level of contractile activation were constant as the result of compensatory activating and inactivating influences; for example, length-dependent increases in activation may be offset by decreases in activation resulting from the active shortening that occurs during an isotonic contraction. It is well established in both skeletal and cardiac muscle that as a muscle shortens during a twitch contraction it loses some of its ability to produce force during the remainder of the contraction. Recently, Brutsaert et al. (1978) found that the extent of this deactivation associated with muscle shortening varies considerably in different types of cardiac tissue. It is most marked in cats, and is almost absent in the frog. It seems reasonable to expect that the time-dependent effects of length-dependent activation would be most pronounced in a preparation which has very little deactivation associated with active sarcomere shortening; perhaps this is the situation which exists in the isolated frog atrial cell.

In the previous discussion we suggested that much of the data presented in this paper could be explained if the sarcomeres within the single cell were operating during the twitch contraction on the ascending limb of an isometric force ($P_0$)-sarcomere length relationship which is time-dependent. In a recent publication (Tarr et al., 1981b), we presented a mathematical model for computing the time course of sarcomere length changes and force development during simulated auxotonic twitch contractions using standard numerical methods. In this model it was assumed that the velocity of sarcomere shortening was governed by the classical Hill's force-velocity relationship according to the following equations:

\[
\frac{\text{d}L}{\text{d}t} = \frac{P_0}{bL} - \frac{aL}{1 + \left(\frac{P}{a}\right)^n}
\]

where $L$ is the sarcomere length, $P$ is the force, $P_0$ is the isometric force, $a$ is the velocity constant, $b$ is the force constant, and $n$ is the Hill coefficient. The time course of sarcomere shortening and force development during simulated twitch contractions beginning from four different initial sarcomere lengths is shown in Figure 5. The symbols give the computed values of sarcomere length and force at time intervals of 33.33 msec. The solid lines were fitted by eye to the computed values. The simulations were computed for a tissue length containing 50 sarcomeres contracting against a force beam that had a compliance of $0.33 \text{ cm}^2/\text{N}$. For all contractions, the time constant of activation ($\tau$) was 0.25 sec, $P_{\text{max}}$ at 2.4 $\mu$m was 400 $\mu$N, $a$ was 100 $\mu$N, and $b$ was 5 $\mu$m/sec.

![Figure 5](http://circres.ahajournals.org/)
The isometric force ($P_0$) was assumed to be a time-dependent linear function of sarcomere length according to the following equation:

$$P_0 = P_{0\text{max}} \left[ 1 - \exp\left(-t/\tau\right) \right] \frac{(S - R)}{(2.4 - R)}.$$ 

This equation describes an instantaneous straight line relationship for $P_0$ as a function of sarcomere length ($S$) which rotates with time about a fixed sarcomere length $R$: $P_{0\text{max}}$ is the maximum value of $P_0$ at a sarcomere length of 2.4 $\mu$m. The time course of force ($P$) development in the simulated contraction is related to the stiffness of the force beam ($K$), the number ($N$) of sarcomeres in the simulated length of tissue, the initial sarcomere length ($S_0$), and the sarcomere length ($S$) at any given time during the contraction by the following equation:

$$P = (K)(N)(S_0 - S).$$

The time course of sarcomere shortening and force development during simulated auxotonic twitch contractions initiated from different initial sarcomere lengths is shown in Figure 5. In many respects, the results obtained from the simulated contractions are very similar to the experimental results (Fig. 1): an increase in initial sarcomere length resulted in an increase both in the rate and magnitude of force development and in an increase in the velocity of sarcomere shortening. Also, during contractions from different but fairly close initial sarcomere lengths (e.g., 2.6 $\mu$m & 2.8 $\mu$m), there were regions of overlap in the sarcomere length-time relationships where the sarcomere velocity at a given sarcomere length was the same or greater when the contraction began from the longer sarcomere length even though the force developed by the sarcomere was increased. The explanation for this finding is quite simple. The contraction which begins from the longer sarcomere length (e.g., 2.8 $\mu$m) reaches a given sarcomere length (e.g., 2.4 $\mu$m) later in time than the contraction which begins from the shorter sarcomere length (e.g., 2.6 $\mu$m). The increased time allows $P_0$ to increase at all sarcomere lengths, and, thus, the sarcomere velocity at a given sarcomere length can increase even in the face of an increased force according to Hill's force-velocity relationship. Thus, the force-sarcomere velocity-sarcomere length interrelation in this model is time-dependent and the sarcomere velocity at any given sarcomere length and load depends on the time during the contraction in which the sarcomere passes through any given sarcomere length.

The simulated contractions as given in Figure 5 can be used to interpret some, but not all, of the experimental findings. In Figure 2, it is apparent that a portion of the sarcomere length-time relation-
traction initiated from the long sarcomere length has greater force. This finding suggests that the time course of activation also depends on initial sarcomere length. The results of a simulation in which it was assumed that the time constant of the activation process decreased with an increase in initial sarcomere length produced a result similar to that seen experimentally (see Fig. 6.)

In summary, the results presented in this paper indicate that length-dependent alterations in contractile activation play a role in the improved contractile performance which accompanies increases in initial sarcomere length. In the single frog atrial cell, an increase in sarcomere length over a length range of about 2 μm to greater than 3 μm produces an increase in contractile activation such that the force-generating capability of the sarcomere (i.e., Pₒ) during a twitch increases as sarcomere length increases. As a result of the increase in force-generating capability, the velocity of sarcomere shortening in an auxotonic contraction increases as the initial sarcomere length increases. The length-dependent increase in contractile activation (i.e., Pₒ) in conjunction with the rather slow time course of contractile activation causes the velocity of sarcomere shortening at any given sarcomere length and load to depend on the time in which the sarcomere reaches any given length. Since an auxotonic twitch contraction initiated from a long sarcomere length generally will reach a given sarcomere length at a later time than a contraction initiated from a short sarcomere length, it is possible that the velocity of sarcomere shortening will be increased at any given sarcomere length even though the force supported by the sarcomere may also be increased. The data presented in this paper also suggest that an increase in initial sarcomere length increases the rate of contractile activation during the contraction. Thus it is possible that, during an auxotonic contraction initiated from a long sarcomere length, the sarcomeres may pass through a given sarcomere length at the same time as a contraction initiated from a short sarcomere length, yet support a greater load at that time. The present findings suggest that it may be difficult to distinguish those changes in contractile performance resulting from changes in the overlap of the thick and thin filaments from those resulting from length- and time-dependent alterations in contractile activation, since the level and rate of contractile activation appear to depend on initial sarcomere length.

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