Spontaneous and Propagated Contractions in Rat Cardiac Trabeculae

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ABSTRACT Sarcomere length measurement by microscopic and laser diffraction techniques in trabeculae of rat heart, superfused with Krebs-Henseleit solution at 21°C, showed spontaneous local sarcomere shortening after electrically stimulated twitches. The contractions originated in a region of several hundred micrometers throughout the width of the muscle close to the end of the preparation that was damaged by dissection. The contractions propagated at a constant velocity along the trabeculae. The velocity of propagation increased from 0 to 10 mm/s in proportion to the number of stimuli (3–30) in a train of electrically evoked twitches at 2 Hz and at an external calcium ion concentration ($[Ca^{++}]_o$) of 1.5 mM. At a constant number of stimuli (n), the velocity of propagation increased from 0 to 15 mm/s with $[Ca^{++}]_o$ increasing from 1 to 7 mM. In addition, increase of n and $[Ca^{++}]_o$ led to an increase of the extent of local sarcomere shortening during the spontaneous contractions, and the occurrence of multiple contractions. Spontaneous contractions with much internal shortening and a high velocity of propagation frequently induced spontaneous synchronized contractions and eventually arrhythmias. Propagation of spontaneous contractions at low and variable velocity is consistent with the hypothesis that calcium leakage into damaged cells causes spontaneous calcium release from the overloaded sarcoplasmic reticulum in the damaged cells. This process propagates as a result of diffusion of calcium into adjacent cells, which triggers calcium release from their sarcoplasmic reticulum. We postulate that the propagation velocity depends on the intracellular calcium ion concentration, with increases with n and $[Ca^{++}]_o$.

INTRODUCTION

Fabiato described the remarkable phenomenon that spontaneous contractions may occur in fragments of single cardiac cells (Fabiato and Fabiato, 1972, 1975). These contractions could be induced by exposing the cell to a solution with an elevated free calcium concentration (> 0.5 μM) and a low concentration of the calcium

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buffer EGTA (< 0.05 mM). Accumulation of calcium in the sarcoplasmic reticulum (SR) resulted in spontaneous repetitive release of calcium, which caused oscillations of sarcomere length and force (Fabiato, 1985). This phenomenon has been denoted as Ca^{++} overload induced Ca^{++} release by the SR and similar spontaneous contractile activity has been reported in enzymatically dispersed single cells (Wier et al., 1986) and in multicellular cardiac muscle preparations (Fabiato, 1985). Spontaneous contractions in enzymatically dissociated cells have been observed to propagate, usually at low velocities (≤ 150 μm/s; Capogrossi et al., 1986). Velocities up to 3 mm/s have been reported after repeated stimulation (Golovina et al., 1986). A similar phenomenon has been observed microscopically in papillary muscle (Kort and Lakatta, 1984) and is characterized by conspicuous random contractions of sarcomeres throughout the preparation, which cause fluctuations of the light scattering properties of the muscle (Capogrossi et al., 1986).

It has been reported that cardiac muscle preparations, such as papillary muscles (Krueger and Pollack, 1975, Fig. 4) and trabeculae (ter Keurs et al., 1980, Fig. 2), exhibit signs of damage at the ends that are attached to the transducers. These signs consisted of spontaneous contractile activity and stretch during the twitch. We observed that during the first hour of equilibration of a preparation after dissecting and mounting, spontaneous contractions occurred as a local wave of sarcomere shortening throughout the width of the muscle encompassing a region of a few hundred micrometers. They originated close to one of the damaged ends of the preparation, and appeared to move at a constant velocity (up to 15 mm/s) along the length of the trabeculae. This suggested that the process that allows movement of the spontaneous contraction can travel easily from cell to cell. This process seemed to depend on the intracellular calcium concentration because both the amplitude of the sarcomere-shortening wave and its velocity appeared to depend on the extracellular calcium concentration and on the stimulus rate. Moreover, when rapidly propagating spontaneous contractions occured, which exhibited extensive sarcomere shortening, arrhythmias frequently developed.

In this study, we have analyzed the properties of propagation of the spontaneous contractions and, in particular, the effect of stimulus rate and the external calcium concentration on propagation velocity. Preliminary results have been published previously (ter Keurs and Mulder, 1984; ter Keurs et al., 1988).

METHODS

Dissection and Mounting of the Preparation

Preparations were dissected from the heart of Wistar rats of either sex, age 2–6 mo, bodyweight 200–400 g. The hearts were excised from animals under ether anesthesia. Dissection was performed under a binocular microscope, while the heart was perfused with a modified Krebs-Henseleit solution. Trabeculae running between the free wall of the right ventricle and directly attached to the atrioventricular ring, or connected to the right ventricle close to the atrioventricular ring were selected. The dimensions of the preparations (n = 17) were: length, 2–5 mm; thickness, 90 ± 15 μm; width, 200 ± 50 μm. In trabeculae with more than one damaged region, we often observed that spontaneous contractions started independently at both damaged regions. If they traveled in opposite directions and collided, no propagation beyond the region of collision was observed. Reversal of propagation direction could be

observed in such muscles when the stimulus conditions were changed. The variability that is inherent to the presence and extent of damage to the trabeculae evidently influenced the quantitative aspects of the propagation of the spontaneous contractions. We minimized this variability by selecting trabeculae of at least 2 mm in length and by careful dissection of the right ventricle at least 250 μm away from the insertion site of the muscle so that the resulting damage did not invade the trabecula.

The muscles were mounted in a glass-covered chamber; the volume of the chamber was 0.5 ml. The preparation was superfused at a flow rate of 4 ml/min. The solutions used during dissection and experiments contained (in millimolar): 147.9 Na^+, 5.0 K^+, 127.5 Cl^-, 1.2 Mg^{2+}, 2.0 PO_4^{3-}, 1.2 SO_4^{2-}, 28.0 HCO_3^-, 11.0 glucose, and $CaCl_2$ as specified in the results section. The solutions were in equilibrium with 95% O_2 and 5% CO_2; pH was 7.4.

Lowering the temperature to ~20°C appeared in preliminary experiments to prolong the period in which the properties of the spontaneous contractions (i.e., the extent of sarcomere shortening and the propagation velocity) were stable, hence the temperature of the fluid in experimental chamber, measured with a thermistor, was kept constant in a range between 19.5 and 21.0°C.

Experimental Apparatus

A region of the muscle that was illuminated by a He-Ne laser beam (cross section, 400 μm) was observed using an inverted microscope and television system (Sony Video monitor model PVM-90CE). Sarcomere length in the illuminated area was measured from diffraction patterns generated by the muscle in laser light. We have made use of diffraction methods as described previously (Daniels et al., 1984). Sarcomere behavior of different areas of the trabecula could be recorded and compared by translating the microscope stage that supported the preparation in a direction parallel to the longitudinal axis of the specimen. The displacement of the microscope stage was measured from a linear potentiometer by means of a bridge amplifier (13-4615-50; Gould Inc., Oxnard, CA). The muscle was connected to a silicon strain gauge force transducer (AE801; Sensonor, Horton, Norway) via a stainless steel hook and a stainless steel ring around a small block of myocardial wall to which the trabecula was attached. The valvular end of the preparation was connected to a stainless steel stationary hook. The dynamic properties of the system have been described previously (Daniels et al., 1984).

Stimulus Protocol

The preparations were studied during the first hour after excision. They were stimulated by means of parallel platinum electrodes; the stimulation protocol consisted of trains at a rate of 2 Hz, interspersed by 12-s rest periods. Two stimulus protocols were used; one in which the number of stimuli in a stimulus train was varied between 1 and 30 stimuli; the resulting spontaneous contraction, after the last electrically stimulated contraction, was studied at a calcium ion concentration of 1.0 mM. In the second protocol, the number of stimuli in the train was kept at 20, and the effect of varied calcium concentrations between 0.5 and 7.0 mM on the spontaneous contraction was studied. Spontaneous contractions were studied at a sarcomere length between 2.1 and 2.3 μm; passive force at the selected length was 5% of total force. Force and sarcomere length of the last stimulated contraction and of the spontaneous contraction after it were recorded on an oscilloscope and a hard copy unit (5103 and 613, 4621; Tektronix, Inc., Beaverton, OR). The measurement was repeated two to four times in each region of the muscle. The microscope stage was then moved to allow study of other regions of the trabecula. Usually, the regions that were studied, were as far apart as possible to allow the accurate calculation of the velocity of movement of spontaneous contractions (v) from the ratio of the distance between the regions at which the spontaneous contractions were

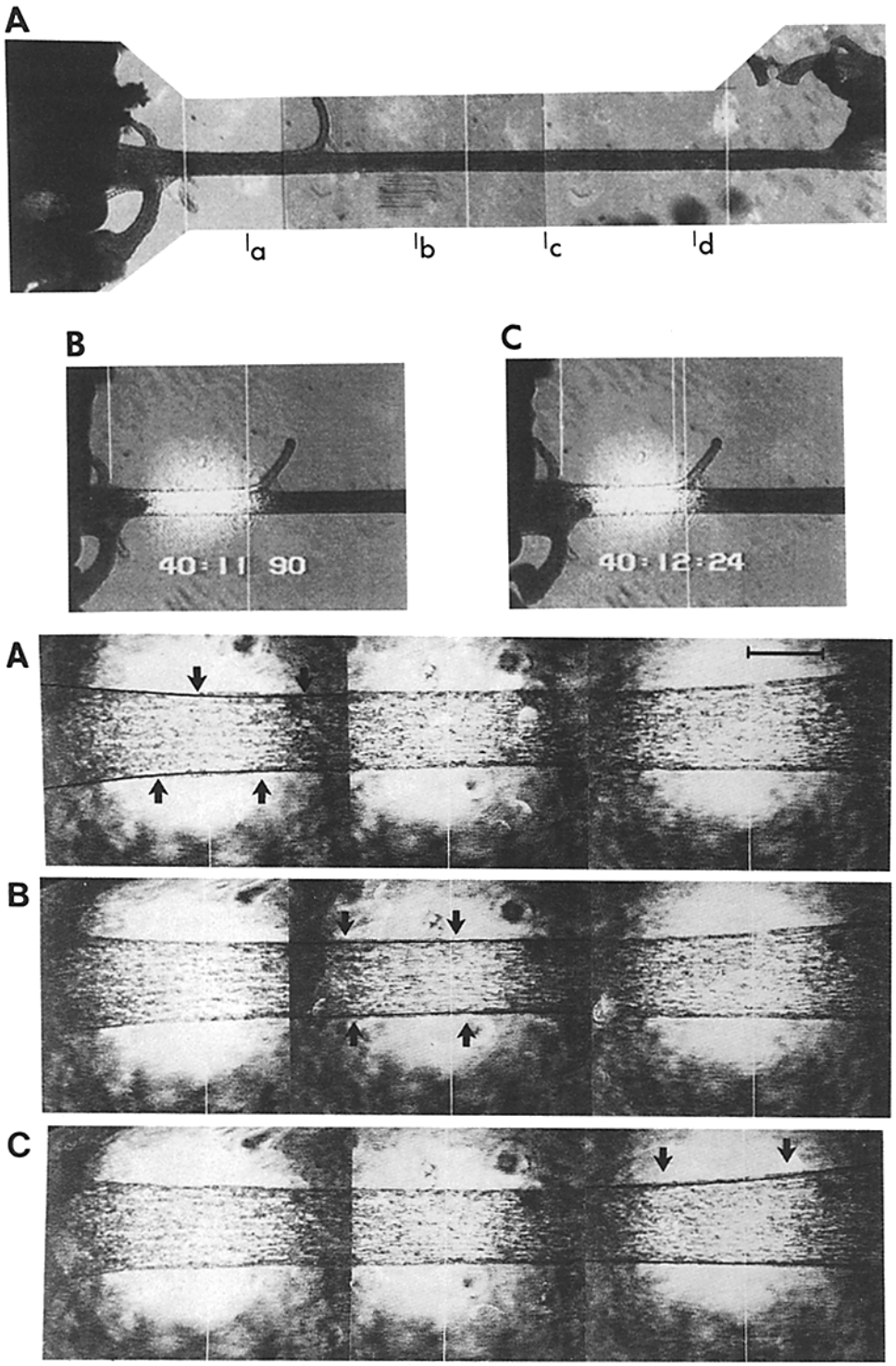

FIGURE 1.

recorded, and the difference between their coupling interval. Control measurements were taken between the measurements of both protocols. Data were accepted only if v at $[Ca^{2+}]_o$ = 1.0 mM and n = 20 varied < 10%. In some experiments the shortening waves were studied by frame-by-frame analysis of video recordings of the microscopic image of the muscles.

The effect of low external potassium concentration ($[K^+]_o$) and low sodium concentration ($[Na^+]_o$) (Allen et al., 1985; Wit and Rosen, 1986) on spontaneous contraction and the velocity of propagation was tested in preliminary experiments. We did not use lowering of $[K^+]_o$ or $[Na^+]_o$ however, as these interventions caused synchronous aftercontractions throughout the preparations.

RESULTS

The trabeculae could typically be observed over most of their length, but usually remnants of the right ventricle prevented observation of the region of insertion itself. When this region could be observed, spontaneous asynchronous contractile activity could be seen microscopically in cells of this region as has been reported before (Krueger and Pollack, 1975, p. 631; ter Keurs et al., 1980). Asynchrony of the spontaneous activity caused broadening of the first order band of the laser-diffraction pattern and reduction of its intensity, thereby often obviating the electronic measurement of sarcomere length. The spontaneously active region was stretched severely (up to 50%) during the electrically induced twitches (Fig. 1). Upon repetitive stimulation twitches were followed by (often multiple) spontaneous

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FIGURE 1. (*Opposite*) Reproductions of the video images of two muscles, which developed spontaneous contractions, after electrically elicited twitches. The top panel illustrates that the twitch is accompanied by stretch of the attachment of the muscle to the remnant of the right ventricle. The bottom panels show propagation of a spontaneous contraction. The top panels show a trabecula (3.22 mm long, 107 μm wide, and 80 μm thick) in which the spontaneous contraction started at the left side of the preparation. The trabecula was attached to the right ventricle with two small side branches and one thin long branch which was cut; the contracted remnant of this branch serves as a marker near *a*, indicated by the white marker line to the right of *a*. The trabecula was inserted directly into the atrioventricular ring and was attached to the apparatus via the tricuspid valve. It is visible that the region near the right ventricular myocardium was stretched during the twitch, as indicated by the rightward movement of the line that marks the branch near the right ventricle (see *B*, taken at rest, and *C*, taken 340 ms later during maximal contraction); simultaneous leftward movement of the marker line, which indicates the insertion of the small branch, shows that the area *a* shortened (18%). The spontaneous contractions following this twitch were recorded by measurement of sarcomere length in areas *a*, *b*, *c*, and *d*. The corresponding recordings are shown in Fig. 2 (*top*). The bottom panels show propagation of a spontaneous contraction, manifest as a localized increase of the width of the muscle (indicated by the arrows and recognizable by comparing the tracing of the diastolic silhouette of the muscle on the frames with the actual width of the muscle). The first moment at which the regional spontaneous contraction could be observed was 38 ± 2 videoframes (1,266 ± 66.7 ms) after the onset of the preceding twitch at the left edge of the laser beam illuminating area *A*. (*A*) localization of the contractile wave 1,366 ms after the twitch. (*B*) Taken 466 ms later; the wave has propagated by ~480 μm. (*C*) The wave arrived near the valvular end of the preparation 533 ms later at a distance of 560 μm from the contracting region in *B*. Propagation velocity 1.05 mm/s. Calibration bar 100 μm. The thickness of the muscle was 70 μm and the length was 2.05 mm.

contractions of increasing amplitude that appeared to originate in the stretched area, and could be observed microscopically as a region with an increased diameter (up to 7%) over a few hundred micrometers that moved along the trabecula (Fig. 1). Measurement of sarcomere length at various positions along the trabecula (Fig. 2) confirmed the microscopic observation that the contractions started in the region that was stretched during the regular twitch (see Fig. 1) and moved at a constant rate, even though the velocity of movement could vary substantially.

The first regional spontaneous contraction occurred during the final part of the

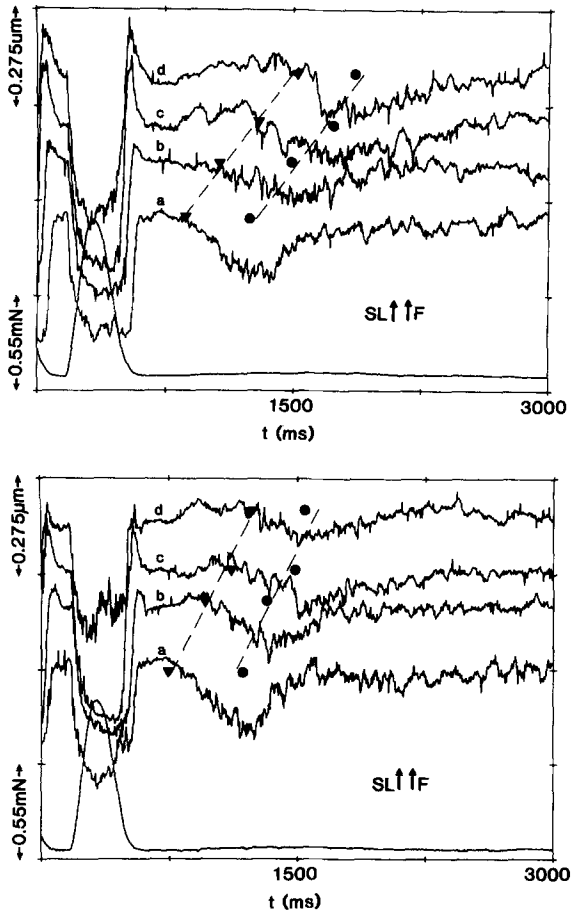

FIGURE 2. Recordings of force and sarcomere length in regions *a*, *b*, *c*, and *d* in the muscle depicted in the top of Fig. 1. Both panels show twitch force and sarcomere shortening during the twitch and during a propagating spontaneous contraction. The sarcomere tracings have been displaced by an amount that was proportional to the distance between the recording sites. Prior to each record the muscle had been stimulated electrically at 2 Hz (10 stimuli in the top panel and 16 stimuli in the bottom panel). Each sarcomere tracing is the average of two recordings; the force traces of eighth recordings have been superimposed. Note that a small force-transient is noticeable during the presence of the propagating contraction. Calibrations as indicated; the resting sarcomere length was 2.15 µm. (*Top*) The twitch is followed by spontaneous contraction that moves at a constant rate along the trabecula. Both the time of onset of local shortening (filled triangles) and the moment at which sarcomere length (filled circles) was minimal (during the spontaneous contraction), were linearly related to the position along the muscle. Calculated velocity of the spontaneous contraction was 3.5 mm/s. (*Bottom*) (Same format) the velocity of propagation has increased with the increase in the number of preceding stimuli, but the movement of the spontaneous contraction was still a linear function of position along the muscle. Calculated velocity of propagation was 5.5 mm/s. Note that the force-transient is larger than in the top panel.

relaxation phase of the preceding twitch (1.3 ± 0.2 s after the onset of contraction in the muscles in Fig. 1). The coupling interval between multiple regional spontaneous contractions was inversely related to the amplitude of the spontaneous contractions, but it was considerably shorter than the coupling interval between the first spontaneous contraction and the twitch (~ 660 ± 70 ms, also see Fig. 3).

When the muscle had stabilized at 25°C, contractions were normally followed by a period of ~1-s duration without any sarcomere length fluctuations. Small spontaneous sarcomere length fluctuations could be measured later by means of the diffraction technique during the interstimulus interval, while at the same time contrac-

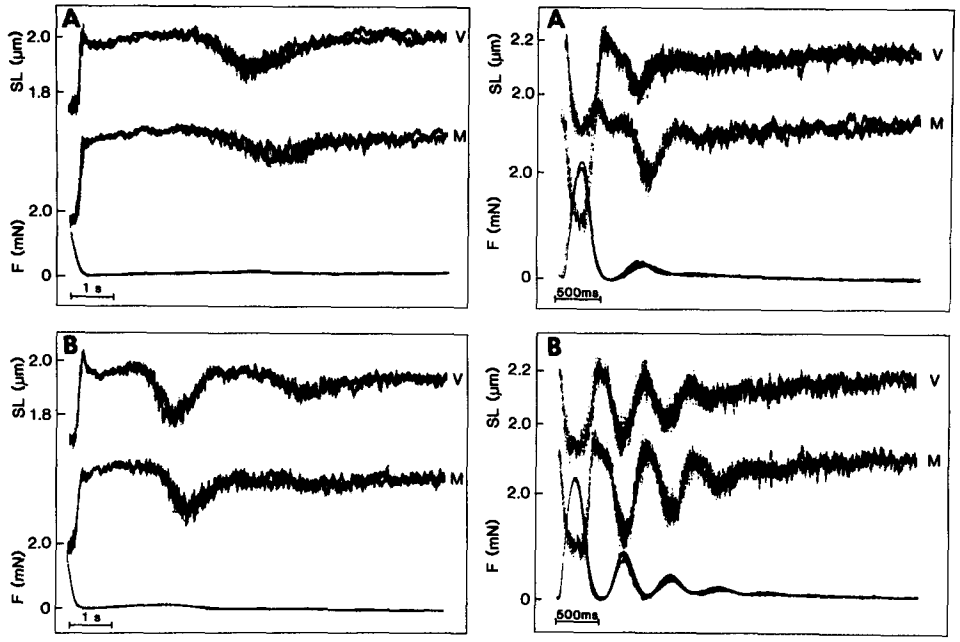

FIGURE 3. Variation of the velocity of propagation of the spontaneous contractions is coupled with the amplitude and the number of spontaneous contractions. Twitch force (bottom trace of each panel), and sarcomere length measured at the myocardial end (top trace) and at the valvular end (second trace) of the trabeculae are displayed. The sarcomere length recordings have been displaced vertically to allow comparison of the shortening transients. Calibrations as indicated. Temperature was 21.5°C. Frequency of stimulation was 2 Hz. The left panels show the effect of increasing the number of stimuli from five (*A*) to ten (*B*). The propagation velocity increased from 1.3 to 2.5 mm/s. The distance between the recording sites of sarcomere length was 1 mm; $[Ca^{++}]_o$ = 1.0 mM. The right panels show the effect of variation of $[Ca^{++}]_o$. The number of conditioning stimuli was 20 and the distance between the recording sites of sarcomere length was 1.0 mm. The propagation velocity of the first spontaneous contraction increased from 4.3 mm/s at $[Ca^{++}]_o$ = 1.5 mM to 14.8 mm/s at $[Ca^{++}]_o$ = 3.0 mM. Together with the increase of the propagation velocity it is noticeable that the same time course of the force transient of the spontaneous contraction becomes similar to that of the twitch. The number of spontaneous contractions increased with an increase of $[Ca^{++}]_o$, which is similar to that of a damped oscillation. The amplitude and the propagation velocity of the second and third spontaneous contractions decreased progressively (*B*).

tions could be noted microscopically within individual cells in the preparation. These contractions extended over ~ 10–20 μm and moved longitudinally through the cells as has been described (Capogrossi et al., 1986). The velocity of movement, measured by frame-by-frame analysis of video recordings of the microscopic image of these muscles, varied from 50 to 125 μm/s (eight muscles).

Propagation of Spontaneous Contractions after Trains of Stimuli

The velocity, number, and amplitude of sarcomere shortening waves during spontaneous contractions at 21°C depended strongly on $[Ca^{++}]_o$ and on the stimulus rate (Fig. 3). Their coupling interval to the onset of the twitch at any position along the

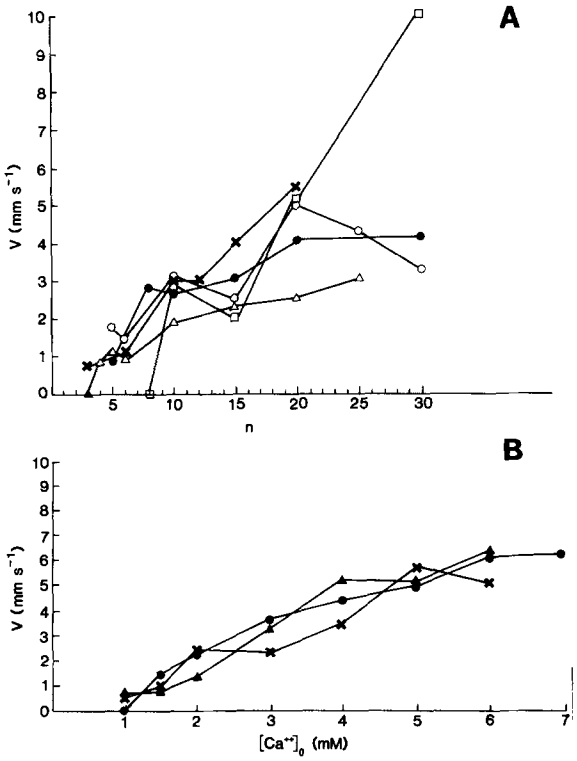

FIGURE 4. The relation between propagation velocity and number of stimuli or $[Ca^{++}]_o$. *A* shows the relationships between v and the number (n) of preceding stimuli during a stimulus train at 2 Hz in five muscles; $[Ca^{++}]_o$ was 1.0 mM. *B* shows the relationships between v and $[Ca^{++}]_o$ after a conditioning train of 20 stimuli at 2 Hz in three muscles at varied $[Ca^{++}]_o$.

muscles was highly reproducible (Fig. 3). This allowed repeated measurements along a trabecula. The velocity of propagation of the contractions observed at a $[Ca^{++}]_o$ of 1.0 mM increased from 0 to 10 mm/s (Fig. 4 *A*) with the number of stimuli (0–30) during the stimulus train. Although all muscles exhibited this behavior, variability between the muscles was manifest at higher numbers of stimuli.

It is likely that the increasing number of stimuli increased the amount of Ca^{++} that accumulated in the cells (Allen et al., 1984). On the other hand, Na^+ ions (Cohen et al., 1982) must have accumulated as well. We, therefore, tested separately the effect of varied $[Ca^{++}]_o$. Because propagation of the sarcomere shortening was

always observed with 20 stimuli in the conditioning train we studied the effect of $[Ca^{++}]_o$ on v under these conditions in other experiments. Figs. 4 *B* and 5 show the effect of $[Ca^{++}]_o$ on the propagation velocity in a series of nine trabeculae; an increase of $[Ca^{++}]_o$ caused a proportional increase of the velocity of motion of the contraction waves. Three preparations showed a gradual increase of v from 0 to 5.5 mm/s when the external calcium concentration was increased to 7.0 mM (Fig. 4 *B*). In five other preparations v increased from 0.5 to 15 mm/s at external calcium concentrations between 1.0 and 4.0 mM (Fig. 5). At higher higher calcium concentrations these muscles developed either arrhythmias (see below; Figs. 5 and 7), or the difference in coupling intervals at different locations along the trabeculae became too small to validly estimate the velocity of motion. In two muscles the direction of propagation reversed.

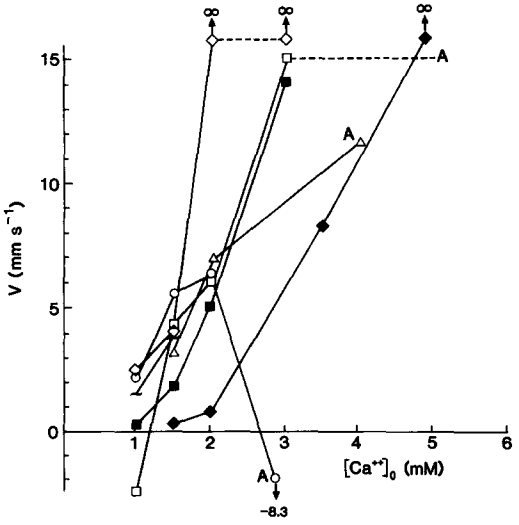

FIGURE 5. The relation between the velocity of propagation of the first contraction after the twentieth twitch of a train at 2 Hz at varied $[Ca^{++}]_o$ of six other muscles. The velocity increased steeply in four muscles with $[Ca^{++}]_o$ until an arrhythmia developed (*A*) or until the difference in coupling intervals at the areas of measurement became too small to be measured reliably (∞). In two muscles the directions of propagation reversed with increasing $[Ca^{++}]_o$ (open squares and open circles).

Interaction between Spontaneous Contractions and the Electrically Evoked Twitch

Fig. 6 shows that with increasing amplitude of the spontaneous contraction the electrically evoked twitch decreased. Apparently, the next stimulus triggered calcium release from an incompletely replenished SR as the concurrent twitch was small. The subsequent spontaneous contraction was then also small and its propagation velocity was low (not shown). The following electrically elicited twitch was large again and so was the rapidly propagated (not shown) spontaneous contraction that followed it (Fig. 6). The resultant pattern was that of an alternans where pairs of small twitches and small propagated contractions alternated with large twitches and large propagated contractions. When stimulation was stopped the muscle became completely quiescent in a few seconds. The first twitch, after a pause of 15 s, was as large as that during regular stimulation but it was not followed by propagating contractions (not shown).

Twitches and Arrhythmias

In high $[Ca^{++}]_o$ nearly all preparations showed spontaneous contractions, which occurred synchronously throughout the muscle (see Fig. 7). The time-course of force and of sarcomere shortening of the spontaneous synchronous contractions was comparable to the time-course of an electrically elicited twitch; we have denoted these contractions as spontaneous twitches. Spontaneous twitches started during the slow rising phase of the spontaneous propagated contraction. The rapid increase of

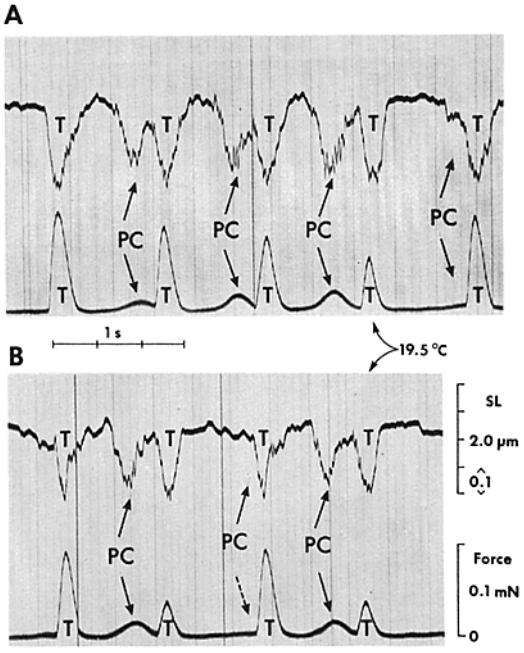

FIGURE 6. In this figure it is demonstrated that spontaneous contractions lead to depression of the force of the following twitch and that the occurrence of a spontaneous contraction depends on the magnitude of the preceding twitch. *A* shows the increase of the amplitude of propagated contractions (*PC*) during continued stimulation at 0.4 Hz. The increase of the propagated contractions is accompanied by a decrease of the amplitude of the subsequent twitch. The fourth twitch of the record elicited only a small spontaneous contraction and was followed by a twitch of the same amplitude as the first twitch of the train. (*B*) This pattern evolved into that of a mechanical alternans in which the large twitch was followed by a large propagated contraction, which in turn was followed by a small twitch with a small subsequent propagated contraction. This sequence of alternatingly large twitches and large propagated contractions and small twitches and small propagated contractions was maintained for many minutes. (Calibration bars as indicated apply to *A* and *B*.)

the rate of rise of force of such a spontaneous twitch is clearly noticeable in Fig. 7. When a spontaneous twitch occurred at a certain combination of the number (n) of stimuli in the train and $[Ca^{++}]_o$, a slight increase of n or $[Ca^{++}]_o$ caused a series of spontaneous twitches, i.e., an arrhythmia (Fig. 7 *B*). The sarcomere length tracing obtained in two areas in Fig. 7 *B* shows synchronous sarcomere shortening during the spontaneous twitches, whereas each synchronized contraction was preceded by a propagated spontaneous contraction as is indicated by the dashed lines in Fig. 7 *B*. It is noteworthy that the intervals between the spontaneous twitches was initially

short, but subsequently increased progressively. Stable spontaneous tachycardias could persist for many minutes before the intervals between the spontaneous twitches increased and the arrhythmia terminated spontaneously, and only a spontaneous propagated contraction was observed. The spontaneous twitches of an arrhythmia were initiated at the moment of arrival of the spontaneous contraction at a fixed point along the trabecula (region *B* in Fig. 7 *B*) irrespective of its propagation velocity. The increase of the interval time between the spontaneous twitches corresponded closely to the decrease of the velocity of propagation of the sarcomere-shortening wave.

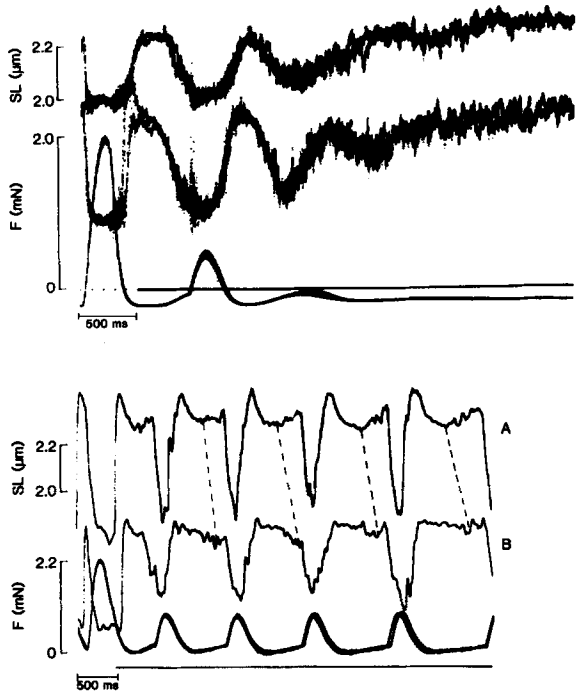

FIGURE 7. Here it is shown that spontaneous contractions cause the development of twitches and arrhythmias. Sarcomere length (top and middle traces) was recorded near the myocardial end (middle trace in the top panel; and top trace in the bottom panel) and near the valvular end of the muscle; the measurement distance was 1.2 mm (*top*) and 1.6 mm (*bottom*). Force is shown in the bottom traces. Temperature was 21°C. 20 stimuli at 2 Hz preceded the recorded twitch. $[Ca^{++}]_o$ was 2.5 mM in the top panel and 3.0 mM in the bottom panel. Calibrations were as indicated. The top panel shows an example of the development of a spontaneous twitch that is triggered by a propagating aftercontraction. Note the acute increase of the rate of rise of force development during the first aftercontraction, and the similarity of the time-course of subsequent twitch and that of the electrically elicited twitch. For further explanation see text. The bottom panel shows an example of a short-lasting triggered arrhythmia. Note the gradual increase of the interval between the spontaneous twitches. The spontaneous contraction started at the valvular end (*A*) of the preparation and traveled toward the myocardial end (*B*). Note that synchronized contractions were triggered at the moment that the propagated contractions arrived at *B*. Note, furthermore, that the propagation velocity decreased while the interval between the synchronized twitches increased.

DISCUSSION

The data presented in this paper show that spontaneous contractions may arise after electrically stimulated twitches in or near recently damaged regions of rat cardiac

trabeculae and that they propagate along the muscle. The spontaneous contractions and propagation thereof were induced by manoeuvers that load cardiac cells with Ca^{++} ions, such as lowering of the temperature, repetitive stimulation, and an increasing calcium concentration in the superfusion medium. Any of the above interventions also increased the amplitude and number of spontaneous contractions and caused an increase of the amplitude and velocity of the propagating contractions. Large propagating contractions caused a decrease of the force of the following electrically triggered twitch, and could themselves elicit twitches and arrhythmias.

Spontaneous Contractions in the Damaged Region

Asynchronous spontaneous contractile activity near the dissected ends of the ventricular attachment(s) of the trabeculae observed here and previously (ter Keurs et al., 1980; Krueger and Pollack, 1975) can be explained by assuming that calcium overload occurs in the damaged cells. Calcium overload has also been invoked as an explanation of the occurrence of spontaneous contractions within the cells of cardiac muscle preparations (Kort and Lakatta, 1984), such as we found after stabilization of the muscles at higher temperatures.

The spontaneous contractions disappeared largely in muscles at 25°C over the course of 1 h. This implies that calcium loading decreased because of decreased entry of Ca^{++} into the myocytes, which was probably a result of the closure of gap junctions between cells in the damaged region and cells that were killed by the dissection process. The balance between calcium extrusion and calcium influx in this region was clearly influenced by temperature since the properties of calcium overload were manifest at lowered temperature in a stable manner over many hours.

Apparently, electrically evoked twitches caused synchronization of oscillatory calcium release by the SR in overloaded cells in these regions, as was manifested by one or more synchronous contractions throughout the cross section of the damaged region; this was evidenced by microscopic observation and by laser diffraction measurement of sarcomere length in these regions. The interval period between the cycles of oscillatory contraction was ~660 ms, which would correspond to the period that has been observed in single cells (Capogrossi et al., 1986) and to the results found by Lakatta's group on spontaneous intensity fluctuations (SLIF) of light scattered by cardiac muscle of rat (Kort and Lakatta, 1984).

Why are spontaneous contractions in the damaged regions synchronized after the twitch even though the latter regions show only focal spontaneous activity during long pauses? Two possible mechanisms should be considered. Firstly, the action potential may trigger calcium release from the calcium-overloaded SR of each sarcomere that was not already engaged in the release process; this release is then followed by the same process that is responsible for recovery of force after the twitch (i.e., the mechanical restitution process; Ragnarsdottir et al., 1982). Therefore, calcium release from the sarcoplasmic reticulum of all sarcomeres will be virtually synchronized and the next release triggered by calcium overload can only occur after recovery of the release process has sufficiently lowered the release threshold. The strength of the coupling process can be judged from the occurrence of multiple regional spontaneous contractions. After longer intervals, however, coupling is not strong enough to allow more than oscillatory behavior at a subcellular level. How-

ever, the observation that the delay between the twitch and the first regional spontaneous contraction was different from the interval between the subsequent regional spontaneous contractions makes this hypothesis less likely.

Another possible source of synchronization is the large transient stretch of the damaged region during the stimulated twitch. Either the stretch itself or, more likely, the rapid release of the damaged end of the muscle at the end of the twitch, which occurs shortly before the spontaneous contraction, may cause synchronization of a calcium release. A possible mechanism may be that dissociation of calcium from the myofilaments accompanies rapid shortening (Housmans, 1983). The calcium release from the myofilaments could, then, act as a trigger for calcium release by the SR. The observation that the spontaneous contractions are elicited by strong twitches (Fig. 6) but not, or much less, by small twitches would be consistent with this hypothesis. It is, on the other hand, unlikely that transient stretch would be required for the second or third spontaneous contraction because the stretch due to the spontaneous propagated contractions was extremely small.

Propagated Contractions

Our results show that the coupling interval of the spontaneous contractions increases in proportion to the distance from the damaged region (Figs. 1 and 2). Furthermore, the velocity at which the spontaneous contraction moved and its amplitude were proportional to the number of stimuli in a train and to the calcium concentration in the medium (Figs. 3–5). The novelty of this observation is that the range of velocities extends to greater values than has been reported before. Golovina et al. (1986), using video techniques, have reported velocities up to 3 mm/s in isolated cells. Their technique allowed the measurement of propagation velocity up to ~3 mm/s. Hence, the results of their and of this study suggest that both phenomena reflect the same mechanism of propagation.

Several hypotheses can be considered as an explanation of this phenomenon. Stern et al. (1983) have postulated that the apparent motion may be caused by a progressive delay of the release of calcium from the SR as a result of a decrease of overloading of the SR at greater distance from the damaged end. Although we have chosen to study the phenomenon of propagated contractions under conditions that favor loading of the SR with calcium, we believe that the following observations plead against such a hypothesis. Firstly, in the case of multiple damaged regions, motion of the spontaneous contractions still occurred at a constant rate, i.e., contractions moved at constant velocities in opposite directions, collided, and then stopped (see Methods). No regions in the muscle were found in which contraction occurred synchronously. However, if we used interventions that are known to cause generalized calcium overload, such as at high $[Ca^{++}]_o$ combined with high stimulus frequencies or exposure to low Na^+ or low K^+ solutions (see Methods), synchronous aftercontractions were observed throughout the whole muscle. Secondly, the decrease of the velocity of motion of the second and third spontaneous contraction would put special and severe constraints on the assumption that calcium overload might be graded along the muscle. For example, the threefold variation of the coupling interval of the propagated contractions (Fig. 3) was clearly larger than the variation (±15%) that we could detect at the site of origin of the regional spontaneous contractions. If the apparent motion would result from a gradient of calcium

overload along the muscle and short coupling intervals would reflect a large calcium overload, then one would expect that lowering the Ca overload, as in the second spontaneous contraction, would increase the interval proportionally. This was clearly not the case. One might object that there could be a nonlinear relation between the coupling interval and the level of calcium loading. In that case, however, the observation of motion at a constant rate at all velocities should result from the fortuitous combination of the relation between coupling interval and loading along the muscle, and the relation between calcium loading and position along the muscle.

An alternative mechanism of propagation of the spontaneous contraction could be the conduction of a membrane potential change that would induce the contraction. Although, admittedly, we did not perform an electrophysiological study, the observed velocities (0.1–15 mm/s) make electrotonic conduction unlikely because the time constant of passive conduction along cardiac muscle is too short to explain

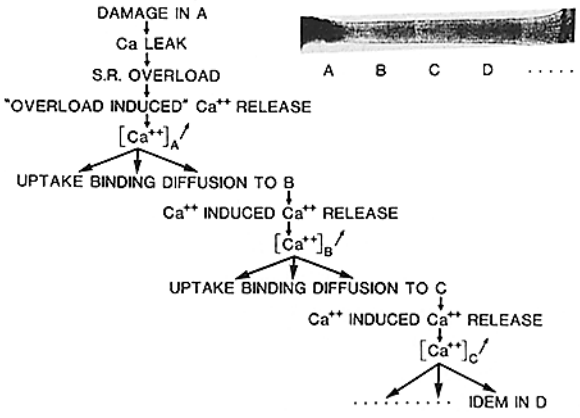

FIGURE 8. The proposed scheme of events that leads to propagation of spontaneous contractions in a trabecula. In or near the damaged end of the muscle (*A*), Ca^{++} overload of the SR occurs. As a result of the Ca^{++} overload of the SR oscillatory calcium release may occur spontaneously or upon an electrically stimulated twitch. The local rise of the calcium concentration in *A* causes diffusion of calcium ions toward *B*, where calcium-induced calcium release is elicited. Repetition of this process in *C*, *D*, and further down the muscle causes propagation of the calcium wave. The propagation velocity will be determined by the properties of the release process, Ca^{++} diffusion, and Ca^{++} binding and sequestration kinetics (see text for further explanation).

this range of velocities (Kaufman et al., 1963). Moreover, no report is known to us to describe a 100-fold variation of the time constant of electrotonic conduction with varied intra- or extracellular calcium levels.

Propagation of contraction as a consequence of stretch-induced calcium release (Pollack, 1974) was considered but we rejected this hypothesis as stretch of the muscles failed to change the velocity of movement of the waves of contractions.

The concept illustrated in Fig. 8 offers a satisfactory explanation for our observations. The process starts in the damaged region of cardiac muscle as a result of calcium overload of the SR, and the electrical and/or mechanical events during the stimulated twitch lead to spontaneous oscillatory calcium release. Calcium ions released during this process will evidently be bound to the binding sites in the cytosol and will diffuse into adjacent regions of the cell and into adjacent cells. The rise of the calcium concentration in the adjacent cell may then induce calcium

release by the adjacent SR. This process will be repeated in cells further down the muscle by calcium-induced calcium release.

The propagation characteristics of the spontaneous contractions depend in this model on three factors: (*a*) the initiating event, i.e., the magnitude and temporal characteristics of the damped oscillation of the calcium level in the calcium-overloaded damaged region of the muscle (see above); (*b*) the threshold for calcium-induced release, the amount of calcium released, and the rate of release by the SR in the central region of the muscle (Fabiato, 1985); and (*c*) the diffusion characteristics of calcium, which depend on diffusion through the cytosol and through gap junctions, together with binding to and dissociation from the cytosolic binding sites calmodulin and troponin (Cannell and Allen, 1984; Gillis et al., 1982).

Simulation of these properties in a model (Backx et al., 1989) in which we have incorporated these properties in so far as they are known, indeed yield propagation velocities that range from 0.1 to 25 mm/s. Two variables influence the properties of the model strongly: firstly, the diastolic calcium level determines the amount of calcium that is accumulated by the SR, and thereby controls the amount of calcium that is released and the rate of release. The diastolic calcium level also dictates the level of saturation of the calcium binding sites and hence influences the rate of rise of the calcium concentration near the adjacent release sites on the membrane of the SR.

The observed dependence of the propagation velocity of the contractions on the number of stimuli in a train and on the $[Ca^{++}]_o$ in this study is consistent with the model since both interventions influence the free calcium concentration in the cytosol. Furthermore, the model would predict that the duration of contractile activity during the spontaneous contraction is comparable to that of the normal contraction. This is in agreement with the observation in Fig. 3 that with rapid propagating waves, in which the observed sarcomere shortening transient is not protracted by travel of the shortening wave across the laser beam, the shortening wave of the twitch lasts as long as that of the spontaneous contraction.

Force Development during the Spontaneous Contractions

The limited extent of the waves explains partially why the concomitant force was small. The contracting sarcomeres shortened during the spontaneous contraction at the expense of a much longer series elastic element than during the twitch, thus developing less force. The observation that force development concurrent with the spontaneous contraction lasted as long as the propagation (Figs. 2 and 3) is in agreement with this explanation. If the duration of spontaneous contractions would change little while their propagation velocity increased (Backx et al., 1989) the spatial extent of the contraction would increase and hence the concomitant force development should increase, which is indeed the case (Fig. 3). Furthermore, the force developed during the spontaneous contraction must have depended on the level of activation, which must have been lower than that of the regular twitches for at least two reasons. Firstly, the spontaneous contractions occur shortly after the regular twitch at a moment in which the process of mechanical restitution is "incomplete," hence the amount of calcium released by the SR is presumed to be less than at the longer intervals (Ragnarsdottir, 1982; Bers, 1985). Secondly, the amount of calcium

released should be less than following a trigger by the action potential because the amount released depends on the rate of rise of the calcium concentration that triggers the release (Fabiato, 1985). The latter is evidently lower in a spontaneously propagated contraction than during the stimulated contraction.

The Negative Inotropic Effect of Spontaneous Contractions

The results shown in Fig. 7 of this study and those of other studies (Allen et al., 1985) have shown that a spontaneous contraction depresses the force developed during a subsequent twitch. This behavior is consistent with current models of excitation-contraction coupling (Wohlfart and Noble, 1982; Fabiato, 1983; Morad and Cleeman, 1987; Schouten et al., 1987) and the assumption that the spontaneous contraction is induced by calcium release from the SR without accompanying calcium entry from the extracellular space. The force of a contraction depends on the amount of calcium released by the SR. After release, the SR is replenished with the calcium that has entered the cell during the action potential and a fraction of the calcium that returns from the contractile filaments (Wohlfart and Noble, 1982; Morad and Cleeman, 1987; Schouten et al., 1987). Calcium release, and thus force development, of each contraction are then proportional to the amount of calcium released during the previous contraction and the amount of calcium that has entered during the preceding action potential. Moreover, many studies have provided evidence that there exists a negative feedback between the force of contraction and the amount of calcium that enters during the action potential of the same twitch (Wohlfart and Noble, 1982). Small twitches are therefore accompanied by a larger calcium entry. It follows that if a spontaneous contraction is not accompanied by calcium entry from the extracellular space, a substantial fraction of the calcium that is released during the spontaneous contraction will leave the cell through extrusion mechanisms in the membrane. Thus, any contraction that occurs after a spontaneous contraction will be reduced in inverse proportion to the amplitude of the spontaneous contraction.

Spontaneous Contractions and Arrhythmias

Force of the spontaneous contraction was generated as long as the wave of sarcomere shortening traveled along the muscles. This contrasted the time-course of the spontaneous twitches that were observed under conditions of a high calcium load (Fig. 7 *A*). This observation is consistent with the assumption that the spontaneous twitch would be elicited by a transient depolarization that leads to an action potential which we have observed (unpublished observations) and has been described in other studies (Aronson, 1981; Hiraoka et al., 1981). We frequently found that spontaneous arrhythmias occur under these circumstances as has been described previously (Cranefield, 1977; Karagueuzian and Katzung, 1982; Eisner and Lederer, 1979; Kass and Tsien, 1982; di Gennaro et al., 1983).

Kass and Tsien (1982) postulated that spontaneous aftercontractions as a result of calcium oscillations in the presence of elevated cytosolic calcium concentration underlie the delayed transient inward current and depolarization (Kass et al., 1978; Matsuda et al., 1982), which may evoke action potentials. Calcium influx during these action potentials may again add to the calcium load such that the sequence

calcium release depolarization and action potential, is perpetuated in a triggered arrhythmia. The transient inward current and depolarization are known to take place (Ferrier, 1976) with a long (i.e., 300–1,000 ms) coupling interval after the last electrically stimulated contraction. Transient depolarizations (Mary-Rabine et al., 1980) have been described occasionally in normal fibers but usually occur under a variety of conditions that lead to elevated cytosolic calcium concentrations such as exposure to digitalis glucosides, catecholamines, high $[Ca^{++}]_o$, or low $[K^+]_o$ or $[Na^+]_o$ (Wit and Rosen, 1986).

The termination of the arrhythmias is of particular interest (Fig. 7 *B*). Interval time increased between the twitches exponentially from 750 to 2,500 ms before termination of the tachycardia. This is consistent with the hypothesis that (Fig. 8) the spontaneous contraction that leads to the synchronized twitch starts at the damaged end of the preparation, then travels through the preparation, and when it reaches a region with a low threshold for action potential generation an action potential is elicited as a result of the concomitant transient depolarization. The interval between spontaneous twitches should depend on both the threshold for action potential generation and on the propagation velocity of the spontaneous contraction, which depends on the intracellular calcium concentration.

These observations suggest that propagating spontaneous contractions in calcium-loaded cells are intimately linked with arrhythmias of cardiac muscle; this may be of clinical importance. The results suggest that recent focal damage of myocardium such as in a heart with a recent myocardial infarction may lead to the development of triggered arrhythmias.

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