Intrinsic Tension and Shortening Velocity of Intact Segment without Damaged Cells in Bullfrog Cardiac Muscle

Moto MATSUMURA and Keiji TAMURA*

Department of Physiology,
** Division of Cardiology, Department of Medicine,*
Kawasaki Medical School, Kurashiki 701-01, Japan

Accepted for publication on December 7, 1994

ABSTRACT. The tension and length change of the bullfrog atrial trabeculae muscle were measured under control of the central segment length. Damaged cells located at both ends of the muscle preparation were avoided in the segment. Two pieces of thin black tape were placed circumferentially on the central portion of the muscle preparation 5 to 7 mm apart from each other. The movement of each piece of tape was recorded by a photoelectrical device and the distance between the two pieces of tape was measured through a differential amplifier. The output voltage of the amplifier was fed back to an ammeter, so that the length of the central segment could be kept constant during the contraction. Even in the ordinary isometric contraction, the central intact segment shortened by around 10% in compensation for stretching the peripheral damaged segments. The ratio of the segment isometric tension to the muscle isometric tension reached 1.5 to 2.0. The shortening velocity under nominally 0 load measured from the intact segment was 120% of the one measured from a whole muscle.

Key words: cardiac muscle contraction — muscle length control — length-tension relation — force-velocity relation

The mechanical properties of different kinds of cardiac muscles in different animals, such as the length-tension relation and the force-velocity relation, as well as the active state curve have been extensively studied.[1-3] The results were explained based on the findings obtained from the frog skeletal muscle.[4-6] Many previous authors used papillary muscles from cats, rabbits or guinea pigs. In their experiments, the ventricular end together with the ventricular tissue was fixed while the tendinous end was connected to the transducer with a thin thread. The most serious problem in using these muscle preparations to investigate muscle mechanical properties is the additional compliance due to extra tissues and damaged cells caused by the procedures of isolation and preparation, as pointed out by several other authors.[7-11]

Among these authors, Donald *et al*[10] stuck the papillary muscle perpendicularly with two thin stainless pins and used them as markers to separate the central intact segment from the peripheral damaged segments, and then they measured and controlled the intact segment length, SL, instead of the

松村幹郎，田村敬二

whole muscle length, ML. However, their method contained some errors when the pins moved in a diagonally leaning position during contraction. Huntsman *et al*[11] also measured the SL from a change in the cross-sectional area of the muscle, assuming the tissue volume did not change throughout contraction and relaxation. Their assumption seems not to be strictly maintained,[12] especially in multicellular tissues. We have developed a new technique for measuring and controlling the SL which makes determination of the exact length-tension and force-velocity relations of the bullfrog atrial muscle excluding damaged cells attainable. We call this method for controlling muscle length the length clamp method. In the present paper, the procedures and a few results obtained by the segment length clamp method are presented.

METHODS

Atrial muscles of the bullfrog (*Rana catesbeiana*) were used. A muscle preparation 7 to 12 mm long and 0.7 to 1.0 mm in diameter was placed horizontally in the chamber (1.7×7.0×1.0 cm). The sides of the chamber were made of plastic and the base was thin cover glass. The muscle was massively stimulated with pulses of 2×suprathreshold strength and 3 msec duration at a rate of 1/5sec via Ag-AgCl electrodes positioned in the side walls of the chamber. All the experiments were carried out at room temperatures of 20 to 24°C. Ringer solution contained (mM) NaCl 117, KCl 2.0, $CaCl_2$ 3.6, glucose 10.0 and HEPES-NaOH buffer 8.0 adjusted to a pH of 7.4. The Ca^{2+} concentration was doubled to obtain a strong contraction.

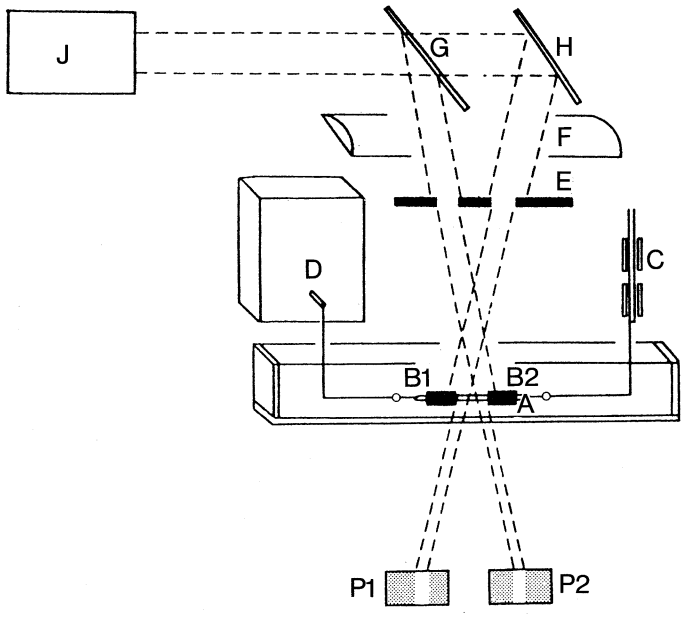

Fig 1. Arrangement of experimental instruments. A: muscle preparation, B1, B2: black tape as a marker, C: strain gauge, D: length transducer, E: slit, F: cylindrical lens, G: beam splitter, H: surface reflecting mirror, J: halogen lamp house, P1, P2: photodiode arrays

The arrangement of the experimental equipment is shown in Fig 1. Small hooks made of silver wire (0.1 mm diameter) were firmly tied to both ends of the muscle preparation with thin silk thread. The hook at one end was connected to a strain gauge tension transducer and the one at the other end to a length transducer (JCCX-101, Mass. Cambridge), which was reconstructed so that the input for negative pulse might be added. Each transducer was mounted on a micromanipulator. In the isotonic condition, the length transducer was replaced by an ammeter, the arm of which was supported by steel rods, and the rotation of the arm following muscle shortening was optically detected. For measurement of the SL, two pieces of 0.5 mm wide pasted black tape, which was usually used for drawings, were firmly attached circumferentially to the muscle. Each piece weighed about 5 mg. When the muscle shortened, the edge of each piece of tape moved along the longitudinal direction of the muscle preparation. The distance between two markers was 5 or 7 mm in the initial condition, and its change during contraction was measured by an optical device. The light source was a 50 watt halogen lamp driven by a 12 V battery. The light beam was made parallel through a convex lens and was split into two directions to illuminate the two markers separately. The beam splitter and the surface mirror were cemented to the axis of each galvanometer of a two-pen recorder. Thus, the light beams could be accurately directed toward each of the two markers. A cylindrical lens (f=6.0 cm) and two narrow slits (0.5×1.5 mm) which focused the light beam to each of the markers, were placed between the mirror and muscle. The width of the light beam that illuminated the photodiode arrays (S-1592, Hamamatsu Photonics) changed as the marker moved during the contraction, and the change in the position of illuminated portion of the arrays was converted to the voltage. The test of the optical system demonstrated that the distance could be measured

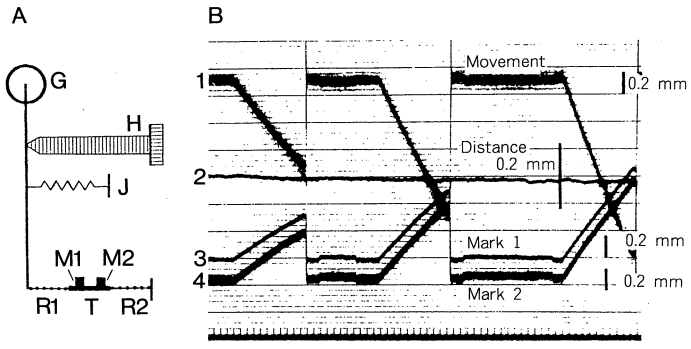

Fig 2. Test experiment to show the constant distance when each marker is displaced with the different speeds

A: A schematic diagram of the arrangement for the test. G: ammeter, H: micromanipulator, J: spring, M_1 and M_2: markers, T: stainless wire of 10 mm long, R_1 and R_2: thin rubber threads of 10 mm long. Rubbers are stretched to about twice as long as a relaxed length.

B: Trace 1: movement of a tip of galvanometer arm; downward deflection indicates the rightward movement. Trace 2: change in distance between M_1 and M_2; downward deflection indicates the elongation of the distance. Traces 3 and 4: displacement of markers; upward deflections indicate the rightward displacement. Rubber threads are allowed to shorten at different speeds by adjusting a micromanipulator.

accurately, as shown in Fig 2. Two pieces of black tape were pasted to a stainless wire, to both ends of which thin rubber threads were attached. An end of one rubber thread was fixed and of another rubber thread was tied to a tip of the arm of an ammeter (Fig 2A). A position of the arm was changed manually with a micromanipulator. Records in Fig 2B show the constancy of distance between markers even though each of markers was displaced.

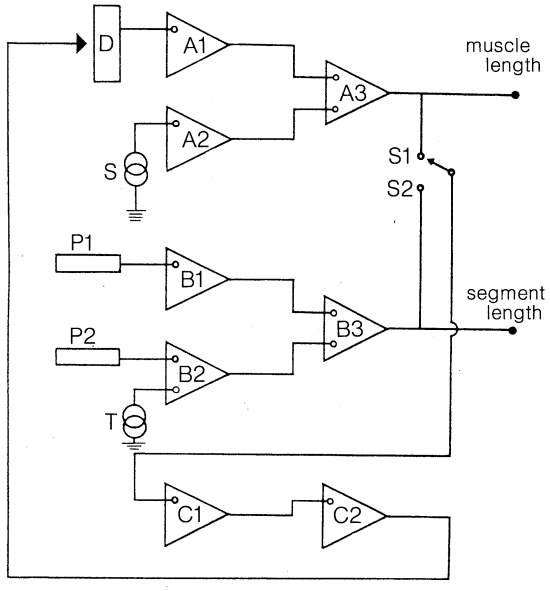

Fig 3. Electric circuit for measurement and control of the central segment length. D: length transducer, A1, A2, A3: amplifiers, S: voltage source for adjustment of ML, P1, P2: photodiode arrays, B1, B2, B3: amplifiers, T: voltage source for adjustment of SL, C1, C2: converting and power amplifiers. At position S1, ML is controlled by a pulse into A2, and at position S2, SL is controlled by a pulse into B2.

The diagram for the electrical feedback circuit is shown in Fig 3. When the switch was at position S1, the difference in the output voltage of each of the photodiode amplifier corresponded to SL. If the switch was turned to position S2, the output voltage of the amplifier B3 was fed to the length transducer, so that the SL was kept constant by controlling ML throughout muscle contraction. If the electrical pulse was injected into T, the initial SL was controlled voluntarily in proportion to the pulse. The feedback amplifiers consisted of operational amplifiers (3500 A, 3500 E, Burr Brown) into which a differential or an integration circuit was inserted to reduce the voltage oscillation. Movements of the two markers, changes in SL and ML and tension development were simultaneously displayed on a cathode ray oscillograph or an electromagnetic UV recorder (Type 2901 Yokogawa, Tokyo). The natural frequency of the strain gauge tension transducer with the muscle preparation was 400 Hz, which was enough to record the time course of the tension changes accurately. However, the response time of the length transducer to the displacement of 0.5 mm was about 0.1 sec. This response seemed to be slow but was enough to hold the ML or SL constant throughout nearly the entire phases of contraction and relaxation, except at the beginning

of contraction. Before starting the experiment, the relative positions of the light beam, muscle and photodiodes were adjusted so that the SL would not change even if the muscle was displaced, as shown in test experiment of Fig 2.

RESULTS

1. Tension development under muscle isometric and segment isometric contractions.

The muscle was first kept in an isometric condition during contraction and the developed tension was recorded together with the movement of each marker and the distance between the two markers (Fig 4A). Then, the electric switch was turned to position S2 in Fig 3 to obtain the segment clamp condition (Fig 4B). It was also necessary that the SL would not be changed by switching from S1 to S2, and this was attained by injecting positive or negative voltage into T in Fig 3. In the ordinary muscle isometric contractions, as shown in Fig 4A, the right hand marker moved leftward (trace 3), the lefthand marker moved rightward (trace 4), and the distance between markers shortened. The change in the distance, which indicated shortening of the intact segment, attained 0.8 mm (Fig 4 A1) and 0.7 mm (Fig 4 A2), roughly around 10% of the muscle length. During the segment isometric contractions, as shown in Fig 4B, the righthand and lefthand markers both moved in the same direction,

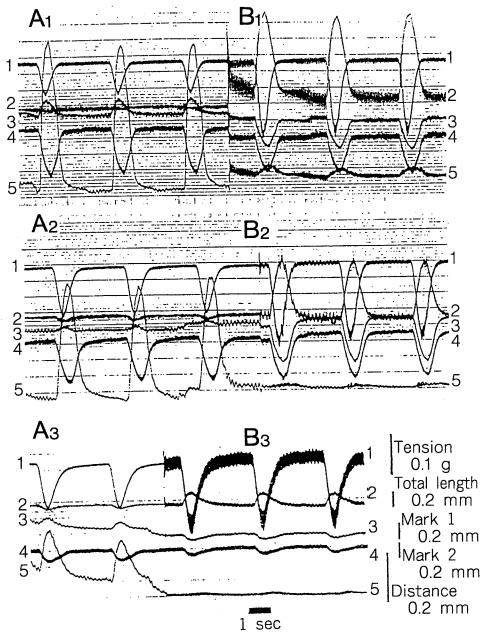

Fig 4. The changes in tension, muscle length and segment length in muscle isometric (A1-A3) and segment isometric (B1-B3) contractions. Muscle was stimulated at the rate of 1/5 sec. Trace 1: tension, downward deflection indicates positive tension development. Trace 2: total muscle length, upward deflection indicates elongation of the muscle. Traces 3 and 4: displacement of markers. Trace 5: distance between two markers, upward deflection indicates segment shortening. The muscle length was 0.85 Lmax in A, 1.0 Lmax in B and 0.93 Lmax in C, where Lmax was the muscle length at which the maximum tension was obtained. 21°C. Stimulation rate; 1/5 sec.

resulting in a constant distance between the markers. Meanwhile, the total muscle length was elongated (traces 2, Fig 4 B1 - B3). Larger tension was developed under the segment clamp condition than under the ordinary isometric condition. The ratios of peak tension in both conditions were 2.0 in records A1 and B1, 1.6 in A2 and B2 and 1.5 in A3 and B3. Actually, small shortening of the segment length was noticed during the length clamp, because of the incomplete feedback amplification. When the gain of the amplifier was augmented the segment was well clamped but the small oscillation of the muscle length took place and it resulted in the oscillatory tension change. In, Fig 4 B3, the oscillation thus produced was superposed upon the tension trace.

2. Force-velocity relation

In the ordinary afterload contraction, muscle shortening, displacement of the two markers and segment shortening as well as tension development were simultaneously recorded (Fig 5). The segment shortening started prior to the onset of whole muscle shortening, and progressed linearly during the initial phase, which was followed by decreasing velocity. The peak of the segment shortening came to the relaxation phase of the whole muscle. The result suggested that contraction and relaxation took place in a non-uniform manner among the segments. Under nominally 0 load, the shortening velocity of the intact segment was measured to be about 1.2 times larger than the relative muscle velocity.

Fig 6 shows the tension development when the shortening was made constant by injecting a saw-tooth pulse into T of a B2 amplifier of Fig 3.

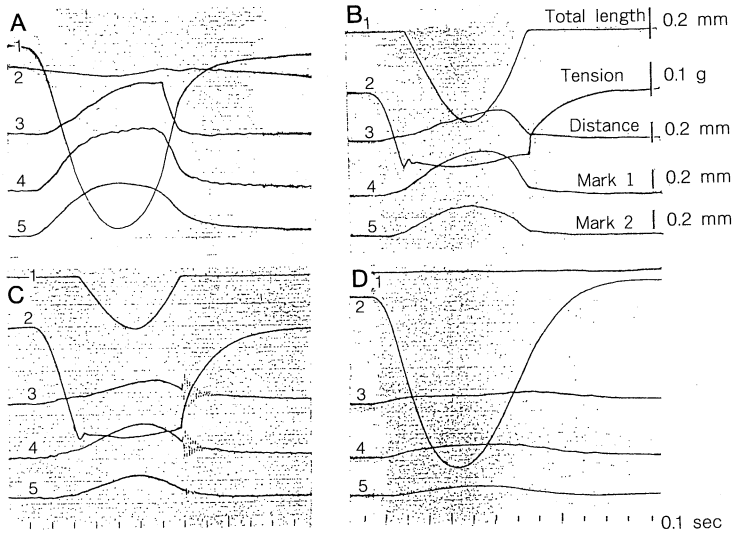

Fig 5. The changes in tension, muscle length and segment length in an afterloaded contraction. Preload was 16 mg, and afterload was 0 mg in A, 0.22 g in B, 0.32 g in C and 0.58 g (isometric) in D. Trace 1: total muscle length, downward deflection indicates the muscle shortening. Trace 2: tension, downward deflection indicates the positive tension development. Trace 3: distance between markers Traces 4 and 5: movement of markers. 22°C. Stimulation rate; 1/5 sec.

Using this procedure we could obtain the force-velocity relation by measuring the force as a function of the velocity. The muscle was held under a preload of 0.2 g and the central segment was allowed to shorten simultaneously with the stimulating pulse. At the start of the segment shortening, if it was fast, the tension decreased transiently because the speed of movement of the transducer arm exceeded the intrinsic muscle shortening velocity (Fig 6A, B). When the velocity was low, the central intact segment shortened actively stretching the peripheral damaged segments and one marker moved along the direction of shortening while the other marker moved in the opposite direction (Fig 6C, D). After the time of peak tension, the segment shortening progressed without whole muscle shortening (Fig 6C, D). The results showed that the intact segment was shortened by releasing the peripheral damaged segments keeping the mechanical equilibrium with their elastic resistance.

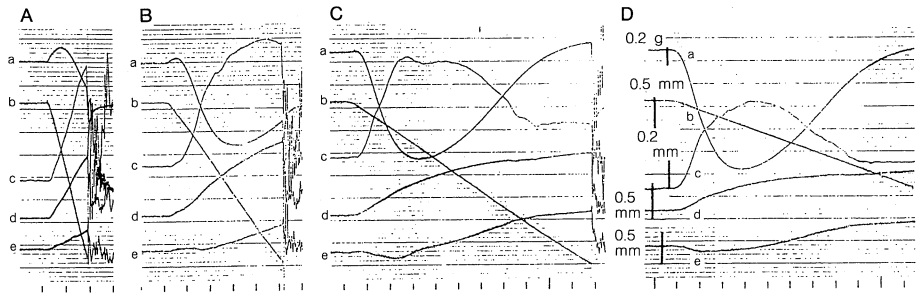

Fig 6. Controlled segment length shortening. Shortening velocities were 1.9 SL/sec in A, 0.7 SL/sec in B, 0.4 SL/sec in C and 0.25 SL/sec in D. Traces a : tension development downward, b : segment shortening, shortening downward, c : muscle shortening, shortening upward, d and e : movement of two markers. $P_0 = 2.3$ g. ML was 10.0 mm, SL was 5.0 mm. Stimulation rate ; 1/5 sec. 24°C.

DISCUSSION

The present studies demonstrated that, even though the muscle preparation was held in the isometric condition, the central intact segment shortened appreciably against the elastic resistance of the peripheral damaged segments and that both contraction and relaxation took place in a non-uniform manner.

Some limitations were inevitable for the measurement and control of the SL. One of the disadvantages was that the muscle shortened carrying these markers which might act as a load against contraction. However, each marker weighed 5 mg and might be well negligible when compared to the isometric tension of around 1 g. Another difficulty was that the movement of the marker reflected the contraction of neither the superficial nor the interior cell layer separately but an averaged contraction of the whole. The preparation used in the present study was a small strip of the atrial muscle, but was still a multicellular tissue. It should be reminded that the length or the velocity recorded here was not a simple sum of the contraction of each cardiac cell arranged in series but was of the whole muscle tissue which contained the intercellular visco-elastic components. Therefore, it was not necessary to distinguish the superficial layer from the interior one.

The tension under the segment isometric condition was always larger than

that under the muscle isometric one (Fig 4). More marked increases in segment isometric tension than our results have been presented by Donald *et al*[10] and Huntsman *et al*[11] They used the papillary muscles of cats or ferrets, and the differences between their results and ours seem to consist in the different species used in the experiments. Qualitatively, however, the effects of the damaged cells on tension development are the same among the muscles of different animals.

When the muscle was allowed to shorten under an afterload contraction, the maximum shortening velocity of the central segment was always higher than that of the whole muscle. The limiting factor to the free shortening velocity under a nominally 0 load may be internal viscous resistance.[12] The present study demonstrated that, in multicellular tissue like our muscle preparation, it played a considerable role in determining shortening velocity whether the muscle shortening was uniform or not. The force-velocity curve obtained by measuring tension during forced shortening (Fig 6) was nearly consistent to the curve for afterload contraction. In other words, the force-velocity relation was independent of the procedure of measurement, whether the shortening velocity was measured as a function of a load or tension was measured as a function of velocity.

In summary, about 20% of the muscle preparation was damaged at both ends of muscle preparation after usual isolation procedures. These damaged cells resulted in the considerable underestimation of both tension at any length and shortening velocity under any load.

ACKNOWLEDGMENTS

Supported by a Project Research Grant from Kawasaki Medical School (No. 2-615).

REFERENCES

1) Sonnenblick EH : Implications of muscle mechanics in the heart. Fed Proc **21** : 975-990, 1962
2) Brady AJ : Active state in cardiac muscle. Physiol Rev **48** : 570-600, 1968
3) Brutsaert DL, Sys SU : Relaxation and diastole of the heart. Physiol Rev **69** : 1228-1315, 1989
4) Hill AV : The heat of shortening and the dynamic constants of muscle. Proc Roy Soc **B126** : 136-195, 1938
5) Wilkie DR : Facts and theories about muscle. Prog Biophys **4** : 283-324, 1954
6) Huxley AF : Muscle structure and theories of contraction. Prog Biophys and Biophys Chem **7** : 255-318, 1957
7) Krueger JW, Pollack GH : Myocardial sarcomere dynamics during isometric contraction. J Physiol **251** : 627-643, 1975
8) Jewell BR : A reexamination of the influence of muscle length on myocardial performance. Circ Res **40** : 221-230, 1977
9) Krueger JW, Forletti D, Wittenberg BA : Uniform sarcomere shortening behavior in isolated cardiac cells. J Gen Physiol **76** : 587-607, 1980
10) Donald TC, Reeves DNS, Reeves RC, Walker AA, Hefner LL : Effect of damaged ends in papillary muscle preparations. Am J Physiol **238** : Hl4-H23, 1980
11) Huntsman LL, Rondinone JF, Martyn DA : Force-length relation in cardiac muscle segments. Am J Physiol **244** : H701-H707, 1983
12) Baskin RJ, Paolini PJ : Muscle volume changes. J Gen Physiol **49** : 387-404, 1966
13) de Tombe P, ter Keurs HEDJ : An internal viscous element limits unloaded velocity of sarcomere shortening in rat myocardium. J Physiol **454** : 619-642, 1992