

Sarcomere Dynamics in a Spontaneous Contraction Wave and Its Effect on the Following, Electrically Triggered Twitch in Rat Myocyte

Comparison with the Rested State Twitch

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ABSTRACT A spontaneous contraction (SC) wave propagates among sarcomeres in heart muscle by the mechanism of Ca^{++} -induced release of Ca^{++} from sarcoplasmic reticulum (SR). In the present study, some characteristics of unloaded shortening during the SC and its effect on a subsequent, electrically triggered twitch (Tsc) were examined at a sarcomere level in isolated rat myocytes. The results were compared with those of a rested state twitch (RS), which was accompanied by an action potential. Average shortening velocity from onset to peak of shortening was 3.74 ± 1.25 (mean \pm SD, $n = 18$) and 5.35 ± 2.30 $\mu\text{m/s}$ per sarcomere ($n = 54$) in SC and RS, respectively. That the former was smaller than the latter ($P < 0.01$, t test) suggests that Ca^{++} are released from the SR more slowly in the SC than the RS. There were no differences in either the extent or area of shortening between SC and RS. The extent of shortening increased significantly as shortening velocity increased in all the SC ($P < 0.05$), RS, Tsc, and triggered twitch (Trs) after the RS ($P < 0.001$ in the last three). The slope of the line for the regression of the extent upon the velocity of shortening in the SC was ~ 1.5 times greater than the other three. This suggests that the SC has a different time course of change of myoplasmic [Ca^{++}] and therefore a different mode of the causal SR Ca^{++} release from the electrically triggered twitches (RS, Trs, Tsc). There were positive correlations between the extent and the area of shortening in each of the RS ($P < 0.01$), the Trs ($P < 0.05$), and the Tsc ($P < 0.001$), but not in SC. The slope of the line for the regression of the extent upon the area of shortening in the Tsc was about three times greater than those in the RS and the Trs, suggesting characteristics of the Tsc from different

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those of the RS and the Trs. An SC inhibited a Tsc in an interval-dependent manner. The shortening velocity in the Tsc recovered fully at a test interval of ~ 0.6 s between the onsets of the two successive contractions. The velocity increased further with further increasing the test interval (up to 0.9 s). At a test interval of 0.8–0.9 s, the shortening velocity in the Tsc was greater than those in the preceding SC and the corresponding Trs by 1.17- and 1.80-fold, respectively, as compared in the same five sarcomeres. This may be taken to indicate that the SC accelerates the SR Ca^{++} release in the subsequent, Tsc. There was little difference in either the extent or area of shortening between the Tsc and the Trs, aside from the shortening area at a test interval of 0.3–0.4 s. At such short intervals, the shortening area was 17.6 ± 7.2 ($n = 5$) and 30.4 ± 9.1 $\mu\text{m}\cdot\text{ms}$ ($n = 14$) in the Tsc and the Trs, respectively. The difference was significant ($P < 0.05$). This result is in agreement with the idea that a part of Ca^{++} entering the myocyte during an action potential is sucked up by the SR and used for the next twitch. In conclusion, the SC has different effects from the RS on the subsequent, action potential-mediated twitch.

INTRODUCTION

It is widely accepted that in mammalian heart muscle the transsarcolemmal Ca^{++} influx during an action potential causes a transient release of Ca^{++} from the sarcoplasmic reticulum (SR), leading to a twitch contraction (for recent reviews, see Reiter, 1988; Fabiato, 1989). Magnitude of the twitch alters in response to changes in amounts of both the Ca^{++} influx (London and Krueger, 1986; Cannell, Berlin, and Lederer, 1987; Beucklemann and Wier, 1988; Talo, Stern, Spurgeon, Isenberg, and Lakatta, 1990) and the releasable Ca^{++} in the SR in a complex way (Fabiato, 1985*a*; Lewartowski and Pytkowski, 1987; Bouchard and Bose, 1992). In order to interpret the graded nature of the twitch in terms of the Ca^{++} -induced Ca^{++} -release mechanism of the SR (Endo, 1977) for the excitation–contraction coupling in the mammalian heart muscle, Fabiato (1985*a,b*) assumes that the amount of SR Ca^{++} release depends on the rate of increase of $[\text{Ca}^{++}]$ in close proximity of the SR and on an inactivation process in the Ca^{++} release mechanism of the SR. Both positive and negative evidence has been reported on the inactivation of the SR Ca^{++} release. The inactivation mechanism operates in skinned cardiac muscle (Fabiato, 1985*a*), in isolated cardiac SR vesicles (Chamberlain, Volpe, and Fleischer, 1984; Meissner and Henderson, 1987), and in intact skeletal muscle fibers (Schneider and Simon, 1988; Simon, Klein, and Schneider, 1991) when the myoplasmic $[\text{Ca}^{++}]$ is increased to a level $> 10^{-6}$ M. It has also been reported, on the contrary, that the Ca^{++} release channels in isolated SR vesicles incorporated into planar lipid bilayers have lost their Ca^{++} -induced inactivation by $[\text{Ca}^{++}] < 10^{-3}$ M (Smith, Colonado, and Meissner, 1986; Anderson, Lai, Liu, Rousseau, Erickson, and Meissner, 1989). Nabauer and Morad (1990) have reported with an intact, single rat myocyte that a submaximal level of $[\text{Ca}^{++}]$ does not operate the inactivation mechanism. The amount of releasable Ca^{++} in the SR is regulated by Na-Ca exchange across sarcolemma during a diastole and the transsarcolemmal Ca^{++} influx during the action potential (Rueter and Seitz, 1968; Glitsch, Reuter, and Scholz, 1970; Eisner and Lederer, 1985; Bridge, Spitzer, and Ershler, 1988; Egan, Noble, Powell, Spindler, and Twist, 1989). The Na-Ca exchange during the action potential may promote the Ca^{++} influx and

trigger Ca^{++} release from the SR (Leblanc and Hume, 1990), though Niggli and Lederer (1990) reported that the depolarization itself had no effect on the Ca^{++} -induced release of Ca^{++} from the SR.

In an isolated, single rat ventricular myocyte, it is often seen that a phasic contraction occurs spontaneously in a few sarcomeres located at a longitudinal end (Berry, Friend, and Scheuer, 1970; Vahouny, Wei, Starkweather, and Davies, 1970). The spontaneous contraction (SC) propagates over the other end as a contraction wave. The propagation of the contraction wave is not mediated by any change of membrane potential and is therefore ascribed to a succession of the Ca^{++} -induced release of Ca^{++} from the SR from one sarcomere to another (Fabiato and Fabiato, 1977; Rieser, Sabbadini, Paolini, Fry, and Inesi, 1979). For individual sarcomeres, the SC is a twitchlike contraction without any action potential. Comparison of Ca^{++} dynamics, i.e., rates of Ca^{++} release from and uptake into the SR and time course of replenishment of releasable Ca^{++} in the SR, between phasic contractions with and without an action potential may provide useful information about the excitation-contraction coupling in both physiological and pathological states of heart muscles. Though sarcomere dynamics has been studied both during a SC (Rieser et al., 1979; Krueger, Danton, Siciliano, 1992) and an action potential-mediated twitch (Krueger, Forletti, and Wittenberg, 1980; Roos, Brady, and Tan, 1982), a systematic comparison of the characteristics of the SC and the action potential-mediated twitch has not been made at a sarcomere level. The change in myoplasmic $[\text{Ca}^{++}]$ underlying the sarcomere motion has been measured using Ca^{++} -sensitive dyes as a Ca^{++} transient during both the SC (Grouselle, Stuyvers, Bonoron-Adele, Bease, and Georgescauld, 1990) and the action potential-mediated twitch in isolated myocytes (Cannell et al., 1987; Wier, Cannell, Berlin, Marban, and Lederer, 1987; O'Rourke, Reibel, and Thomas, 1990). Thus far, however, it is difficult to compare the Ca^{++} transient between the SC and the action potential-mediated twitch at a sarcomere level because of the limited space and time resolutions of the Ca^{++} transient. Capogrossi, Kort, Spurgeon, and Lakatta, (1986) reported that a SC inhibited velocity and extent of shortening of a subsequently induced, action potential-mediated twitch (Tsc) in rat myocytes. These authors noted, however, that it takes about 1 s for the contraction wave to travel throughout the myocyte, with the effect that the interval between the SC and the Tsc varies considerably among sarcomeres within a myocyte. In order to elucidate more accurately the inhibitory effect of the SC on the Tsc, therefore it is necessary to examine the motion of the same sarcomere in the two successive contractions.

In the present study, the time course of sarcomere shortening in the SC and its effect on the Tsc have been examined with isolated rat myocytes by tracking the motion of the same sarcomere during the two successive contractions. The results were compared with those of the RS. The main findings are (a) the SC may accelerate shortening velocity in the Tsc at a test interval of 0.8–0.9 s between the two and (b) the shortening area of the Tsc immediately after the SC completes relaxation is significantly smaller than that of the corresponding triggered twitch (Trs). The results will be discussed in relation to Ca^{++} dynamics in the myocyte. Correlations among the velocity, extent, and area of shortening have also been examined in each of the SC, the Tsc, the rested state (RS), and the Trs, with the result that the mode of the

correlations was different between the SC and the three electrically triggered twitches (Tsc, RS, and Trs).

MATERIALS AND METHODS

Preparation

Hearts were isolated from 6–8 wk-old male Whister rats (260–300 g) anesthetized with diether-ether. Intact, single myocytes were isolated by a conventional method with solutions equilibrated with a room temperature (22–25°C). The isolated heart was perfused retrogradely through the aorta at a pressure of 90 cm H₂O for 10–15 min with a standard Krebs solution (in mM: NaCl 118, KCl 2.5, CaCl₂ 1.8, NaHCO₃ 25, KH₂PO₄ 1.2, MgSO₄ 1.2, glucose 10, pH 7.2–7.4). The perfusate was switched to a Ca-free Krebs solution made by omitting CaCl₂ from the standard Krebs solution. Immediately after the heart beat stopped, the perfusate was changed to a digesting solution made by adding 0.036 mM CaCl₂, 0.4 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO, type 1), and 0.1% trypsin to the Ca-free Krebs solution. The heart was digested until entire surface of the heart became whitish, usually for 15–20 min. After washing away the digesting solution by perfusing the heart with 20 ml of the Ca-free Krebs solution, the ventricles were cut into several pieces in the Ca-free Krebs solution. The resulting fragments were agitated with a pipette. Ca concentration in the bathing solution was increased in a stepwise manner to 1.8 mM in 1 h. A few drops of this solution contained several rod-shaped, single myocytes with clear striation among many damaged, round cells. All of the solutions used were well oxygenated with 95% O₂/5% CO₂ gas. Isolated myocytes were stored in 100–200 ml of the standard Krebs solution at the room temperature and used within the day, i.e., within several hours after isolation.

Scan of the Striation Image

A few drops of the cell suspension were placed in an experimental chamber (10 × 30 × 2 mm) with a 150- μ m-thick glass bottom on a mechanical stage of an inverted microscope (model TMD; Nikon Inc., Tokyo, Japan). The microscopic image ($\times 40$ objective) of several sarcomeres in central region of a myocyte was projected onto a photomultiplier (Hamamatsu Photonics Inc., Hamamatsu, Japan) with a slit via an optical scanner (model G120D; General Scanning Inc., Watertown, MA) consisting of a front surface mirror equipped to the servo-motor (Fig. 1A). Change of the mirror angle was detected with a sensor incorporated in the servomotor. A rotational movement of the mirror translated the striation image along the slit, which is equivalent to an area of $\sim 0.2 \times 3 \mu\text{m}$ on the specimen, generating an intensity profile of the striation image as the output of the photomultiplier (Fig. 1B). High-frequency noise in the photomultiplier signal was cut off with a 1 kHz filter. The mirror was usually oscillated by a sawtooth wave at 123 Hz. A 3- μ m-wide segment was thus scanned by 8–10 μ m along the length of a myocyte. Outputs of both the photomultiplier and the servomotor were fed into an dual beam oscilloscope (model VC-10; Nihon Kodens Inc., Tokyo, Japan) for monitor. The amplified analog signals from the oscilloscope were digitalized at 5 kHz using a 8-bit analog-to-digital converter (model AD-1240; Nihon Phoenix Inc., Tokyo, Japan) and stored in a disk of a microcomputer (PC98XL; NEC Inc., Tokyo, Japan) for later analysis.

Data Analysis

The intensity profile of the striation image of a myocyte is shown in Figs. 1B and 2A together with the change of the mirror angle. Sarcomere length (SL) was defined as the distance between centroids of two adjacent I-bands (Fig. 1B). Though a translational movement of the intensity profile against a reference in the mirror signal was unavoidable during a contraction, the

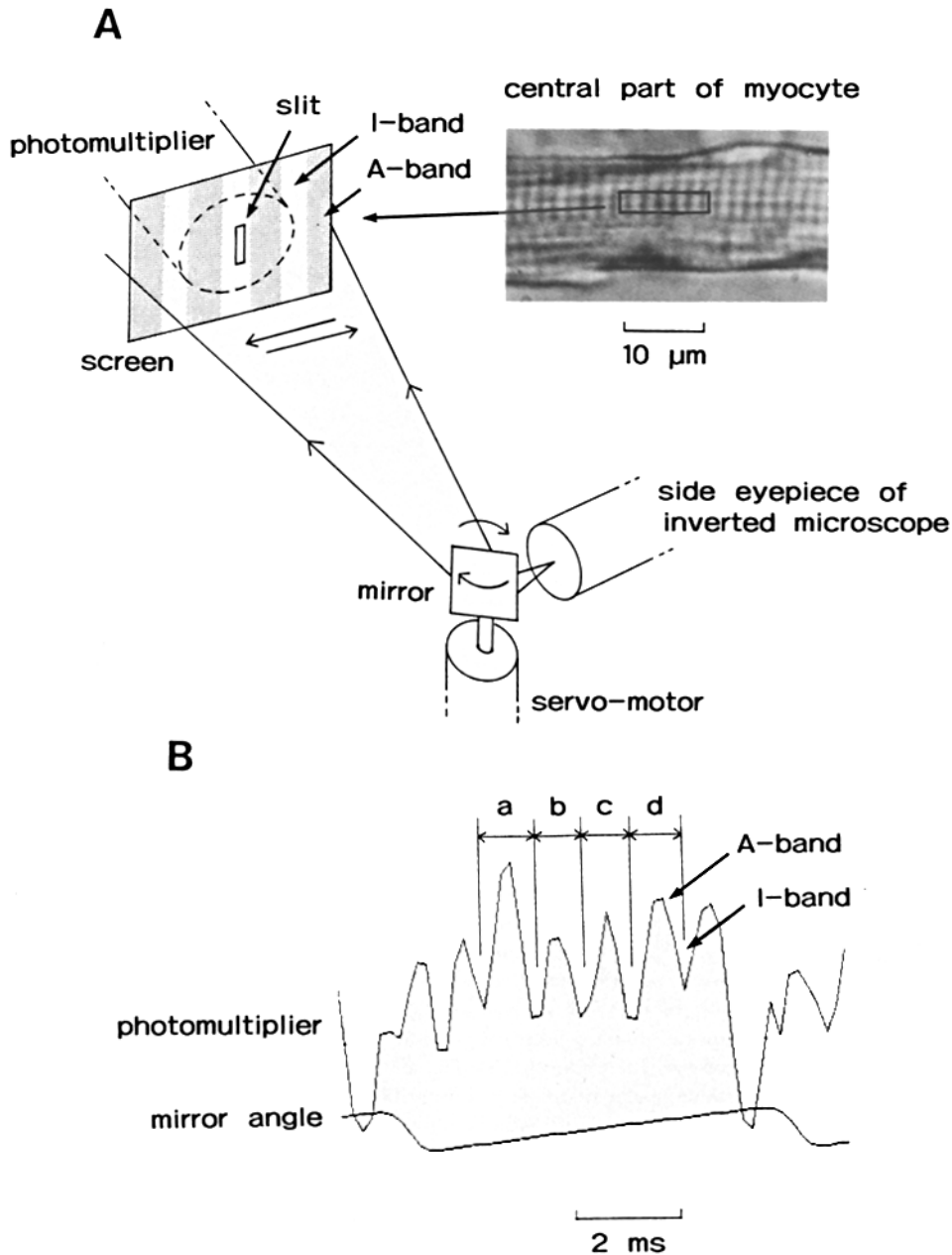


FIGURE 1. (A) Arrangement of apparatus. The microscopic image of striation in central part of an isolated rat myocyte is reflected by an optical scanner consisting of a front-surface mirror equipped to a servomotor and projected onto a photomultiplier through a rectangular slit. (B) Digitalized outputs of the photomultiplier (upper) and the servomotor representing the mirror angle (lower). Change of the mirror angle at a constant velocity translates the image over the slit, resulting in an intensity profile of the striation image. The mirror is oscillated in a sawtooth manner. SLs *a-d* are determined by measuring the time between adjacent two centroids of valleys representing I-bands in the intensity profile. In this and the following figures, the light intensity increases downwardly in the upper trace; arbitrary units in ordinate in the lower.

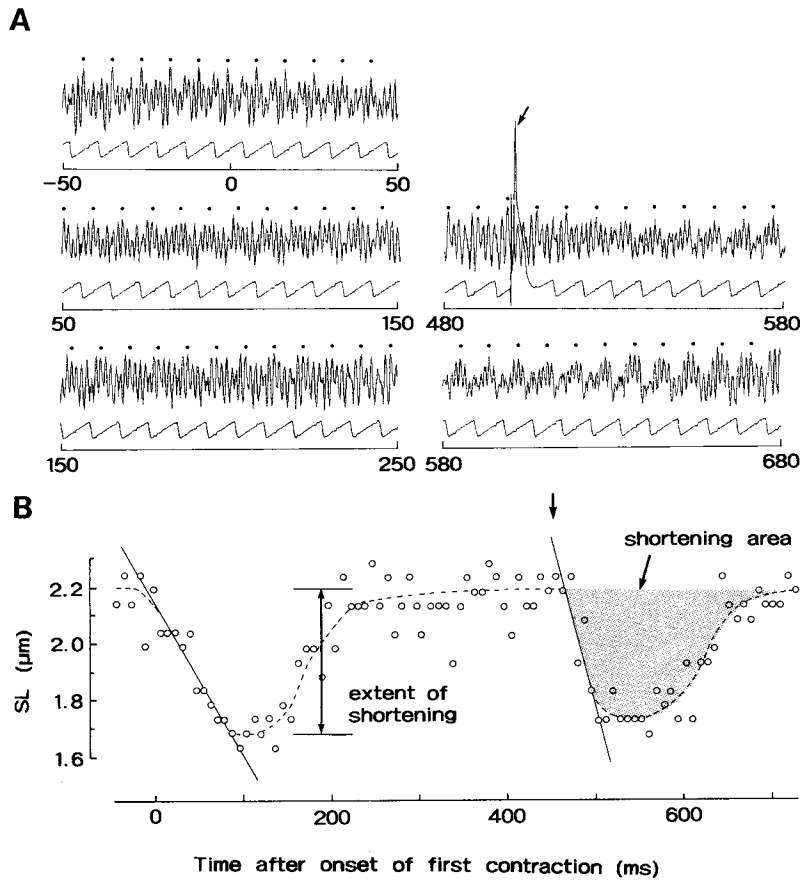


FIGURE 2. (A) An example of change of the intensity profile of a striation image during a contraction wave and the following Tsc in a segment of a myocyte and (B) the resulting change of the length of a sarcomere. A, upper trace shows intensity profile; lower trace shows change of mirror angle. Records *a-c* are continuous. *d* is shortly after *c*. *e* is a continuation of *d*. Time is given in msec from onset of the first contraction, as in B. Signal is distorted by a spike indicating the time of application of an electrical pulse to the myocyte (arrow). Dots indicate the A-band of the same sarcomere (preparation 17b). The length change of the sarcomere is shown in B. In B, average shortening velocity (lines), extent (arrows), and area of shortening (shading) are represented. The shortening velocities estimated from the slope of the line are 5.2 and 11.5 $\mu\text{m/s}$ per sarcomere in the SC and the following Tsc, respectively. Dashed line indicates a possible time course of unloaded shortening. Arrow points to the time of electrical stimulation.

direction of the translation could be determined by tracking the movement of a characteristic feature in the intensity profile from scan to scan, so that lengths of the same sarcomeres were tracked throughout a series of contractions in selected myocytes (Fig. 2). By plotting SL change against time, as shown in Fig. 2 B, velocity, extent, and area of shortening in a contraction of a sarcomere were measured. The shortening velocity was given from slope of the line which was obtained by fitting to the data points in the shortening phase of the contraction by a least square method, while the extent of shortening by averaging the SLs at peak of shortening. The

shortening area was determined by weighing a piece of a high-quality paper with the same shape as the shortening form of a sarcomere, such as shown by a shadow in Fig. 2 *B*. A 95% confidence limit was used to determine statistical significance.

Experimental Protocol

The isolated myocytes were initially quiescent without stimulation. Rod-shaped myocytes with a clear striation which responded to application of 5-ms current pulses through a pair of Ag-AgCl wire electrodes (1 mm in diameter and 8 mm apart each other) with vigorous twitches were selected for the present purpose. The myocyte responded to the current pulse with a twitch independently of the pulse duration examined (5–20 ms) and in an all-or-none manner with a stepwise increase of current intensity of the pulse. Therefore, the electrically triggered twitch is thought to be an action potential-mediated one. This was confirmed by means of a conventional microelectrode. In the present study, strength of the electrical pulse was kept at slightly above the threshold level. A twitch contraction brought about a large translational movement at the edge of myocyte and less extent at the center. Segmental region with a least translational movement was found out by observing the microscopic image of myocyte during twitches. Such a region was scanned as described previously. After an application of a long series of stimulating pulses, some of myocytes began to initiate spontaneously a contraction wave at a low frequency, which originated at a longitudinal end and traveled to the other. The effects of RS and SC on the following twitches were examined with the myocytes that were generating spontaneously the contraction wave at a frequency <0.1 Hz. Because it was difficult to set an optical apparatus for detection of an initiation of the contraction wave in addition to the one for scanning the striation image, a stimulating pulse to cause a twitch after a SC was triggered manually. The timing of the application of pulse in the record was determined by introducing a signal into the mirror angle signal (an arrow in Fig. 2 *A*). This caused some noise in the mirror angle signal (Fig. 2 *A*). The actual velocity of the scan of the striation image was quite constant, as demonstrated in Fig. 1 *B*. Myocytes producing contraction waves more frequently than 0.1 Hz were discarded. All experiments were made at room temperatures of 22–25°C.

RESULTS

Sarcomere Dynamics in RS and SC

Twitch force–stimulus frequency relations in rat heart muscle, which exhibits a negative staircase (Henderson, Brutsaert, Parmley, and Sonnenblick, 1969; Forester and Mainwood, 1974), show that a maximal twitch force is reached at a stimulus interval > ~10 s. Therefore, an RS was evoked with a preceding rest period of >10 s in the present study. The time course of sarcomere shortening in a contraction wave was examined with the myocytes that were spontaneously generating cyclic, contraction waves with intervals >10 s. That is, an SC of a sarcomere examined had a preceding rest period comparable to that of the RS. The contraction wave originated at one end of a myocyte and reached the other end within 1 s or so, as has been characterized already (e.g., Capogrossi et al., 1986). Examples of the time courses of a RS and a SC are shown in Figs. 2 and 3. The resting SL in the myocytes used was 1.7–2.1 μm .

In unloaded contractions of isolated myocytes, both maximum velocity and extent of shortening have usually been used as indices of the contractility (for a review, see Brady, 1991). In that it was difficult to determine the maximum shortening velocity accurately with the present method, an average velocity of shortening from its onset

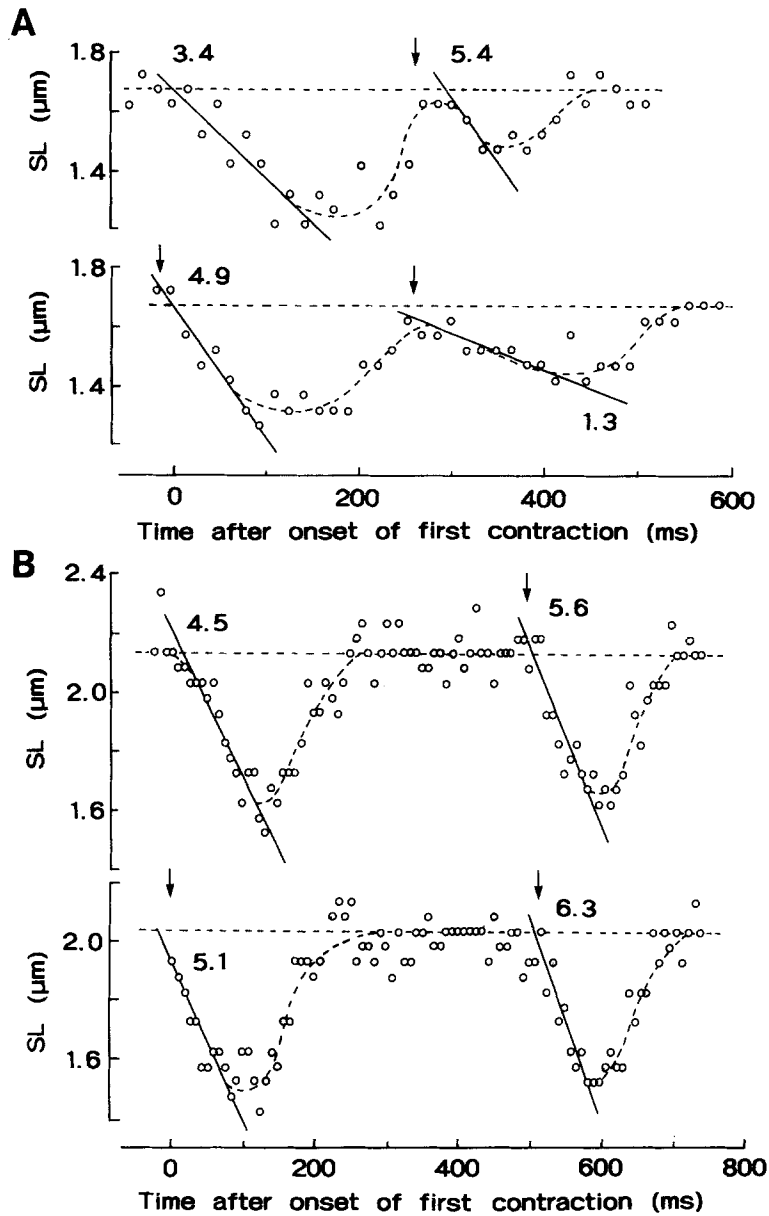


FIGURE 3. Examples of double contractions with (A) short and (B) long test intervals. In both A and B, the upper panel shows SC and Tsc, while the lower RS and Trs. Dashed lines represent resting SLs and possible time courses of shortening. Solid lines represent estimation of the shortening velocity. The shortening velocity estimated from slope of the line is given near each line in the figure in the unit of $\mu\text{m/s}$ per sarcomere. Arrows show the time of electrical stimulation. A, preparation 16c; B, preparation 17b.

to a peak was measured together with extent and area of shortening at a sarcomere level (Fig. 2). The definitions of the velocity, extent, and area of shortening are indicated in Fig. 2 B.

All of the velocity, extent, and area of shortening in the RS and the SC varied to some extent among sarcomeres in different and even in the same myocytes and to a lesser extent in a series of contractions of a given sarcomere (cf. Fig. 2 B and upper trace in Fig. 3 B; Table I). The velocity, extent, and area of shortening were $3.74 \pm 1.25 \mu\text{m/s}$ per sarcomere (mean \pm SD, $n = 18$ in eight sarcomeres from four different myocytes), $0.38 \pm 0.12 \mu\text{m}$, and $40.7 \pm 11.5 \mu\text{m}\cdot\text{ms}$, respectively, in the SC and were $5.35 \pm 2.30 \mu\text{m/s}$ per sarcomere ($n = 54$ in 18 sarcomeres from six different myocytes), $0.42 \pm 0.12 \mu\text{m}$, and $44.8 \pm 11.2 \mu\text{m}\cdot\text{ms}$, respectively, in the RS. The shortening velocity was significantly smaller in the SC than the RS ($P < 0.01$, t test), though there was a large variation in the shortening velocity of the SC among different sarcomeres in comparison with the RS. The velocity was greater in the SC than the RS in some sarcomeres, whereas the opposite was the case in the others (see

TABLE I
Shortening Velocities ($\mu\text{m/s}$ per Sarcomere) in Tsc and Trs at Test Intervals of 0.8–0.9 s with Those in the Preceding SC and RS in Five Sarcomeres

Preparation*	Tsc	SC	Tsc/SC	Trs	Tsc/Trs	RS	Tsc/RS
		$\mu\text{m/s}$		$\mu\text{m/s}$		$\mu\text{m/s}$	
10c	4.88	4.09	1.19	2.13	2.29	2.51	1.94
10d	3.38	3.00	1.13	1.32	2.56	1.43	2.36
10e	2.14	1.91	1.12	1.95	1.10	2.63	0.81
16c	5.39	5.39	1.00	2.78	1.94	3.70	1.46
16e	4.85	3.45	1.41	4.31	1.13	5.39	0.90
mean	4.13	3.57	1.17	2.50	1.80	3.13	1.49
\pm SD	± 1.34	± 1.29	± 0.15	± 1.14	± 0.67	± 1.50	± 0.67

*Numbers and letters define myocytes and individual sarcomeres, respectively.

Table I). There were no significant differences in either the extent or the area of shortening between the SC and the RS.

Recovery of Contractility after RS and SC

The effect of the SC on the following twitch was examined by triggering electrically a twitch after a SC at test intervals between the onsets of the two successive twitches in the range of 0.3–0.9 s. The result was compared with that of the RS as the control.

Examples of successive RS and Trs are shown in the lower traces in Fig. 3, A and B. An RS depressed a Trs in such a manner that the shorter the test interval, the smaller the values in all the velocity, extent, and area of shortening in the Trs as expected from the negative staircase phenomenon. The all recovered so slowly, as shown in Fig. 4, A–C, that they did not recover fully within the test interval examined (up to 0.9 s).

Examples of successive SC and Tsc are shown in upper traces in Fig. 3, A and B. An SC depressed the extent and area of shortening in a Tsc in the entire range of the test interval up to 0.9 s (Fig. 4, E and F). The effect of the SC on the shortening

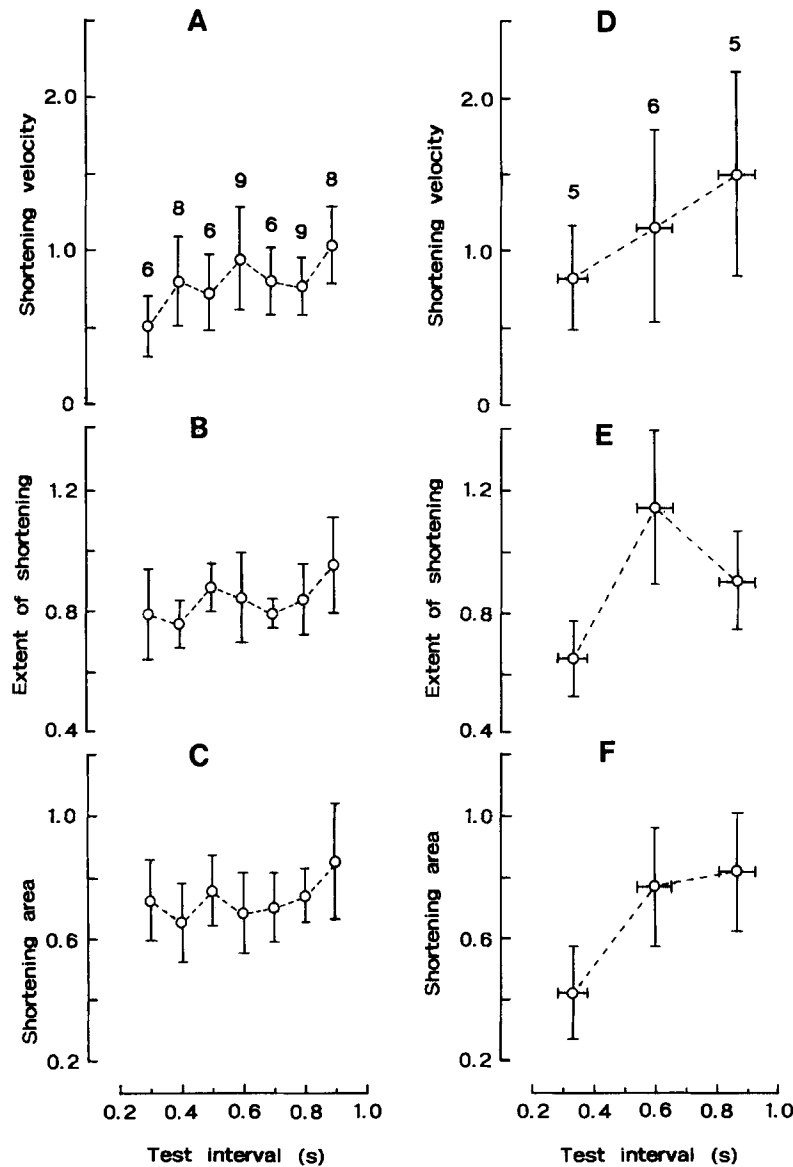


FIGURE 4. Recovery of the velocity, the extent, and the area of shortening in Trs (A–C) and Tsc (D–F). Ordinates are relative values to the preceding contraction except for D. In D, the shortening velocity is relative to the mean value in the RS, not the preceding SC, since the velocity in the SC is smaller than in the RS. Mean (circles) \pm SD (bars) are shown. Data number is shown on each bar in A and D.

velocity in the Tsc was complex. The shortening velocity in the Tsc was smaller than the mean velocity in the RS at test intervals < 0.6 s, was equal to that at 0.6 s, and appeared to exceed that at the intervals > 0.6 s (Fig. 4 D). To clarify whether or not the SC really has an accelerating effect on the shortening velocity in the Tsc at a test

interval of 0.8–0.9 s, the velocity in the Tsc at this long interval was statistically compared with those in the corresponding Trs, the SC, and the RS. Because, however, a large variation of the shortening velocity among different sarcomeres in each of the four types of contractions made the statistical comparison difficult, the shortening velocity in the Tsc was compared with those in the SC, the Trs, and the RS obtained from the same sarcomere. Individual values of the shortening velocity in the Tsc's at the test interval of 0.8–0.9 s are listed in Table I together with those in the preceding SC, the corresponding Trs, and the associated RS in the five sarcomeres examined. In all the five sarcomeres the shortening velocity in the Tsc is equal to or greater than that in the SC. The average ratio of the shortening velocity in the Tsc to the SC was 1.17 in the range of 1.00–1.41, whereas the ratio of the Tsc to the Trs was 1.80 in the range of 1.10–2.56 (Table I). This analysis suggests that the shortening velocity in the Tsc at the test interval of 0.8–0.9 s is meaningfully greater than the velocities in both the SC and the corresponding Trs. It was not clear whether the shortening velocity in the Tsc in this test interval is greater than that in the RS or not, since the ratio of the velocity in the Tsc to the RS varied considerably; the average ratio was 1.49 in the range of 0.81–2.36 (Table I).

Both the recoveries of the extent and the area of shortening after the SC were quick during the test interval of the initial 0.6 s in comparison with those thereafter (Fig. 4, *E* and *F*). The extent of shortening was $0.200 \pm 0.010 \mu\text{m}$ ($n = 3$) and $0.380 \pm 0.108 \mu\text{m}$ ($n = 6$) in the Tsc at a test interval of 0.3 s and the corresponding Trs, respectively; the former is smaller than the latter significantly ($P < 0.05$). The extent of shortening appears to recover fully at a test interval of ~ 0.6 s. On the other hand, the shortening area in the Tsc at the test interval of 0.3 s was $12.7 \pm 3.5 \mu\text{m}\cdot\text{ms}$ ($n = 3$), the value significantly ($P < 0.001$) smaller than that of $33.7 \pm 8.4 \mu\text{m}\cdot\text{ms}$ ($n = 6$) in the corresponding Trs. The typical Tsc and Trs at the test interval of 0.3 s obtained from the same sarcomere are shown in Fig. 3 *A*. As data number was increased by pooling the data from twitches at test intervals of 0.3–0.4 s, the shortening area was $17.6 \pm 7.2 \mu\text{m}\cdot\text{ms}$ ($n = 5$) and $30.4 \pm 9.1 \mu\text{m}\cdot\text{ms}$ ($n = 14$) in the Tsc and the Trs, respectively; the difference between the two is significant ($P < 0.02$). There was no significant difference in the extent of shortening between the two when such pooled data were examined however.

Correlation among Velocity, Extent, and Area of Shortening

Because all of the velocity, the extent, and the area of shortening should be in someway related to the causal change of myoplasmic $[\text{Ca}^{++}]$, correlations among them were examined in each of the RSs, SCs, Trs's, and Tsc's. In RSs, the extent of shortening increased with increases in both the velocity ($P < 0.001$; Fig. 5 *A*) and the area of shortening ($P < 0.01$; Fig. 5 *C*); there was no correlation between the area and the velocity (Fig. 5 *B*). In SCs, on the other hand, the extent of shortening increased with an increase of the shortening velocity ($P < 0.05$; Fig. 5 *D*); there were no correlations either between the area and the velocity (Fig. 5 *E*) nor between the extent and the area (Fig. 5 *F*).

The extent of shortening increased with an increase of the shortening velocity in both Trs's and Tsc's ($P < 0.001$ in both; Fig. 6, *A* and *D*). The slope of the line for the regression of the extent upon the velocity of shortening was 0.037 and 0.039 s in

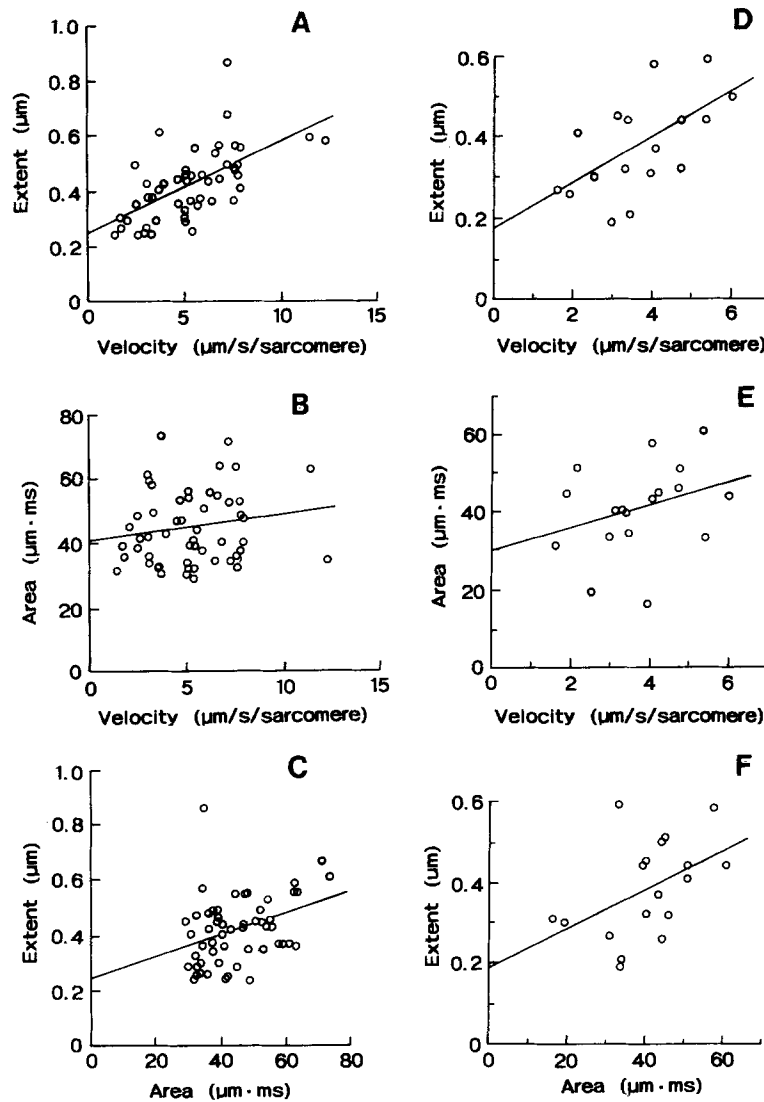


FIGURE 5. Correlations among the velocity, the extent and the area of shortening in RS (*A–C*) and SC (*D–F*). Plots of the extent vs. the velocity (*A* and *D*), of the area vs. the velocity (*B* and *E*) and of the extent vs. the area (*C* and *F*). Regression lines: *A*, $y = 0.244 + 0.033x$, $r = 0.6157$ ($P < 0.001$); *B*, $y = 40.8 + 0.74x$, $r = 0.1522$ (insignificant); *C*, $y = 0.242 + 0.004x$, $r = 0.3627$ ($P < 0.01$); *D*, $y = 0.173 + 0.056x$, $r = 0.5891$ ($P < 0.05$); *E*, $y = 29.8 + 2.92x$, $r = 0.3156$ (insignificant); *F*, $y = 0.350 + 0.005x$, $r = 0.4651$ (insignificant).

the Trs's and Tsc's, respectively; the two are almost the same. The slopes in these twitches were also similar to the corresponding slope (0.033 s) in the RSs but much smaller than that (0.056 s) in the SCs (Table II). This suggests that mode of dependency of the extent on the velocity of shortening in the electrically triggered twitches is different from that in the SC.

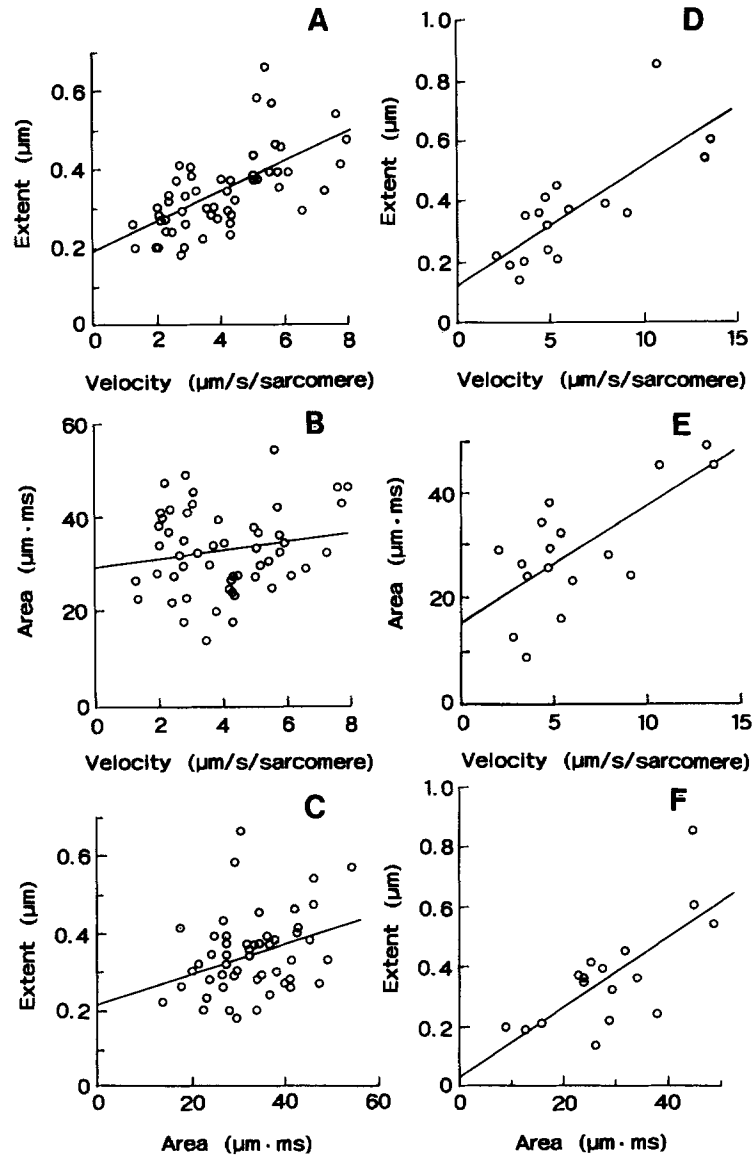


FIGURE 6. Correlations among the velocity, the extent and the area of shortening in Trs (A–C) and Tsc (D–F). Plots of the extent vs. the velocity (A and D), of the area vs. the velocity (B and E) and of the extent vs. the area (C and F). Regression lines: A, $y = 0.186 + 0.037x$, $r = 0.6296$ ($P < 0.001$); B, $y = 54.1 + 1.65x$, $r = 0.1724$ (insignificant); C, $y = 0.212 + 0.004x$, $r = 0.3428$ ($P < 0.05$); D, $y = 0.120 + 0.039x$, $r = 0.7773$ ($P < 0.001$); E, $y = 15.2 + 2.20x$, $r = 0.6995$ ($P < 0.01$); F, $y = 0.028 + 0.012x$, $r = 0.7255$ ($P < 0.001$).

The extent of shortening increased with an increase of the shortening area in both the Trs's ($P < 0.05$; Fig. 6 C) and the Tsc's ($P < 0.001$; Fig. 6 F). This indicates a close correlation between the extent and the area of shortening in the triggered twitches. However, the slope of the line for the regression of the extent upon the area

of shortening in the Tsc's (0.012/ms) was much greater than those in the RSs (0.004/ms) and the Trs's (0.004/ms). Table II summarizes the correlations among the velocity, extent, and area of shortening in the SC, Tsc, RS, and Trs.

A close correlation was observed between the area and the velocity of shortening in the Tsc's ($P < 0.01$; Fig. 6 *E*), but not in the Trs's (Fig. 6 *B*) nor in the RSs as described previously. Thus, the Tsc had characteristics different from those of the Trs and the RS. There was no apparent differences between the characteristics of the Trs and those of the RS.

TABLE II
Summary of Correlations Among Velocity, Extent, and Area of Shortening in
SC, Tsc, RS, and Trs

	SC	Tsc	RS	Trs
Extent vs. velocity	1.44	1.00	0.85	0.95
Area vs. velocity	—	1.00	—	—
Extent vs. area	—	1.00	2.14	2.11

Values are slopes of regression lines with more than a 95% confidence limit and are given relative to those of Tsc. Dashes indicate insignificant relationships.

DISCUSSION

Fabiato (1985*b,c*) has pointed out that in skinned heart muscles the Ca^{++} -induced release of Ca^{++} from the SR overloaded with Ca^{++} occurs spontaneously from the longitudinal part of the SR and possibly from the terminal cisternae; the latter is the site from which physiological Ca^{++} release seems to occur in mammalian heart muscle (Moravec and Bond, 1991). In an isolated rat myocyte with intact sarcolemma, a contraction wave is initiated spontaneously at the enzymatically disconnected, intercalated disk which becomes leaky. The SRs in the sarcomeres close to such an intercalated disk will easily be overloaded with Ca^{++} . The sarcomere with the SR overloaded with Ca^{++} may or may not exhibit a stronger twitch than does the one with the SR containing a physiological amount of Ca^{++} . The SRs in a central segment of the myocyte, in which the sarcomere motion was analyzed in the present study, might not be overloaded with Ca^{++} , in that it is somewhat difficult to imagine that Ca^{++} entering from the leaky intercalated disk diffuses over the sarcomeres located in the central region of the myocyte. Supportive evidence of this is the observation that the extent of shortening of the central sarcomeres during propagation of a contraction wave was similar to or rather smaller than that in the RS in the present study. It is thought that the SRs in the central sarcomeres retained their physiological function. Though there is argument about the mechanism of the excitation-contraction coupling in the physiological twitch of the mammalian heart muscle, the following discussions are made in the frame work that the Ca^{++} influx during development of an action potential causes Ca^{++} release from the SR by the Ca^{++} -induced Ca^{++} -release mechanism.

We measured velocity and extent of unloaded shortening of the sarcomere in the rat myocyte in the present study to assess the mode of Ca^{++} release from the SR. The rationale of this is as follows. The velocity of shortening with a light load in a skinned

cardiac muscle fiber increases as $[Ca^{++}]$ in the bathing medium and therefore the level of the thin filament activation rise (De Clerck, Claes, and Brutsaert, 1977; Maughan, Low and Alpert, 1978). In a twitch contraction of an intact cardiac muscle fiber, the shortening velocity after a release from isometric to isotonic state under a given light load reaches maximum about midway in the rising phase of the twitch tension and lowers gradually thereafter (Brady, 1966). The time course of the change of the shortening velocity resembles that of the Ca^{++} transient (Allen and Kurihara, 1980; Wier, 1980). Thus the shortening velocity at a light load is as useful as the Ca^{++} transient for assessment of the level of the thin filament activation. In case of isolated, unloaded myocytes, the shortening occurs against an elastic resistance which acts as a restoring force during relengthening (Niggli and Lederer, 1991). That is, the contractile apparatus consisting of the thick and thin filaments shortens with an internal load. Therefore, despite the argument about whether the velocity of unloaded shortening of the contractile apparatus of a skeletal muscle fiber is sensitive or insensitive to the level of the thin filament activation (Podolsky and Teichholz, 1970; Julian, 1971; Podolin and Ford, 1986), the velocity of sarcomere shortening in an unloaded rat myocyte is useful for assessment of the thin filament activation level, which is a function of myoplasmic $[Ca^{++}]$. As to the myoplasmic $[Ca^{++}]$, it is in equilibrium with binding sites in troponin complexes on the thin filaments. Considering that there are four Ca^{++} binding sites in a troponin complex, two troponin complexes per 40 nm along the 1- μ m-long thin filament and one thin filament per 0.7×10^{-9} -mm² cross-sectional area as estimated from the lattice structure of the myofilaments (Squire, 1981), the concentration of the Ca^{++} binding sites amounts to $\sim 5 \times 10^{-7}$ M. Because the thin filaments are fully activated at $\sim 10^{-5}$ M $[Ca^{++}]$, the amount of Ca^{++} bound to the troponin complexes would be less than one-tenth of the myoplasmic $[Ca^{++}]$ at any activation level. The myoplasmic $[Ca^{++}]$ may not be influenced significantly by the interaction with the troponin complexes. Thus the myoplasmic $[Ca^{++}]$ at any moment is determined essentially by the amounts of Ca^{++} released from and taken up by the SR per unit time. In that the amount of Ca^{++} taken up by the SR per unit time is merely a function of the myoplasmic $[Ca^{++}]$ at that time, the time course of increase of the myoplasmic $[Ca^{++}]$ during shortening phase of a twitch contraction would be primarily determined by the mode of the SR Ca^{++} release. Consequently, it is considered that the time course of shortening phase of a twitch contraction of an unloaded myocyte and therefore the average velocity of shortening during this phase reflects the absolute amount and the change of the SR Ca^{++} release. In agreement with this argument, O'Rourke et al. (1990) have reported that the unloaded velocity of shortening of an isolated myocyte is functions of both rate of increase of the Ca^{++} transient and its maximal level. Theoretically, the shortening velocity is a time derivative of the extent of shortening. Provided that the extent of shortening is closely related to the maximal level of the Ca^{++} transient as reported by Spurgeon, duBell, Stern, Sollott, Ziman, Silverman, Capogrossi, Talo, and Lakatta, (1992), the shortening velocity should be related to the rate of change of the myoplasmic $[Ca^{++}]$. Thus, it is reasonable to assume that the shortening velocity is a useful index of the mode of change of the myoplasmic $[Ca^{++}]$ and the causal change of the amount of the SR Ca^{++} release.

The present study showed that the slope of the line for the regression of the extent

upon the velocity of shortening was nearly the same among the electrically triggered twitches (RS, Trs, and Tsc; Fig. 6, *A* and *D*); the values in these twitches are smaller than that in the SC (Table II). This suggests that (*a*) there is no difference in the mode of dependency of the extent on the velocity of shortening among the electrically triggered and therefore the action potential-mediated twitches, (*b*) the mode is independent of the length of the preceding rest period, (*c*) the mode is also independent of whether the twitch is preceded by the SC or the RS, and (*d*) the mode in the action potential-mediated twitch is different from that in the SC, the twitch without an accompanying action potential. As discussed in the previous section, the velocity and the extent of shortening are functions of the rate of increase and the maximal level of the myoplasmic $[Ca^{++}]$. It then follows that the relationship between the rate of increase and the maximum level of the myoplasmic $[Ca^{++}]$ in the SC is different from that in the action potential-mediated twitches.

The extent of shortening had a correlation with the shortening area in the action potential-mediated twitches (Table II). The Tsc, however, is different from the RS and the Trs in the point that the slope of the regression line in the Tsc is about three times greater than those in the RS and the Trs. In other words, the shortening area increases more slowly as the extent of shortening increases in the Tsc than in the RS and the Trs. No difference was observed in the slope of the regression line between the latter two. The shortening area would be related to a whole time course of the change of the myoplasmic $[Ca^{++}]$, since a phase of relengthening of a myocyte during relaxation is reported to be a function of the myoplasmic $[Ca^{++}]$ which is decreasing (Spurgeon et al., 1992). The Ca^{++} uptake activity of the SR in the Tsc, which determines the speed of decrease of the myoplasmic $[Ca^{++}]$, may be different from that in the RS and the Trs.

Capogrossi, Suarez-Isla, and Lakatta, (1986) have indicated that a spontaneous contraction wave inhibits a extent and a maximum velocity of shortening of the following, electrically triggered twitch at test intervals < 1.8 s in isolated rat myocytes. The inhibition is qualitatively the same as does the action potential-mediated twitch. Partially confirming this, the present study further demonstrated that the shortening velocity in the Tsc at a test interval of 0.8–0.9 s was greater than those in the preceding SC and the corresponding Trs (Table I). The dependency of twitch magnitude on stimulus frequency in rat heart muscle is at present ascribed to an interval-dependent change of the amount of releasable Ca^{++} in the SR. The change is attributed mostly to slow movement of Ca^{++} from the uptake to the release site in the SR (Wier and Yue, 1986; Morad and Cleemann, 1987; Schouten, van Deen, de Tombo, and Verveen, 1987; Bers and Bridge, 1989; Bouchard and Bose, 1989; Frampton, Frampton, Harrison, Boyett, and Orchard, 1991), not to depletion of the SR Ca^{++} content. That there were no differences in either the extent nor the area of shortening between the Tsc and the Trs at the test interval of 0.8–0.9 s (Fig. 4) therefore suggests little difference in the amount of releasable Ca^{++} in the SR between the Tsc and the Trs. In supposing that the amount of SR Ca^{++} release is determined by the product of open probability of Ca^{++} release channels in the SR membrane and amount of the releasable Ca^{++} , the difference in the shortening velocity between the Tsc and the Trs at the test interval of 0.8–0.9 s may be taken to indicate that the channel open probability is different between these Tsc and Trs.

The channel open probability would be changeable independently of the amount of the releasable Ca^{++} in the SR. In agreement with this, that the amount of the SR Ca^{++} release changes independently of the amount of the releasable Ca^{++} in the SR is suggested by the observation that the Ca^{++} transient changes in correlation with change of the Ca^{++} influx into the cell during an action potential in mammalian heart muscle (Barcenas-Ruiz and Wier, 1987; Beucklemann and Wier, 1988; Callewaert, Cleemann, and Morad, 1988; Trautwein and Hescheler, 1990). It is proposed by Fabiato (1985*b*) that fast component of the Ca^{++} influx induces the Ca^{++} release from the SR, whereas the slow component is used for loading of the SR. If so, the supposed difference in the open probability of the Ca^{++} release channels in the SR between the Tsc and the Trs at the test interval of 0.8–0.9 s would be ascribed to some differences in the fast component of the Ca^{++} influx and/or in the sensitivity of the Ca^{++} release channels to the change of $[\text{Ca}^{++}]$ in their close vicinity between the two.

It was found in the present study that the shortening area in the Trs at a test interval of 0.3–0.4 s was clearly greater than that in the corresponding Tsc without any difference in the shortening velocity between the two. This finding suggests that the myoplasmic $[\text{Ca}^{++}]$ stays at a higher level longer in the Trs than the Tsc. This may be interpreted by assuming an accelerated Ca^{++} uptake activity of the SR and/or a slow movement of Ca^{++} from the uptake to the release site in the Tsc, compared with the Trs. Another possibility is that, as suggested by Fabiato (1985*b*), the slow component in the Ca^{++} influx during an action potential is taken up by the SR for the next contraction during a RS. Such a recruitment of releasable Ca^{++} during the RS would result in an increased Ca^{++} release from the SR in the immediately after Trs, in comparison with a Tsc after a SC without that kind of Ca^{++} recruitment.

The shortening velocity in the RS was generally greater than that in the SC in the present study. In a physiological twitch such as the RS, Ca^{++} which enter a myocyte through L-type Ca^{++} channels trigger the SR Ca^{++} release (Barcenas-Ruiz and Wier, 1987; Callewaert et al., 1988; Nabauer, Callewaert, Cleemann, and Morad, 1989; Lederer, Berlin, Cohen, Hadley, Bers, and Cannell, 1990). The L-type Ca^{++} channels distribute over surface and transverse (T)-tubule membranes. Consider a typical rat myocyte of 7–10 μm thickness consisting of laterally aligned myofibrils of 1 μm in diameter. The terminal cisternum of SR, from which Ca^{++} are released, runs continuously through the myofibrils transversely along the T-tubule with a tight connection between them. Then it is possible that Ca^{++} entering through the surface L-type Ca^{++} channels trigger Ca^{++} release from the superficial, terminal cisternae. This Ca^{++} release in turn triggers Ca^{++} release from the more inner, terminal cisternae, resulting in a wavelike propagation of the Ca^{++} release from the terminal cisternae along the T-tubule. Another possibility is that the inner SR is activated by Ca^{++} entering through the L-type Ca^{++} channels in the T-tubule membrane. Recent structural studies have suggested that the L-type Ca^{++} channel (dihydropyridine receptor) is connected to a Ca^{++} release channel (ryanodine receptor) in the terminal cisternum of the SR through a 12-nm-long structure called a "foot" expanding between them (Block, Imagawa, Campbell, and Franzini-Armstrong, 1988; Takeshima, Nishimura, Matsumoto, Ishida, Kangawa, Minamino, Ueda, Hanaoka, Hirose, and Numa, 1988). Ca^{++} entry through such L-type Ca^{++} channels may

quickly raise $[Ca^{++}]$ in the narrow space between the membranes of the T-tubule and the terminal cisternae, bringing about a rapid release of Ca^{++} from this region of the terminal cisternae. Therefore, the latter type of the activation of the inner SRs would result in a greater velocity of sarcomere shortening than the former. Though it is uncertain which type of the Ca^{++} release from the inner SR is dominant in the physiological twitch, the two could work complementarily. The latter type of the activation in the RS, if works essentially in the RS, will imply the observed difference in the shortening velocity between the SC and the RS. A different mode of the SR activation is also suggested by the observation that the mode of dependency of the extent on the velocity of shortening in the SC is different from that in the electrically triggered twitches (Table II).

A spontaneous contraction wave both abbreviates duration of an action potential in the following twitch and depresses an extent of cell shortening in an interval-dependent manner in isolated rat myocytes (Capogrossi et al., 1986). On the contrary, the decrease of twitch magnitude, when caused by varying stimulus frequency or $[Ca^{++}]$ in the bathing medium, is associated with increased duration of the action potential in rat and other mammalian heart muscles (Boyett and Jewell, 1980; Schouten, 1986; Bouchard and Bose, 1992). Thus the action potential duration changes independently of the twitch magnitude. It has been thought that an increase of the myoplasmic $[Ca^{++}]$ inactivates Ca^{++} channels in the sarcolemma, bringing about an abbreviation of the action potential duration in a twitch contraction; but it is apparent that this mechanism cannot reflect the bidirectional change of the action potential duration accompanied by the decrease of the twitch magnitude. The observed differences in the characteristics of twitch between the Tsc and the Trs in the present study would not be ascribed to the change of the action potential duration, if any.

In conclusion, the SC has different effects from the RS on the subsequent, action potential-mediated twitch.

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