Evidence That the Velocity of Sarcomere Shortening in Single Frog Atrial Cardiac Cells is Load Dependent

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SUMMARY Recent experiments using laser diffraction techniques to determine the time course and extent of sarcomere shortening in thin bundles of cardiac tissue have given results which suggest that the velocity of sarcomere shortening in cardiac muscle is independent of the developed force (Nassar et al., 1974; Krueger and Pollack, 1975). However, the anatomical complexity of the intact tissue precludes a definitive interpretation of the data, since the exact relationship between the force being borne by the total tissue to the force being borne by any observed group of sarcomeres is uncertain. The single frog atrial cell provides a simple cardiac preparation in which the relationship between sarcomere velocity and sarcomere force is well defined, since these cells are only 1-2 myofibrils wide. The purpose of the present investigation was to determine if sarcomere shortening in the single frog atrial cell is dependent on force by measuring the time course of sarcomere shortening in single cells under conditions in which the cell developed markedly different forces. The results presented in this paper give direct evidence that the velocity of sarcomere shortening in the single cardiac cell depends on the force being developed by the sarcomeres. Thus, cardiac sarcomeres have a type of force-velocity relationship, although the exact nature of this relationship could not be determined in these experiments. Circ Res 48: 200-206, 1981

IN preliminary experiments on single frog atrial cells we found that the rate of force development during auxotonic twitch contractions was relatively constant for a significant portion of the rising phase of force development. Since the rate of force development in these experiments was directly proportional to the average sarcomere shortening velocity within the cell, the data indicated that the velocity of sarcomere shortening remained relatively constant even though force was increasing dramatically. Other investigators have found similar results in intact cardiac tissue (Nassar et al., 1974; Krueger and Pollack, 1975). However, the interpretation of the data derived from intact tissue is complicated by uncertainties with regard to the distribution of forces within the tissue (see Manring et al., 1977); i.e., the relationship between the force being borne...
by the total tissue to the force borne by any groups of sarcomeres is uncertain. The finding of a constant sarcomere velocity in the face of an increasing force raises the possibility that sarcomere velocity in cardiac muscle is independent of force (see Nassar et al., 1974).

The purpose of the present investigation was to determine if sarcomere velocity is dependent on force by measuring the time course of sarcomere shortening in single isolated frog atrial cells under conditions where the single cell developed markedly different forces. These cells are 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 also be the load–bearing sarcomeres. The results presented in this paper give direct evidence that the velocity of sarcomere shortening in the single cardiac cell depends on the force being developed by the sarcomere.

Also presented are the results of a computer simulation in which the time course of sarcomere shortening during a simulated auxotonic contraction was computed by numerical methods. In this simulation it was assumed that (1) the velocity of sarcomere shortening governed by Hill’s force–velocity relationship and (2) isometric force ($P_0$) varied in a relatively simple manner with time and sarcomere length. The results of the simulation demonstrate that a constant velocity of sarcomere shortening can occur in the face of a significant increase in force due to rather simple interactions of time and sarcomere length on isometric force.

**Methods**

Isolated single frog atrial cells were prepared by enzymatic digestion of intact frog (*Rana catesbeiana*) atrial tissues (Tarr and Trank, 1976). In some experiments trypsin (0.5 mg/ml) alone was used to disperse the cells from the tissue. The dispersed cells were harvested without centrifugation by removing the dispersed cells from the undigested tissue by means of a pipette. A small number of cells then were placed in a culture dish containing 2.5 ml of Ringer’s solution. The Ringer’s solution had the following composition: NaCl = 111 mM, KCl = 5.4 mM, CaCl$_2$ = 1.8 mM, tris(hydroxymethyl)aminomethane = 10 mM, glucose = 4 mM, and HCl as required to adjust the pH to 7.3. Experiments were done at room temperature ($\approx$25°C).

Conventional bright field light microscopic techniques were used to view the cell, the sarcomere pattern within the cell, and the position of the force beam (see below). Synchronized stroboscopic illumination was used to "freeze" the motion of the sarcomeres and the force beam. The data from most experiments were recorded on a closed circuit TV-video tape system and analyzed, using the stop frame (pausemode) capability of the video tape recorder in combination with a double TV cursor (Tarr et al., 1979). Each TV frame was identified by a six-digit real-time clock and a two-digit frame count. Both the digital clock and the digital frame identification were recorded on the video tape along with the microscopic image of the preparation, and they allowed a precise analysis of the timing of the cell’s mechanical events. In the experiments dealing with auxotonic contractions in which the compliance of a given force beam was altered (see below), the data were recorded photographically on a Grass C-4 camera.

For sarcomere length determinations, the length occupied by a small group of sarcomeres (generally 10) was determined and an average sarcomere length was calculated. The total length resolution was limited by the microscope optics to approximately 0.5 μm. Thus, the precision of an average sarcomere dimension determined from a group of 10 sarcomeres is no worse than about 0.05 μm.

To measure the force developed by the cell during twitch contractions the cell was attached to a poly-L-lysine-coated cantilevered force beam: the methods for preparation and calibration of the cantilever force beams have been presented previously (Tarr et al., 1979). The position of the force beam when the cell was completely slack was taken as the zero force position. The developed force in the cell at any given time during a twitch contraction was determined from the displacement of the force beam relative to its position prior to the initiation of the contraction. The total force in the cell was the sum of the resting force and the developed force. The force resolution is on the order of 2.5 nN for a force beam having a compliance of 0.2 μm/nN and 1.0 nN for a force beam having a compliance of 0.5 μm/nN.

Several different types of experiments were performed to determine whether the velocity of sarcomere shortening depended on the force being developed by the cell, and these are depicted schematically in Figure 1. In some experiments the time course of shortening (cell segment of sarcomere) first was determined during twitch contractions under very lightly loaded conditions and then was determined on the same cell during auxotonic contractions in which the cell developed significant force. The lightly loaded condition was accomplished by attaching one end of the cell to a flexible cantilevered force beam and drawing the other end of the cell into the end of a fluid-filled pipette with controlled suction (Fig. 1A). The suction pipette had an internal opening of about 15 μm and was filled with Ringer’s solution containing bovine albumin (1 mg/ml). The albumin decreased the tendency of the cell to stick to the inside of the pipette and thereby reduced the drag forces on the cell when the cell moved through the pipette opening during a contraction. Under these conditions the cell developed very little force when it contracted, since the unattached end of the cell was free to move in and out of the stimulus pipette (Fig. 1B).
FORCE
BEAM
SUCTION
PIPETTE

FIGURE 1 Schematic representation of the lightly loaded, auxotonic, and quick release experiments. The lightly loaded contraction is represented in A and B. The auxotonic contraction is represented in C and the contraction immediately after detachment of the cell from the stiff beam (i.e., quick release to small load) is represented in D. See text for further discussion.

Following the lightly loaded experiments, a stiff poly-L-lysine glass beam was attached to the cell surface at a point close to the opening of the suction pipette (Fig. 1C), and the cell then was allowed to shorten and develop force in an auxotonic fashion. The forces developed by the cell and the sarcomere performance during the auxotonic twitch contractions then were compared to those during the lightly loaded twitch contractions under identical conditions of stimulus strength and stimulus duration. In some of these experiments, a fortuitous event occurred. As the cell began to develop force during the rising phase of the auxotonic contraction, it became detached from the stiff beam. In a few cases this detachment did not appear to injure the cell. Thus, these experiments were unintentional quick releases to very light loads (Fig. 1D), and the performance of a given group of sarcomeres in response to a sudden change in load could be determined. In all of these experiments, electrical stimulation of the cell was provided by passing current between a small-bore fluid-filled pipette (anode) placed in close proximity to the cell as it passed around the outside of the stiff beam and an Ag/AgCl electrode (cathode) placed in the bathing medium.

Results

Lightly Loaded vs. Auxotonic Contractions

In these experiments the time course of sarcomere shortening during very light loaded twitch contractions was compared to that during auxotonic twitch contractions. Figure 2 gives a typical result for the time course of shortening of a group of 10 sarcomeres determined under lightly loaded and auxotonic conditions. These sarcomeres were close to the force beam and could be identified easily under both conditions. It is obvious that the velocity of shortening was markedly slower during the auxotonic contraction than during the lightly loaded contraction (upper part of Fig. 2). Also, the force developed during the auxotonic contraction was much greater than the force developed during the lightly loaded contraction (lower part of Fig. 2).

Auxotonic Contractions against an Adjustable Compliance Force Beam

In these experiments the cell was allowed to contract auxotonically and flex a force beam having an adjustable compliance. Figure 3 presents the data obtained from one cell when this cell contracted against a force beam at two different compliances. The sarcomere length determined for a group of 10 sarcomeres was markedly slower during the auxotonic contraction than during the lightly loaded contraction (upper part of Fig. 2). Also, the force developed during the auxotonic contraction was much greater than the force developed during the lightly loaded contraction (lower part of Fig. 2).

Quick Release Experiments

In these experiments, sarcomere performance was first determined under lightly loaded conditions and then the cell was attached to a second stiff
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FIGURE 2  Time course of sarcomere shortening (upper) and force development (lower) during lightly loaded (circles) and auxotonic (triangles) twitch contractions. The same group of 10 sarcomeres was measured in both contractions.

A typical result obtained from one cell is shown in Figure 4. The sarcomere length-vs.-time and force-vs.-time relationships obtained during a lightly loaded contraction are given by the circles. The data obtained during the auxotonic-quick release contraction are given by the triangles. The arrow indicates the time at which the cell became detached from the stiff beam. It is apparent that prior to detachment the velocity of sarcomere shortening during the auxotonic contraction is significantly slower than the velocity during the lightly loaded contraction. In contrast, the velocity of sarcomere shortening after detachment approximates the sarcomere velocity during the constant velocity phase of shortening during the lightly loaded contraction. These results give dramatic evidence within a given contraction that sarcomere velocity depends on the force developed by the sarcomeres.

Discussion

The data presented in this paper clearly demonstrate that sarcomere velocity in the single cardiac cell is influenced by the force developed by the sarcomeres.

FIGURE 3  Time course of sarcomere shortening and force development during contractions in which the cell shortened against a force beam of adjustable compliance. See text for further discussion.
cell developed significant force. Second, during auxotonic contractions in which a given cell contracted against an adjustable compliance force beam, the sarcomere velocity decreased as the compliance of

Figure 4 Time course of sarcomere shortening and force development during lightly loaded (circles) and auxotonic (triangles) contractions. The arrow indicates the time during the auxotonic contraction when the cell detached from the stiff glass beam and the cell suddenly became lightly loaded. The small drop in force just prior to detachment suggests that the attachment of the cell to the stiff beam was beginning to weaken prior to complete detachment. The same group of sarcomeres was measured throughout the experiment.

Figure 5 Time course of sarcomere shortening (upper) and force development (lower) during simulated twitch contractions. The circles give the computed values of sarcomere length at time intervals of 16.67 msec during a simulated unloaded ($P = 0$) contraction. The triangles give the computed values of sarcomere length and developed force at time intervals of 16.67 msec during a simulated auxotonic contraction. The solid lines were fitted by eye to the computed values. The phases of the contractions during which the velocity of sarcomere shortening was relatively constant are designated by the arrows.
the force beam decreased. Third, quick release of a cell to very light loads during the rising tension phase of an auxotonic contraction resulted in an immediate increase in sarcomere velocity. All of these observations clearly indicate that the sarcomere velocity in cardiac muscle is load dependent; that is, the cardiac sarcomere has some type of a force-velocity relationship.

The observation in the single cell that the rate of tension development (proportional to velocity of sarcomere shortening) during auxotonic contractions is fairly constant during a significant portion of the rising phase of tension development (see Figs. 1-4) is similar to the findings in intact tissue that the velocity of sarcomere shortening remains fairly constant during the rising phase of tension development (Nassar et al., 1974; Krueger and Pollack, 1975). Also, data have been presented previously by Pollack and Krueger (1976) that could be interpreted as indicating that, in intact rat papillary muscle, the unloaded sarcomere velocity is greater than the sarcomere velocity during a so-called isometric contraction (see Fig. 7 of their paper). However, the authors did not discuss their findings in this regard. Thus, the performance of sarcomeres within the single cardiac cell appear to be similar to those in intact cardiac tissue, although the distribution of force is better defined in a cell only 1-2 myofibrils wide than in the multicellular intact tissue.

The findings in the intact cardiac preparation, as well as in the single cardiac cell of a constant velocity of sarcomere shortening under conditions of dramatically changing force, raise the question as to how this constant sarcomere velocity occurs. If we assume that the velocity of sarcomere shortening is governed by the classic Hill force-velocity relationship, then one would expect that the velocity of shortening (V) should decrease as force increases (P) according to the equation V = b(P₀ - P)/(P + a). This would be true if the only parameter changing with time were P. However, isometric force (P₀) depends on the level of contractile activation and therefore is time dependent. Also, P₀ depends on sarcomere length, and it is possible that the time-dependent (i.e., the level of contractile activation) and length-dependent effects of P₀ interact to produce a phase of sarcomere shortening during a twitch contraction in which the velocity of sarcomere shortening appears to be independent of force. Manring et al. (1977) entertained such an explanation of the data relating the time course of sarcomere shortening to force in intact frog atrial tissue (Nassar et al., 1974). Furthermore, Hill (1970) has demonstrated that even skeletal muscle does not obey the classic force-velocity relationship during the early phase of an isometric contraction, presumably because the so-called active state is not fully developed. Rather, sarcomere velocity appears to be independent of force until P/P₀ approaches a value of about 0.4 (see Hill's Fig. 3.3). Thus, it seems reasonable to anticipate that an explanation for the phase of constant velocity of sarcomere shortening during a twitch contraction might reside within a consideration of possible time- and length-dependent interactions.

To determine if a relatively simple time and length interaction could produce a constant velocity of sarcomere shortening under conditions in which the sarcomere velocity was governed at all times by Hill's force-velocity relationship, the time course of sarcomere shortening during simulated twitch contractions was computed on a digital computer using standard numerical methods (see Appendix.). Isometric force (P₀) was assumed to be dependent on time and sarcomere length in a very simple fashion: at every instant of time, P₀ increased as a linear function of sarcomere length and the magnitude of P₀ varied with time according to a simple I-Exp(-t/τ) relationship.

The results of two different simulations are shown in Figure 5. The circles (upper half of figure) give the time course of sarcomere shortening during a twitch contraction in which the sarcomeres developed no force (i.e., an unloaded contraction). The triangles give the time course of sarcomere shortening during an auxotonic twitch contraction in which the sarcomeres developed significant force. The time course of force development during the auxotonic contraction is shown in the lower half of Figure 5.

These computer simulations gave results similar to those obtained experimentally. First, the time course of sarcomere shortening during the unloaded contraction had a phase of constant velocity over a significant range of sarcomere lengths, a result similar to that observed experimentally during lightly loaded contractions (Figs. 2 and 4). Second, the velocity of sarcomere shortening during the auxotonic contraction was significantly less than that during the unloaded contraction (Figs. 2 and 4). Third, there was a significant portion of the auxotonic contraction during which sarcomere velocity and rate of force development were relatively constant even though force was increasing dramatically. These simulations indicate that the findings presented in this paper are consistent with the hypothesis that the constant velocity of shortening results from an interaction of time- and length-dependent changes in isometric force. However, this statement should not be construed to mean that this is the only possible interpretation of the data. Rather, the simulations demonstrate that a constant velocity of sarcomere shortening in the face of an increasing force can be explained rather simply in terms of an interaction between time- and length-dependent effects.

The data presented in this paper clearly demonstrate that the velocity of shortening of the cardiac sarcomere is dependent on force. However, the ex-
act nature of the force-velocity relationship remains to be determined.

**Appendix**

The time course of sarcomere shortening during simulated twitch contractions were computed by a digital computer using Euler's iterative method; a time interval of 1.667 msec was used in all calculations. The length and time dependency of isometric force during the contraction was assumed to be governed by the following equation where $P_{\text{max}}$ is the maximum value of $P_0$ at a sarcomere length of 2.4 $\mu$m and $\tau$ is the time constant of contractile activation.

$$P_0 = (P_{\text{max}}) \frac{1 - \exp(-t/\tau)}{1} (S-R)/(2.4 - R)$$

This equation describes an instantaneous straight line relationship for $P_0$ as a function of sarcomere length ($S$). The line rotates with time about a fixed sarcomere length $R$. The time course of force development during the contraction was computed from the equation, $P = (K)(N)(S_0 - S)$, where (1) $K$ = the stiffness of the force beam in nN/$\mu$m; (2) $n$ = the number of sarcomeres within the simulated length of tissue; (3) $S_0$ = the initial sarcomere length prior to the onset of sarcomere shortening; and (4) $S$ = the sarcomere length at any given time during the contraction. Hill's force-velocity relationship as given by the following equation was used to compute the velocity of sarcomere shortening at the end of each computation time interval based on the calculated values of $P_0$ and $P$ at that time.

$$V = b(P_0 - P)/(P + a) \quad \text{at} \quad t = 0, S = S_0$$

The following values chosen for the various parameters used in the calculations were based on reasonable values derived from experimental data. The value of 400 nN chosen for $P_{\text{max}}$ was based on the observation that a typical single cardiac cell can develop auxotonically forces in the range of 100-1000 nN. A value of 1.3 for $R$ was based on the observation that during lightly loaded contractions many cells can shorten to this sarcomere length. A value of 3 nN/$\mu$m was chosen for the force beam stiffness, since this is a typical value for our cantilevered force beams. For the unloaded contraction, $K = 0$. An n value of 50 was used, since this was typical of the number of sarcomeres in the cell segment lengths often used experimentally. A value of $a = 100$ nN was used, since this gave a $P_{\text{max}}$ ratio of 4 at a sarcomere length of 2.4 $\mu$m; a $P_{\text{max}}$ ratio of 4 is a typical value for striated muscle. A value of $b = 5$ $\mu$m/sec was used, since this would be equivalent to a b value of approximately 1.9 initial muscle lengths/sec; again this is a value typical of striated muscle. A time constant ($\tau$) for activation of 250 msec was chosen to approximate the time course of tension development observed experimentally. The use of an isometric force-sarcomere length relationship in which $P_0$ increases in a linear fashion as sarcomere length increases was based on our experiments (unpublished observations) which indicate that the single cardiac cell is operating on a relatively linear ascending limb of a sarcomere length-auxotonics tension relationship up to a sarcomere length of at least 2.8 $\mu$m. Also, relatively linear force-length relationships have been reported for (1) twitch contractions in skeletal muscle (Rack and Westbury, 1969), (2) tension development in partially activated skinned frog cardiac cells (Fabiato and Fabiato, 1978), and (3) twitch contractions in rat trabeculae bathed in low calcium (ter Keurs et al., 1980).

**References**


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