Sarcomere Length-Resting Tension Relation in Single Frog Atrial Cardiac Cells

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SUMMARY  It generally has been thought that the relatively high resting tension characteristic of cardiac tissue resides in structures (collagen, elastin) external to the individual cardiac cells, but the evidence to support this conclusion has been indirect, since the resting tension of intact single cardiac cells has not been determined previously. The purpose of the present investigation was to determine the resting tension (stress)-sarcomere length relationships of single intact frog atrial cells. For tension determinations, a single cell was attached between two poly-L-lysine-coated glass beams: one beam served as a compliant calibrated cantilevered force beam, and length changes were imposed on the cell by movement of the other beam. Conventional bright-field light microscope techniques were used to view the cell, the sarcomere pattern within the cell, and the position of the force beam. The resting tension of the intact cell increased from a value of about 10 nN at a sarcomere length of 2.35 μm to a value of about 130 nN at a sarcomere length of 3.45 μm. Lagrangian and Eulerian resting stress-sarcomere length relationships were computed from the resting tension-sarcomere length relationships. The Lagrangian stress increased from a value of about 0.6 mN/mm² at a sarcomere length of 2.35 μm to a value of about 7 mN/mm² at a sarcomere length of 3.45 μm. These values of stress are about 8- to 30-fold less than those previously reported for intact frog atrial tissue and indicate that the resting tension of intact frog atrial preparations resides primarily in structures external to the individual cardiac cell. Circ Res 45: 554–559, 1979

IT IS well established that the resting tension (stress)-sarcomere length relationships of a variety of intact preparations of cardiac muscle are different from those of skeletal muscle preparations. In contrast to skeletal muscle in which resting tension is negligible at sarcomere lengths below about 2.3 μm (Gordon et al., 1966; Lännergren and Noth, 1973; Moses and Halpern, 1977), mammalian cardiac preparations have relatively high resting tensions at these sarcomere lengths (Spiro and Sonnenblick, 1964), and the resting tension increases markedly at sarcomere lengths beyond 2.3 μm. In nonmammalian cardiac preparations, variable results have been obtained. Starling (1918) found in intact tortoise ventricle that the resting tension remained relatively small over a wide range (approximately 6-fold) of diastolic volumes, which included the rising phase and the relatively broad range (approximately 2-fold) of the active tension-diastolic volume relationship. In contrast, both Winegrad (1974) and Matsubara and Maruyama (1977) found that the resting tension increased significantly over the sarcomere length range of 2.0–2.6 μm in small bundles of frog atrial tissue. Nassar et al. (1974) found...
of the structures responsible for the resting tension may vary with different cardiac preparations.

The purpose of the present investigation was to determine the resting tension-sarcomere length relationship of isolated intact single frog atrial cells. The resting tension (stress)-sarcomere length relationship of the single frog atrial cardiac cell directly supports the conclusion that the high resting tension that exists in intact frog cardiac preparations resides in structures external to the individual cardiac cells.

Methods

Preparation and Calibration of Cantilever Force Beams

The force generated by the single cardiac cell was measured by a cantilevered glass beam having a compliance such that a measurable movement of the beam could be obtained for a few nanoNewtons (nN) of force. These compliant glass beams were prepared by pulling glass capillary tubing (1 mm o.d.) on a microelectrode puller in such a manner to obtain a section of glass having relatively uniform diameter (10 µm or less) over a length of 2-3 mm. A force beam of appropriate compliance then was fashioned by breaking the tip of the uniform cylindrical region at an appropriate length on a microforge; e.g., a glass beam 10 µm in diameter and 3 mm in length has a compliance on the order of 0.3 µm/nN. The tip of the beam then was heat occluded to remove any sharp edges.

Each glass force beam was calibrated in air indirectly by measuring the deflection of the tip of the beam when it was displaced against a long quartz rod of known compliance. The quartz rod used in this study had a length of about 50 mm, a diameter of about 100 µm, and a compliance of 0.17 µm/nN. The force beams were found to have constant compliance to forces as great as 2000 nN, and the compliance of the force beam was the same in water as in air.

Attachment of Cell to Glass Beams

To measure the force either applied to or generated by a single cardiac cell, it is necessary to attach the cell between two glass beams: a stiff beam, which can be positioned to change the length of the cell, and a calibrated compliant force beam to measure force. The glass surface of both the stiff and compliant beams was coated with poly-L-lysine (Sigma Chemical Co., no. P-2636, mol. wt. ≈ 60,000-80,000) by dipping the beam into a solution of poly-L-lysine (0.1 mg/ml). The excess poly-L-lysine then was washed off by dipping the beam in deionized water. The cell was attached to the two glass beams by touching a glass beam to each end of the cell, and then the cell was secured by performing end-to-end rotation of the cell about the beams with micromanipulative techniques. In this manner, the ends of the cell were wrapped around the glass beams by one to two turns.

Measurement of Cell Dimensions, Sarcomere Length, and Force Beam Displacement

Conventional bright-field light microscope techniques were used to view the cell, the sarcomere pattern within the cell, and the position of the force beam. A beam splitter in the optical path allowed the simultaneous viewing of the microscope field by two TV cameras. One TV camera was positioned to view the force beam; the other camera was positioned to view the sarcomere pattern within a small region of the cell. A split-screen video switch was used to provide a composite TV display of portions of the images from the two synchronized TV cameras. An adjustable TV reticle composed of a grid of horizontal and vertical lines was added to the composite video to provide a dimensional reference for the TV display. The spacing between the grid lines was adjustable, and the reticle line spacing on the TV display was determined by comparison with the spacing of a stage micrometer displayed through the TV microscope system. Each TV frame was identified uniquely by a six-digit real-time clock plus a two-digit count of the TV frames within each 1-second interval.

A typical TV monitor display is shown in Figure 1. The composite image illustrates (1) the split-screen display of a region of the cell with its sarcomere pattern (upper part) and the force beam (lower part), (2) the time and frame identification numbers, and (3) the X-Y TV reticle. The screen dimensions referred to the microscope stage are approximately 38 × 50 µm. During cell length changes imposed on the cell by moving the stiff beam, the sarcomere length changes were observed on the upper screen, and the resulting displacements of the force beam were observed simultaneously on the lower screen. A permanent retrievable store of the TV display for data analysis was recorded on a conventional video tape recorder (Sony AV 3600).

Analysis of the video taped data was possible by using the stop-frame capability (pause mode) of the video tape recorder in combination with a double TV cursor. The double TV cursor displays two independent horizontal pointers on the TV monitor, one originating from the left and one from the right margin of the monitor screen. The vertical position and horizontal length of each cursor is independently adjustable. The horizontal and vertical components of the separation between the pointer tips are presented on two three-digit LED displays. Thus, the digital displays present the horizontal and vertical components of the distance between the cursor tips, and dimensions within the TV display (cell, sarcomere, force beam displacement) can be determined by appropriate placement of the cursor tips. The total length resolution for the sys-
tem was limited primarily by the microscope optics to approximately 0.5 μm. For sarcomere length determinations, the length occupied by 10–15 sarcomeres was measured with the cursors and an average sarcomere length calculated for the group: the precision of an average sarcomere dimension determined from a group of 15 sarcomeres is, therefore, no worse than about 0.03 μm (0.5 μm/15). Applying the same 0.5-μm length resolution to the measurement of the displacement of the force beam gives a force resolution 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.

Preparation of Isolated Frog Atrial Cells

Isolated single frog atrial cardiac cells were prepared by trypsin-collagenase dispersion of intact frog (Rana catesbeiana) atrial tissue as described previously (Tarr and Trank, 1976). After centrifugation, the cells obtained from a 30-minute digestion period were resuspended in a few milliliters of Ca²⁺-free Ringer's solution having the following composition: NaCl = 111 mM, KCl = 5.4 mM, 10 mM tris (hydroxymethyl)aminomethane, glucose = 4 mM, penicillin = 0.1 U/ml, and streptomycin = 100 μg/ml. The pH of this solution was adjusted to 7.3 at 25°C by adding 12.4 N HCl. The viability of cells obtained by this technique has been tested previously, and it was found that about 79% of the cells gave twitch-like contractions in response to electrical stimulation (Tarr and Trank, 1976). Although the electrical excitability of the cells used in this study was not tested directly, in subsequent experiments we have found that cells that survive attachment to the glass beams generally are electrically excitable, can generate twitch tensions on the order of 200 to 1000 nN, and have resting tension-sarcomere length relationships similar to those reported in this paper.

Determination of Cell Length, Sarcomere Length, and Resting Force Relationships

A few drops of the resuspended cells were placed in a culture dish containing 2.5 ml of either Ca²⁺-free Ringer's solution or Ringer's solution having a calcium concentration of 1.8 mM. A cell was attached between two glass beams as discussed previously. The position of the force beam, when the cell was slack between the two beams, was taken as the zero-force position. The force in the cell at any cell length was determined from the displacement of the force beam relative to this zero-force position. The force in the cell at any cell length was determined from the displacement of the force beam relative to this zero-force position as incremental stretches of the cell were performed by movement of the stiff glass beam. The average sarcomere length after each incremental length change was determined as discussed previously. Intermediate or low power magnifications were used to determine the length of the cell segment between the glass beams.

Results

Resting Tension-Sarcomere Length Relationships

The resting tension-sarcomere length relationships were determined for 16 cells in Ca²⁺-free Ringer's solution and 14 cells in Ringer's solution containing 1.8 mM Ca²⁺. For analysis, the data were grouped into sarcomere length intervals of 0.1 μm (e.g., 2.50–2.59 μm), and the mean tension within each length interval was determined. The mean resting tension-sarcomere length relationships obtained from this analysis are presented in Figure 2. It is apparent that no difference exists between the relationships obtained in Ca²⁺-free or Ca²⁺-containing Ringer's solution; the cell diameters of the population of cells investigated in Ca²⁺-free Ringer's solution (5.0 ± 0.5 μm) were not significantly different from those investigated in Ca²⁺-containing...
Ringer's solution (4.5 ± 0.3 μm). The resting tension-sarcomere length relationship could be fitted reasonably to an exponential relationship. The solid line (Fig. 2) describes the equation \[ T = 8.0 \exp(5.26)[(S-2.25)/2.25], \] where \( T \) is tension in nN, and \( S \) is sarcomere length in μm.

**Resting Stress-Sarcomere Length Relationships**

Lagrangian and Eulerian resting stress-sarcomere length relationships (Fig. 3) were computed from the resting tension-sarcomere length data obtained in Ca²⁺-free Ringer's solution. In the computation of Lagrangian stress, the diameter of the observed cell region at the initial sarcomere length \( (S_i) \) was used to compute the initial cross-sectional area \( (A_i) \) of the cell assuming it to be a right cylinder. The Lagrangian stress \( (T/A) \) at each sarcomere length then was computed from the resting tension data; the data again were grouped into 0.1-μm sarcomere length intervals, and the mean Lagrangian stress within each length interval was determined. For the calculation of Eulerian stress, the diameter of the observed cell region at each sarcomere length \( (S) \) was measured, and the cross-sectional area \( (A) \) was calculated assuming the cell to be a right cylinder. The Eulerian stress \( (T/A) \) at each sarcomere length then was computed from the resting tension data, and the mean Eulerian stress

within the sarcomere length intervals of 0.1 μm was determined. Semilog plots of Lagrangian and Eulerian stress vs. sarcomere length. The mean values of stress within sarcomere intervals of 0.1 μm are plotted: either ± 1 SEM also is shown for simplicity. For Lagrangian stress, the solid line describes the equation \[ \sigma = 0.45 \exp(5.26)[(S-2.25)/2.25], \] where \( \sigma \) is the stress. For Eulerian stress, the broken line defines the equation \[ \sigma = 0.45 \exp(6.12)[(S-2.25)/2.25]. \] See text for further discussion.

**Cell Length vs. Sarcomere Length**

In the determination of the resting tension-sarcomere length relationships, the measurement of sarcomere length was restricted to only a small length (less than 50 μm) of the cell. It is possible that some disparity in sarcomere length may occur in the region of the glass beams, since wrapping the cell around the beam would tend to compress the sarcomeres along the inner radius of curvature and extend the sarcomeres along the outer radius of curvature of the cell. Thus, the regions of the cell close to the beams may have a compliance different from that of the cell region under observation. It therefore was necessary to determine how representative the average sarcomere length in the section of cell under observation was of the average sarcomere length in the entire cell segment between the glass beams. To answer this question, the fractional change in overall length of the cell segment
between the glass beams was compared to the fractional change in sarcomere length in the observed region of the cell. The fractional change in overall cell segment length \((L/L_i)\) should equal the fractional change in sarcomere length \((S/S_i)\) if the cell were attached between the glass beams such that no new cellular material (sarcomeres) was added between the beams as the cell was stretched, and the resting tension-sarcomere length relationship was uniform along the length of the cell segment between the points of attachment. Cell length-sarcomere length determinations were performed on 12 cells (10 in Ca\(^{2+}\)-free Ringer's and 2 in Ca\(^{2+}\) = 1.8 mM). For simplicity, representative data obtained from only six of the cells are shown in Figure 4. In this figure, the ratio \((L/L_i)/(S/S_i)\) is plotted as a function of \(S/S_i\); \(L_i\) and \(S\) are the initial lengths prior to stretch. The ratio \((L/L_i)/(S/S_i)\) should equal one for all values of \(S/S_i\) if the fractional change in cell length equals the fractional change in sarcomere length. In experiments on 12 cells with \(L_i\) ranging from 92 to 342 \(\mu\)m, the ratio never deviated by more than 8%, and in most cases, the deviation was only 1–2%. Thus, the observed sarcomeres extended during cell stretch as though they were part of a population of uniformly extended sarcomeres, and the resting tension-sarcomere length relationships obtained from a small population of observed sarcomeres are representative of the entire cell segment between the beams.

Discussion

It is well established that preparations of intact cardiac muscle can have considerable resting tension (stress) at relatively short sarcomere lengths. For example, stresses on the order of 0.3 kg/cm\(^2\) (29 mN/mm\(^2\)) at a sarcomere length of about 2.2 \(\mu\)m have been reported for cat papillary muscle (Spiro and Sonnenblick, 1964); at 20% greater lengths than 2.2 \(\mu\)m, the resting stress was on the order of \(7 \text{ kg/cm}^2\) (=686 mN/mm\(^2\)). Somewhat smaller values have been reported for small bundles of frog atrial tissue (Winegrad, 1974), where the resting stress was on the order of 40 g/cm\(^2\) (3.9 mN/mm\(^2\)) at a sarcomere length of 2.2 \(\mu\)m and 300 g/cm\(^2\) (29 mN/mm\(^2\)) at a sarcomere length of 2.6 \(\mu\)m. In contrast, the single intact frog atrial cell has relatively low resting stress at short sarcomere lengths. For example, the resting stress is on the order of 0.5 mN/mm\(^2\) at a sarcomere length of 2.25 \(\mu\)m, and the stress increases to only about 1 mN/mm\(^2\) at a sarcomere length of 2.6 \(\mu\)m. These values of resting stress for the single cardiac cell are similar to those that have been reported for both single frog semitendinosus muscle cells (Lännergren and Noth, 1973) and intact frog semitendinosus muscle (Moss and Halpern, 1977). Unfortunately, a direct comparison of the data presented in this paper to the resting tension-sarcomere length data on skinned frog atrial cells is not possible, since absolute values of tension for the skinned frog atrial cells were not given by Fabiato and Fabiato (1978); the resting tension was presented as percent of maximum active tension. If the maximum active tension of the 3- to 7-\(\mu\)m-wide, skinned frog atrial cells were on the order of 100 \(\mu\)g (a value similar to maximum twitch tensions we have recorded from single frog atrial cells), then the computed resting tension for the skinned frog cells at a sarcomere length of 3.0 \(\mu\)m would be about 15 \(\mu\)g (147 nN). This value appears to be greater than the value of about 40 nN that we found for intact frog atrial cells (Fig. 2). Although this calculation is uncertain, it does indicate that the intact frog atrial cell probably is not any stiffer than the mechanically skinned cell, suggesting that the sarcolemma contributes little to the resting tension of the single frog cardiac cell.

It recently has been suggested (Maruyama et al., 1976, 1977a, 1977b) that the elastic protein, connectin, makes a contribution to the intracellular elasticity of muscle fibers. In the case of frog cardiac muscle, it has been suggested that connectin makes a significant contribution to the resting tension of the intact tissue at sarcomere lengths greater than about 2.0 \(\mu\)m (Matsubara and Maruyama, 1977). It is possible that connectin plays a role in the resting tension in the intact single frog atrial cell, but it must occupy less than about 5% of the cross-sectional area of the intracellular material. This conclusion is based on the fact that a stress of about 240 mN/mm\(^2\) would be expected for an extension of the cell from 2.2 to 3.5 \(\mu\)m if it were composed entirely of connectin having a Young's elastic modulus of \(4 \times 10^9\) dyn/cm\(^2\) (Maruyama et al., 1976). This value is more than 20-fold greater than the actual stress of about 10 mN/mm\(^2\) at a sarcomere length of 3.5 \(\mu\)m (Fig. 3) found in the intact frog atrial cell. For the resting tension of the intact frog atrial tissue to be due primarily to connectin, as suggested by Matsubara and Maruyama (1977), the connectin must be primarily extracellular, since the

![Diagram](image-url)
resting stress of the intact tissue at a sarcomere length of 2.6 μm is approximately 30-fold greater than the resting stress in the intact cell.

It is well known that the elasticity of living soft tissues is highly nonlinear. Fung (1967) suggested that the relationships between passive tension and length could be described by an exponential relationship of the form \( T = T^* \exp (a) (\lambda - \lambda^*) \), where \( T \) is the tension or stress, \( \lambda \) is the extension ratio (length divided by nonstressed length), \( a \) is an exponential index, and \( T^* \) is the tension (or stress) at a specific extension ratio \( \lambda^* \). Such an exponential function has been used to describe the passive tension-length relationships of skeletal muscle (Moss and Halpern, 1977) and cardiac muscle (Kitabatake and Suga, 1978). The passive tension-sarcomere length relationship of single frog atrial cells appears to be described adequately by an exponential function over the sarcomere length range of about 2.2 to about 3.5 μm used in the present investigation. The exponential index \( a \), obtained from the Lagrangian stress-sarcomere length relationships, is on the order of 5.3, which is similar to a value of 6–7, which can be calculated from data on semitendinosus muscle (Moss and Halpern, 1977), but it is considerably less than the values of 18–37 that have been reported for intact mammalian cardiac muscle (Kitabatake and Suga, 1978).

It previously has been suggested by some investigators that individual cardiac cells are responsible for very little of the stiffness characteristic of intact cardiac tissue (Gay and Johnson, 1967; Winegrad, 1974), but these suggestions were based on indirect evidence. The data presented in this paper directly support the conclusion that the stiffness of resting intact frog atrial cardiac preparations resides primarily in structures external to the individual cardiac cell, since the resting stress in the isolated frog atrial cell at sarcomere lengths of 2.2–2.6 μm is 8- to 30-fold less than values reported for intact frog atrial tissue (Winegrad, 1974).

References
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