NONSTEADY MOTION IN UNLOADED CONTRACTIONS OF SINGLE FROG CARDIAC CELLS

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ABSTRACT We studied the mode of shortening of enzymatically isolated single frog cardiac cells with a high-speed videosystem to see whether or not shortening is smooth. The segmental shortening of the cell in response to electrical stimulation exhibited a clear pause following the initial shortening over a distance of ~11 nm/half-sarcomere. Several preparations showed a second pause following the initial one. Nonsteady motion with a pause lasted usually a few tens of milliseconds. The duration of nonsteady motion was shorter in cells with large velocities of steady shortening following the pause than those with smaller velocities.

INTRODUCTION

It was unquestionably accepted by muscle physiologists that the sarcomere shortens smoothly in a steady state of contraction until Pollack and his co-workers reported that in vertebrate skeletal and cardiac muscle fibers the sarcomere shortening studied using several optical techniques occurred in a stepwise manner (Pollack et al., 1977; Delay et al., 1981; Jacobson et al., 1983). Although it appears that their stepwise sarcomere shortening has almost been proved by optical artifacts such as the Bragg angle effect (Rüdel and Zite-Ferenczy, 1978) and the change of focal region by lateral and/or longitudinal translations of the fiber (Altringham et al., 1984), this does not necessarily mean that the shortening of the sarcomere is smooth. The mode of the sarcomere shortening still remains to be solved. We studied this problem by using isolated single frog cardiac cells consisting of a small number of myofibrils and by following the movement of exactly the same segmental portions of the cells with a high-speed videosystem.

MATERIALS AND METHODS

Hearts isolated from bullfrog (*Rana catesbeiana*) were perfused for 10 min with the standard experimental solution (in millimoles per liter: NaCl, 111; KCl, 5.4; $CaCl_2$, 1.8; $NaHCO_3$, 10; NaH_2PO_4, 0.5; glucose, 4; pH 7.1) to remove blood. Ventricles were cut into small pieces, and incubated in the digesting solution (Ca-free solution containing 0.1% collagenase and 0.1% trypsin) for 4–5 h. The isolated ventricular cells dispersed into the digesting solution were collected by centrifugation, and resuspended in a thin layer of the experimental solution between a slide and a coverslip. The cells were observed under a Zeiss inverted microscope (objective 40×, NA 0.55; Carl Zeiss, Inc., Thornwood, NY). The resting sarcomere length (*SL*) was 1.8 to 2.1 μm. Most cells appeared to contact with the bottom of the slide at their centers. The cells lying on the bottom were stimulated with 5-ms rectangular pulses at 0.2 Hz through Ag-AgCl wire electrodes. The cells responded to the stimuli in an all-or-none manner. Pulse duration had no effect on the time course of the resulting contraction, which closely resembled that of intact frog ventricular muscles (Brusaert *et al.*, 1978). In preliminary experiments, it was confirmed that the cells continued to have twitch contractions at 0.2 Hz for more than 10 min without significant change of the magnitude of contractions.

A microscopic image of the cell with repeating twitch contractions was recorded with a high-speed videosystem (NAC Inc., Minato-ku, Tokyo; HVC 200) at 200 frames/s (exposure time for each frame, 5 ms). Thus, several contractions were videotaped from a given preparation. The videotaped records were displayed on a television screen (21 × 28 cm) and the spacing between the intrinsic markers within the cell (seemingly clusters of mitochondria or glycogen granules [Staley and Benson, 1968] or the length of 10–15 adjacent striations) was measured directly on each frame by placing a linear scale on the face of the television screen in the still mode with an accuracy of 0.3–1% (Fig. 1 *A*). The markers were usually separated by 10–15 striations (i.e., 100–150 mm on the television screen). To minimize systematic errors spacings between three to five different pairs of markers, between which the same group of sarcomeres was included, were measured and these values were used to compute the mean value of the spacings with the standard error (SE) in each frame. The contraction was analyzed only when the images of markers were so clear that the spacings of the same pairs of markers could be followed during at least the initial 60 ms of the contraction. Thus, 15 contractions were analyzed from 12 among 90 different preparations. To draw the shortening curve, the data were fitted by a binominal for the initial, nonsteady phase of shortening and by a straight line for the subsequent steady shortening phase by using the method of least squares. All experiments were made at room temperature (20–25°C).

RESULTS

Figs. 1 and 2 show examples of the early phase of segmental shortening of the cell in response to electrical stimulation. In 14 out of 15 contractions obtained from 12 different preparations, the initial small amount of shortening was followed by a definite pause, i.e., a period in which the shortening velocity was close to zero. The pause was in many cases followed by a phase of steady shortening lasting 50 ms or more. In five contractions from four different preparations, on the other hand, the initial pause

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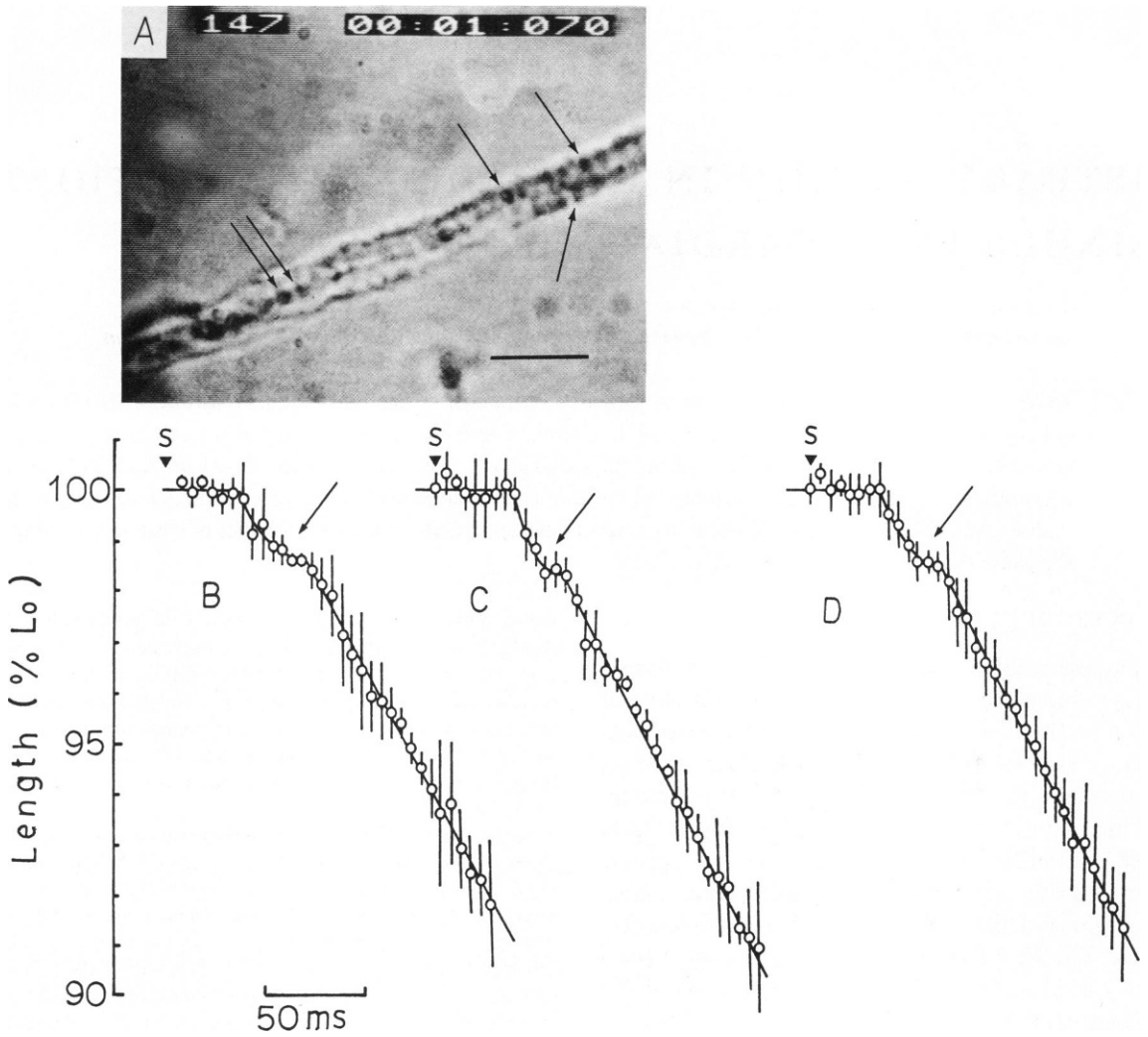

FIGURE 1 (*A*) Photograph of monitor display of a part of a single frog ventricular cell (preparation number 16) just after the onset of contraction on the television screen obtained with a high-speed videosystem. Spacings between the markers (arrows) were measured. Scale bar, 10 μm. (*B*, *C*) Shortening curves obtained from two successive contractions of the cell segment shown in *A*. Circles and bars represent the mean and SE, respectively. 5-ms pulse was applied at *S*. In this and subsequent figures, shortening curves were drawn by fitting to points by method of least squares (see Methods). Ordinate, cell length expressed as percentage of initial length (L_o). Single arrows show the pause. *D* is the average of *B* and *C*.

was followed by a second one (Fig. 2). The third pause was barely detectable in one contraction.

We tried to examine whether or not the pause took place at the same phase of the shortening curve in successive contractions in a given preparation. In most cases, however, the successive contractions could not be analyzed because the microscopic image of one or more of the markers, which were used to analyze one contraction, became obscure in the next contraction; one contraction-relaxation cycle might slightly change the vertical position of the cell segment, moving some of the markers from the focal plane of the microscope. In one exceptional case, where two successive contractions were analyzed, the pause appeared to take place at the same phase in the two shortening curves (Fig. 1 *B* and *C*); the average of the two curves showed the pause clearly (Fig. 1 *D*). This observation suggests that the random fluctuation of the data points is not the origin of the pause.

The velocity of the steady shortening after the pause varied to a large extent for a given external [Ca^{++}] from one preparation to another, and could be changed by changing external [Ca^{++}] in the same preparations (Table I). The pause duration appeared to decrease with increasing velocity of the steady shortening. But, since it is difficult to determine exactly the duration of the pause, we measured the duration (*D*) of the nonsteady motion from the onset of the preceding shortening to the end of the pause. Fig. 3 shows the plots of *D* against both the velocity of the steady shortening (*V*) and the mean shortening velocity during the nonsteady motion (*V'*), which was given by dividing the amplitude (*A*) of the nonsteady motion by *D*. This figure suggests that the nonsteady

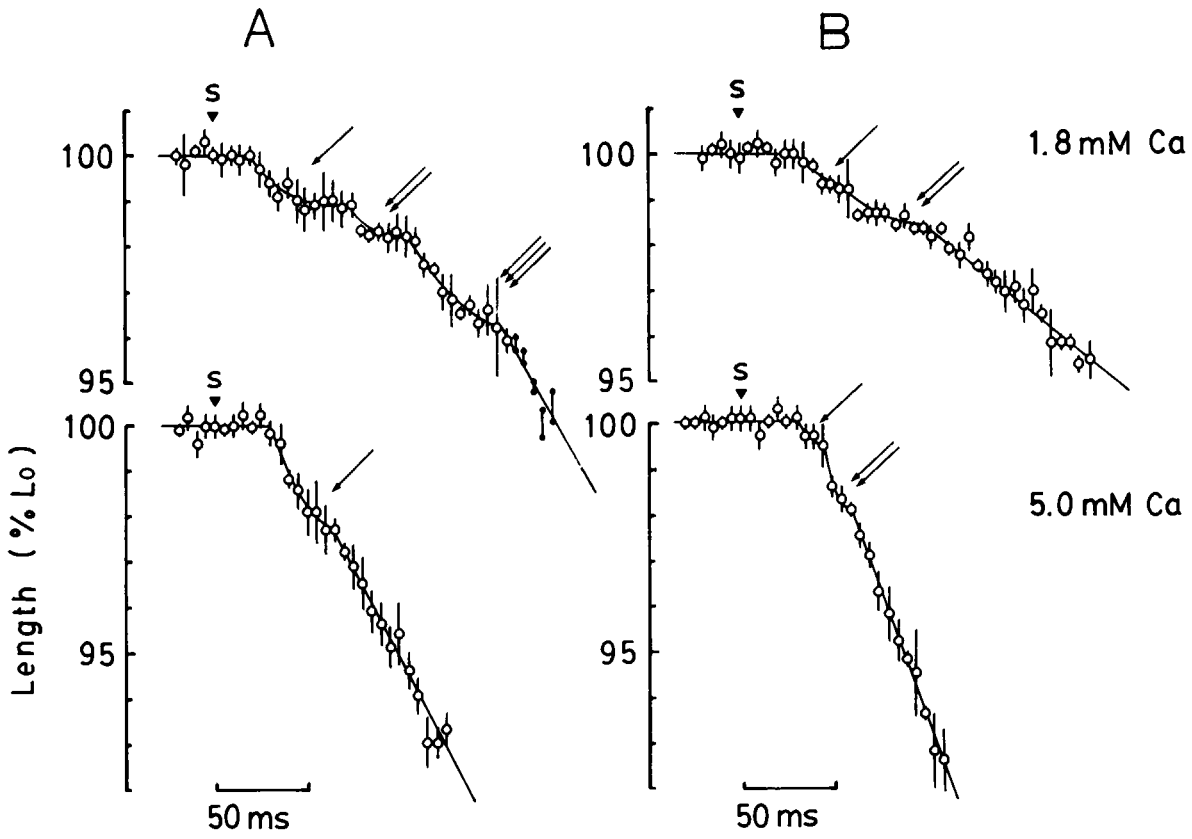

FIGURE 2 Effect of changing external Ca on the shortening curve. *A* shows shortening curves in 1.8 mM Ca (*upper*) and 5.0 mM Ca solutions (*lower*) in the same cell (preparation number 85). The required amount of Ca was replaced with osmotically same amount of $NaHCO_3$. The *upper* curve shows initial (single arrow), second (double arrows), and third pauses (triple arrows), while only one pause was detectable in the *lower* curve. After the third pause, two marker spacings were measured in this case only. Double-filled circles combined by a bar show individual values of the spacings. (*B*) Another cell (preparation number 86). Double pauses were detected in both the *upper* and *lower* curves.

TABLE I
SUMMARY OF THE DURATION (*D*) AND THE AMPLITUDE (*A*) OF NONSTEADY MOTION, THE VELOCITY OF THE STEADY SHORTENING (*V*), AND *A/D* (*V'*) IN INDIVIDUAL PREPARATIONS

Date	Preparation number	*D*			*A*			*A/D* (*V'*)			*V*	[Ca]
		First	Second	Third	First	Second	Third	First	Second	Third		
			ms			% L_o			L_o/s		*Lo/s*	*mM*
12/13/82	16 (1)	33	—	—	1.3	—	—	0.39	—	—	0.71	1.8
	(2)	23	—	—	1.7	—	—	0.74	—	—	0.78	—
	(3)	30	—	—	1.5	—	—	0.50	—	—	0.75	—
12/13/82	19	40	—	—	1.0	—	—	0.25	—	—	0.52	1.8
1/11/83	29	30	—	—	0.7	—	—	0.23	—	—	0.46	5.0
1/11/83	32	25	27	—	1.1	1.5	—	0.44	0.56	—	0.75	5.0
1/11/83	33	38	—	—	0.7	—	—	0.18	—	—	0.69	5.0
1/11/83	37	15	—	—	1.1	—	—	0.73	—	—	1.52	5.0
1/11/83	44	25	20	—	0.8	1.3	—	0.32	0.65	—	1.08	5.0
1/11/83	45	15	—	—	0.6	—	—	0.40	—	—	1.18	5.0
1/11/83	46	17	—	—	1.0	—	—	0.59	—	—	1.24	5.0
10/25/83	85	55	32	52	1.1	0.7	2.0	0.20	0.22	0.38	0.40	1.8
		35	—	—	2.3	—	—	0.66	—	—	0.81	5.0
10/25/83	86	27	43	—	0.8	0.8	—	0.30	0.19	—	0.33	1.8
		15	15	—	0.5	1.4	—	0.33	0.93	—	1.12	5.0
10/25/83	90	—	—	—	—	—	—	—	—	—	1.85	5.0

(1) and (2) were obtained from two successive contractions. (3) was obtained from the averaged contraction of the two.

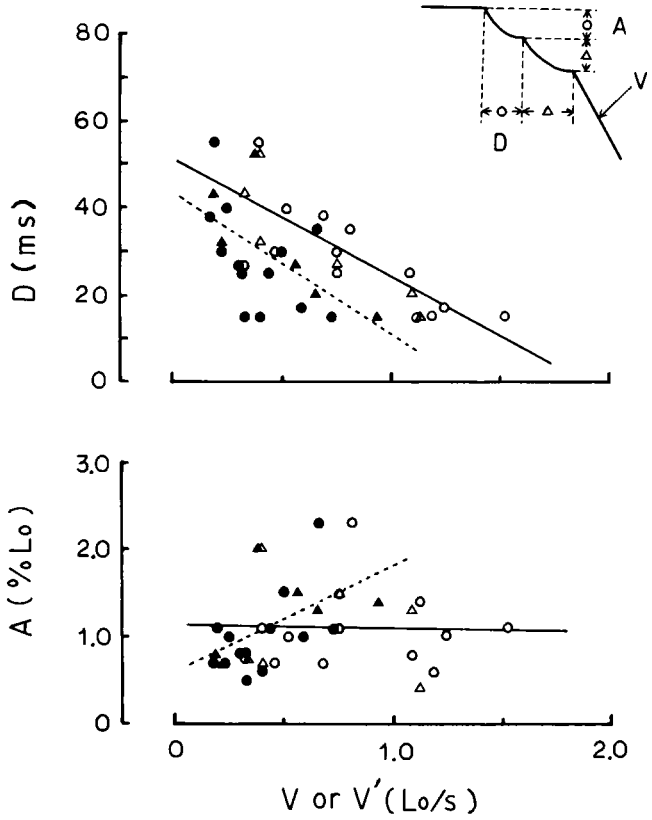

FIGURE 3 Plots of the duration (*D*, *upper*) and the amplitude (*A*, *lower*) of the nonsteady motion against the shortening velocity (*V*) of the steady phase following the nonsteady motion (open symbols with continuous lines; see inset) and against the mean shortening velocity (*V'*) during the nonsteady motion (filled symbols with dotted lines), which was given by *A/D*. Circles and triangles were obtained from initial and subsequent pauses, respectively. Lines were fitted to points by method of least squares.

motion shortens with the increases of both *V* and *V'*. This was confirmed by the experiments, in which the effect of changing external [Ca^{++}] and therefore of changing shortening velocity on *D* was examined using the same preparations (Fig. 2; preparation numbers 85 and 86 in Table I).

On the other hand, *A* appeared to have no significant relation with *V*, though it increased with the increase of *V'* (lower graph in Fig. 3). The average value of *A* was 1.1% L_0 (the initial length of the cell segment), which corresponded to 11 nm/half-sarcomere provided that the resting *SL* was 2.0 μm.

In one preparation (preparation number 90 in Table I), after the onset of shortening, the shortening velocity increased monotonically and reached a steady value, which was the highest among those obtained from 12 different preparations. Taking into account the relationship between *D* and *V*, it is not certain whether the nonsteady motion was too short to be detected with the present time and space resolutions or whether the cell really shortened without any pause.

DISCUSSION

We found that isolated single frog cardiac cells showed nonsteady motion with at least one pause in the early phase of shortening. What is the likely cause of the pause?

(*a*) The cells usually contacted with the glass bottom of the chamber at their central regions, leaving the distal regions free from the bottom. The point of contact was established by observating the point at which there was no movement during at least the initial phase of contraction. We avoided using such regions for analysis, so that the friction between the cell and the bottom had no crucial effect on the time course of shortening. Furthermore, multiple pauses observed in some preparations cannot be explained by friction.

(*b*) As the sarcomere shortens, the tip of the thin filaments begins to cross the center of the A-bands, giving rise to double overlap of the thin filaments. Contrary to frog skeletal muscle fibers, the thin filaments of frog cardiac cells have varying lengths from 0.8 to >1.3 μm (Robinson and Winegrad, 1979). Therefore, even if the double overlap generates internal resistance against shortening, the resistance will increase gradually. Such a gradual increase of the internal resistance would not result in the pause, certainly not in multiple pauses.

(*c*) One may ascribe the pause to the fluctuation of data points. Close examination of the fluctuation in the resting frog cardiac cells showed that the data points did not fluctuate in a systematic way but at random. Whenever the data points deviated greatly from their average value, we found a marked change in the shape of the markers. This might be caused by the intensity fluctuation of the illumination. Such a fluctuation in the data points, however, may not be the cause of the pause since the pause appeared to take place at the same phase in the successive contractions (Fig. 1).

(*d*) In frog cardiac cells, an influx of Ca^{++} during an action potential is the main source of the activator Ca^{++}, although a small fraction of the activator Ca^{++} may have an intracellular origin, i.e., Ca^{++} released from the sarcoplasmic reticulum (for a review, see Chapman, 1979). If a small amount of Ca^{++} is released from the sarcoplasmic reticulum preceding the main Ca^{++} influx, intracellular [Ca^{++}] will initially increase slightly, followed by a rushing increase of [Ca^{++}] due to influx. However, there is no evidence supporting such a stepwise increase of intracellular [Ca^{++}]. Furthermore, the multiple pauses cannot be explained by this mechanism.

The foregoing suggests that nonsteady motion with a pause comes from the molecular mechanism of contraction of single frog cardiac cells. The form of the nonsteady motion followed by the steady phase of shortening resembles the velocity transients caused by suddenly reducing the load on the tetanized frog skeletal muscle fibers from the isometric level to a smaller value (Podolsky, 1960; Civan and Podolsky, 1966); the duration of the nonsteady

motion decreased with increasing velocity of the steady shortening in both cases. It is interesting that in the two cases there is a similarity in the experimental conditions; the nonsteady motion took place at the beginning of the shortening, which was caused by activating the cell by means of electrical stimulation in our case and by suddenly changing the isometric contraction to the isotonic one in the Podolsky's case. The similarities both in the experimental conditions and in the dependency of the duration of the nonsteady motion on the velocity of the following shortening imply that the nonsteady motions in the two cases might result from the same molecular mechanism of contraction.

Many contractions showed single pauses, while others showed double or even triple pauses. We question whether the contractions with single pauses actually had only one pause; perhaps any additional pauses were masked by the scattering of data points due to the limited space resolution. If we take into account the dependency of the duration (D) of the nonsteady motion with a pause on the mean shortening velocity (V') during the nonsteady motion (Fig. 3), a pause in the shortening phase with a relatively high V' might not be detected with the present time and space resolutions. Therefore, it is difficult to discern the exact number of pauses.

Pollack and his co-workers reported that the shortening of single frog skeletal muscle fibers was interrupted by short pauses (see Jacobson et al., 1983). They claim that thousands of sarcomeres may alternate between the rapid shortening period and the pause phase in synchrony. At present, it is not clear whether the pauses observed in isolated single frog cardiac cells have the same origin as those observed in single frog skeletal muscle fibers as documented Pollack and his co-workers (1977).

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 5744-6024) to H. Sugi.

Received for publication 7 June 1984 and in final form 23 April 1985.

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