

Spontaneous Contractions in Rat Cardiac Trabeculae

Trigger Mechanism and Propagation Velocity

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ABSTRACT It has previously been observed that spontaneous contractions start in a region of damage of isolated right ventricular trabeculae of rat, propagate along the muscle, and induce triggered arrhythmias (Mulder, B.J.M., P.P. de Tombe, and H.E.D.J. ter Keurs. 1989. *J. Gen. Physiol.* 93:943–961). The present study was designed to analyze the mechanisms that lead to triggered propagated contractions (TPCs). TPCs were elicited in 29 trabeculae by stimulation with trains (2 Hz; 15-s intervals) at varied number of stimuli (n), lowered temperature (19–21°C), and varied $[Ca^{++}]_o$ (1.5–4 mM) in the superfusate. Length (SL) and shortening of sarcomeres in the muscle were measured at two sites using laser diffraction techniques; twitch force (F_i) was measured with a silicon strain gauge. Time between the last stimulus in the train and the onset of sarcomere shortening due to a TPC at a site close to the damaged end region (latency) and propagation velocity of the contraction (V_{prop}) were correlated with F_i . For 10 trabeculae, TPCs were calculated to start in the end region itself 586 ± 28 ms (mean \pm 1 SEM) after the last stimulus of a train ($n = 15$; $[Ca^{++}]_o$: 1.5 mM), i.e., at the end of or after the rapid release of the damaged end during twitch relaxation. When F_i was increased by increasing either SL prior to stimulation or the afterload during twitches, methods that do not affect intracellular calcium levels, latency decreased, but V_{prop} remained constant. No TPC occurred when F_i was $<20\%$ of maximal F_i . Both increasing $[Ca^{++}]_o$ and n increased F_i to a maximum, increased V_{prop} progressively (maximum V_{prop} , 17 mm/s), but decreased latency. These observations suggest that initiation of TPCs depends on the force developed by the preceding twitch, and therefore on the degree of stretch and subsequent rapid release of damaged areas in the myocardium, while V_{prop} along the trabeculae is determined by intracellular calcium concentration.

INTRODUCTION

Cardiac ventricular arrhythmias are an important cause of mortality and morbidity in humans. The role of reentry mechanisms in (tachy)arrhythmias is well recognized,

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but the contribution of alternative mechanisms of arrhythmogenicity such as involved in triggered arrhythmias is still under study (for review: January and Fozzard, 1988). Triggered arrhythmias were originally described in isolated cardiac muscle as spontaneous contractions following a series of stimuli (Bozler, 1943), and were therefore termed triggered contractions or aftercontractions (Cranefield, 1977). Several observations link these contractions to other manifestations of spontaneous contractile activity in cardiac muscle. Single myocytes, both with (Capogrossi and Lakatta, 1985; Kort et al., 1985) and without intact sarcolemma (Fabiato and Fabiato, 1972; Rieser et al., 1979; Chiesi et al., 1981; Fabiato, 1985), exhibit myofilament activation in the unstimulated state. This activation is thought to result from spontaneous release of calcium ions from the sarcoplasmic reticulum into the myoplasm (Fabiato and Fabiato, 1972; Chiesi et al., 1981; Stern et al., 1983; Fabiato, 1985), and is enhanced by mechanisms that increase intracellular calcium concentration $[Ca^{++}]_i$ (Fabiato and Fabiato, 1972; Rieser et al., 1979; Chiesi et al., 1981; Capogrossi and Lakatta, 1985; Kort et al., 1985). In fact, the spontaneous release of calcium from the sarcoplasmic reticulum occurs locally rather than evenly throughout the cell and causes focal contractions that subsequently propagate within the cell (Fabiato and Fabiato, 1972; Rieser et al., 1979; Chiesi et al., 1981; Capogrossi and Lakatta, 1985; Fabiato, 1985; Kort et al., 1985; Golovina et al., 1986; Wier et al., 1987). This wave-like character is thought to be consistent with a model of calcium diffusing down its concentration gradient, combined with a resulting calcium-induced calcium release from the adjacent sarcoplasmic reticulum (Fabiato and Fabiato, 1972; Rieser et al., 1979; Stern et al., 1984; Golovina et al., 1986; Mulder et al., 1989; Backx et al., 1989). With increasing $[Ca^{++}]_i$, frequency and velocity of propagation of spontaneous contractions within the cell increase (Fabiato and Fabiato, 1972; Rieser et al., 1979; Capogrossi and Lakatta, 1985; Fabiato, 1985) and eventually lead to synchronization of focal contractions (Stern et al., 1983; Capogrossi and Lakatta, 1985; Capogrossi et al., 1987). Similar phenomena have been observed in multicellular preparations; random contractions in "resting" papillary muscle which cause fluctuations in intensity of scattered light were described by Lakatta's group (e.g., Lappe and Lakatta, 1980), while microscopical observations showed wave-like motions (Stern et al., 1983; Kort et al., 1985). Also, unstimulated atrial trabeculae (Glitsch and Pott, 1975) and ventricular muscle preparations (Allen et al., 1984) appeared to exhibit spontaneous tension oscillations. Recently, spontaneous propagated contractions have been observed in stimulated rat right ventricular trabeculae (Mulder et al., 1989) that appeared to start in the damaged end of the muscle and that developed into highly synchronized contractions when calcium concentration of the superfusate ($[Ca^{++}]_o$) was raised. Force development during the propagated contractions was comparable to the previously described aftercontractions (Mulder et al., 1989).

In the present study we examined the mechanisms underlying the initiation and propagation of spontaneous contractions in rat right ventricular trabeculae in more detail. Triggered propagated contractions (TPCs) appeared to start at the end of or after the relaxation phase of the stimulated twitch. We hypothesized that initiation of TPCs might be related to stretch of the damaged end regions of the muscle (which form part of the series elastic element), while propagation results from

another mechanism so that the velocity of propagation of the contraction (V_{prop}) itself is independent of stretch.

Cardiac muscle preparations have been shown to consist of a central region in which normal cells contract uniformly, and a region of damaged cells near the end of the muscle (Krueger and Pollack, 1975; ter Keurs et al., 1980). The damage results from dissection and mounting (Krueger and Pollack, 1975; ter Keurs et al., 1980; Mulder et al., 1989). When the muscle is stimulated, the cells in the central regions contract and, in turn, stretch the damaged region (Krueger and Pollack, 1975; ter Keurs et al., 1980; Mulder et al., 1989). Residual contractile activity together with the elasticity of the damaged region (the series elastic element) will allow this region to transmit force to a transducer or to an attached load. The magnitude of this force obviously is determined by the degree of stretch of the damaged end (Brady, 1979) and can be modulated by several interventions. An increase in $[\text{Ca}^{++}]_i$ will enhance contractile force of the normal cells and hence induce increased stretch of the damaged region during contraction at constant muscle length (Mulder et al., 1989). Stretch of the muscle will increase force generated by the central sarcomeres (Kentish et al., 1986) and thus increase both the length of the damaged region before stimulation and its stretch during contraction at constant muscle length. By controlling the load during contraction it is possible to modulate the behavior of the damaged end region at a constant inotropic state and at constant initial sarcomere length.

In the present study we tested whether generation of TPCs depends on the $[\text{Ca}^{++}]_i$, the sarcomere length (SL) in the central region of the muscle, or on the force transmitted by stretch of the damaged end during the twitch preceding the TPC. We therefore modulated the force of twitches that preceded a TPC by varying muscle length in order to vary SL, by controlling afterload at constant initial SL, and by influencing $[\text{Ca}^{++}]_i$. Latency of initiation and velocity of the TPCs were studied and related to force of the last stimulated twitch (F_i).

The results of our study are compatible with the hypothesis and suggest that stretch of the damaged end region and the subsequent release during relaxation trigger a contraction that subsequently propagates. The triggering process is modified by $[\text{Ca}^{++}]_i$, but the propagation characteristics of the contraction are independent of stretch, and seem to be dictated by $[\text{Ca}^{++}]_i$ alone, suggesting that triggering and propagation are separate, albeit linked, events. Our findings are consistent with the hypothesis that focal sarcoplasmic reticular calcium release occurs as a result of calcium diffusion along the preparation, mediating a calcium-induced calcium release, and causing propagation of the triggered contraction.

METHODS

Dissection and Mounting of the Preparation

Cardiac muscle preparations were dissected from Wistar rats of either sex and mounted in the experimental setup as described before (Daniels et al., 1984). In summary, trabeculae running between the free wall of the right ventricle and the atrioventricular ring were selected and after careful dissection under a microscope, while perfused with a modified Krebs-Henseleit solution, were mounted horizontally in a bath and covered with a glass slide.

The ventricular side was attached to a silicon strain gauge force transducer and the valve was attached to a hook. Usually a remnant of the ventricle was connected to the valvular tissue. Both attaching devices were controlled by micromanipulators (Narishige, Tokyo, Japan) to change muscle and sarcomere length. Preferentially long and thin trabeculae were used; the length varied between 1.46 and 3.28 mm, width between 45 and 173 μm , and thickness between 25 and 70 μm . The solutions used during dissection and experiments contained (in millimolar): 147.9 Na^+ , 5.0 K^+ , 127.5 Cl^- , 1.2 Mg^{++} , 2.0 H_2PO_4^- , 1.2 SO_4^- , 19.0 HCO_3^- , 11.0 glucose, CaCl_2 was varied as specified below. The solutions were equilibrated with 95% O_2 and 5% CO_2 , yielding a pH of 7.4 at both 25 and 20°C. During the experiments the temperature of the fluid in the bath was measured and controlled.

Experimental Apparatus

The muscle was observed using an inverted microscope (Nikon) and a video system (Panasonic camera WV 3170 and recorder AG 2400). The preparation was stimulated through two parallel platinum wires in the bath with pulses (5 ms; 20% above threshold) from a stimulator (SD9; Grass Instrument Co., Quincy, MA) that was triggered by a computer (IBM PC-AT). Laser diffraction techniques were used (Daniels et al., 1984) to measure sarcomere length in the illuminated area (He-Ne laser, cross section of the beam 350 μm). Force and SL were displayed on a storage oscilloscope (Hitachi V134), recorded with a chart recorder (2800S; Gould Inc., Oxnard, CA) and sampled via an analog to digital converter (Data Translation DT 2801A) installed in the computer. Force and SL recordings were stored on hard-disk for later processing, or plotted with a laser plotter (LN 03; Digital Equipment Corp., Marlboro, MA); the computer program for data analysis allowed display of F_t and SL recordings of the last stimulated twitch and a subsequent period of time on a graphics monitor (model CGA; IBM). In experiments in which the afterload was controlled by releasing the muscle the valvular side was attached to the arm of a servomotor. In between and after the series of stimuli the muscle was restretched to resting length, keeping initial SL constant. Because a minimal amount of force was required to achieve stable feedback control, in three additional experiments the afterload was varied using a muscle length feedback system instead of force feedback, allowing for (quick) releases of the muscle to a previously set length, starting just before and lasting during the time course of each twitch.

Stimulus Protocol

Muscle length was set at a point where passive force was 5% of active twitch force at a bath fluid temperature of 25°C and a $[\text{Ca}^{++}]_o$ of 1.0 mM. Subsequently the temperature was lowered to 19–21°C, which has been reported to prolong the period in which the properties of TPCs remain stable (Mulder et al., 1989). If under these circumstances 15 stimuli at a rate of 2 Hz interspersed with a 15-s rest period failed to produce a TPC after the last twitch, $[\text{Ca}^{++}]_o$ was increased in 0.25 mM steps up to 4.0 mM until TPCs were elicited, which usually occurred at a concentration of 1.5–2.5 mM. Muscles were studied in the first hour after mounting only, since the TPCs appeared to behave most constantly in this period (Mulder et al., 1989). The muscle was moved with respect to the laser beam (see below), and SL or afterload was changed in the 15-s rest interval without interrupting the stimulus protocol. When time was needed to allow the muscle to achieve a steady state in a different $[\text{Ca}^{++}]_o$, or to adapt the computer program to deliver a different number of stimuli (n), the preparation was stimulated at a rate of 0.5 Hz. After such a period data obtained from TPCs after the first two stimulus trains were rejected.

Measurements

Measurements were made from the stored computer wave forms of force and SL. Figs. 1 and 5 A show such a display depicting the last stimulated twitch from the precipitating stimulus train and the subsequent TPC at different sites of the preparation. Due to violent "random" motion, which always resulted from damage, it was not possible to reliably measure sarcomere movements in the end regions, where TPCs invariably started. Moreover, stretch of the damaged area (see Fig. 1) led to substantial longitudinal translation of the observed region along the laser beam. This translation precluded accurate measurement of local sarcomere length. Therefore, we studied an area as close as possible to the site of origin of the TPC where the diffraction pattern still could be interpreted (a distance of 285–570 μm from the attached site of the preparation) and regarded the delay (latency; Figs. 1 and 5 A) between the stimulus of the last twitch and the occurrence of sarcomere shortening due to a TPC at this site representative of the time needed to initiate the contraction. This is a valid assumption because the propagation velocity of the TPC along the muscle has previously been shown to be constant (Mulder et al., 1989). For 10 muscles the actual time of start of the TPC in the damaged end

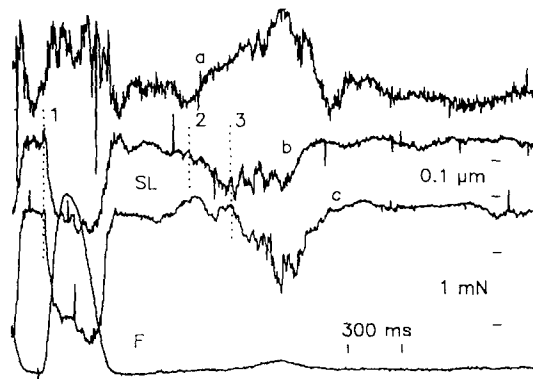


FIGURE 1. Force and SL tracings at three sites of a muscle. The SL tracings were artificially shifted; resting SL was 2.05 μm . Site *a* is located in the damaged end region of the muscle, near the site of origin of the TPC; site *b* and *c* are situated at 0.88 and 1.72 mm from site *a*, respectively. The last stimulated twitch of a series of 15 rapid stimulations is followed by a TPC, reaching site *b* before site *c* and thus

traveling away from site *a*. Both during the twitch and the TPC the damaged area is stretched, suggesting that the TPC started between site *a* and *b*. The interval 1–2 is representative of the latency; the time difference between 2 and 3 allowed calculation of V_{prop} . Temperature, 21°C; $[\text{Ca}^{++}]_o = 1.75 \text{ mM}$.

region was approximated from the known V_{prop} , latency, and the distance between the site where latency was measured and the damaged region, using both the distance to the attachment site of the preparation (x_1) and the distance to the transition from damaged to healthy myocardium (x_2). For this purpose, damaged myocardium was defined as muscular tissue not contracting during a twitch, as judged from video monitoring. To improve the accuracy of these estimates only muscles with small damaged areas ($x_1 < 350 \mu\text{m}$) were selected for these calculations (Fig. 5 A).

V_{prop} was derived from the difference in delay between sarcomere shortening transients of the TPC in two areas and the distance between them, measured by means of a linear potentiometer (Figs. 1 and 5 A). Using time to onset of sarcomere shortening or time to peak sarcomere shortening of the TPC yielded similar values of latency and V_{prop} . The last stimulus in the preceding train appeared to be the most reliable reference for measurements of differences in time of arrival of a TPC. Before another area was studied the measurement was repeated twice. Data were accepted only if force tracings taken during measurements at the

different sites were identical and the measured time of arrival of the TPC obtained from the two consecutive measurements at one spot did not differ by >10%.

Statistical Analysis

For all experiments, the mean effects of the interventions on twitch force, latency, and V_{prop} are graphically represented in the figures, together with typical results of corresponding individual experiments. The grouped data show close to linear relationships for all interventions in the ranges studied; however, this did not necessarily reflect the behavior of individual experiments. We therefore used analysis of variance to test for significant differences ($P < 0.05$) in the ranges studied for all muscles. Because each parameter varied among individual experiments, the analysis of variance was performed on relative values, with reference to the "standard" condition (2.0 mM $[Ca^{++}]_o$, 15 stimuli 2.15 μ m SL, and fully afterloaded). In addition, and more specifically, Spearman rank analysis was performed (Snedecor and Cochran, 1980) on the relationships between F_t and V_{prop} when SL or the afterload was changed to test the null hypothesis that no statistically significant relationship between the variables existed.

RESULTS

Immediately after mounting, small spontaneous sarcomere movements, reflected by diastolic fluctuations in the position of the first order of the diffraction pattern, were visible in the end regions of the muscle preparation. Decrease of the temperature of the superfusate caused this spontaneous activity to become visible in the central portions of the trabecula as well and facilitated induction of TPCs, which could be induced in all trabeculae, although sometimes only if $[Ca^{++}]_o$ was raised to 4.0 mM. As was clear from direct microscopical observation and from the SL measurements, the TPCs usually traveled from the valvular to the muscular attachment of the muscle preparation, unless there was clear evidence of damage near the muscular end (e.g., cut branches) or if the muscle was not attached to a remnant of the ventricle at the valvular side. Direct microscopical observation revealed that the end regions were stretched during the twitch, as has been described previously (Mulder et al., 1989). This stretch was reflected in SL measurements from these regions (Fig. 1). TPCs in general started from the transition zone between damaged and healthy myocardium. Sometimes the direction of the TPC reversed when $[Ca^{++}]_o$ or n were increased (data obtained from these reversed contractions were rejected). Fig. 5 A shows that force development of the TPC lasted as long as it took the contraction to travel along the preparation.

Time of Initiation of Propagated Contractions

The actual initiation of the TPC in the end region was calculated in 10 muscles (see Methods; $[Ca^{++}]_o = 1.5$ mM and $n = 15$ for all muscles) to occur between 586 ± 29 ms (assuming a start at the attachment site, distance x_1) and 641 ± 24 ms (based on a start at the transition from damaged to apparently healthy tissue, distance x_2 ; values as mean ± 1 SEM) after the last stimulus. The observations described above favor the value based on distance x_2 as the more accurate. Peak force of the last twitch and 50% relaxation of SL at the measuring site close to the end region occurred at 185 ± 6 ms and 435 ± 26 ms after the last stimulus, respectively (mean ± 1 SEM). Initiation of the TPC in the end region of each muscle took place

late during or after relaxation. The calculated actual time of initiation of TPCs in one muscle is indicated in Fig. 5 A.

Effects of Changing Initial Sarcomere Length

The response of a single representative preparation to varied SL, which was set before the series of stimuli was delivered, is illustrated in Fig. 2. Fig. 2 A demon-

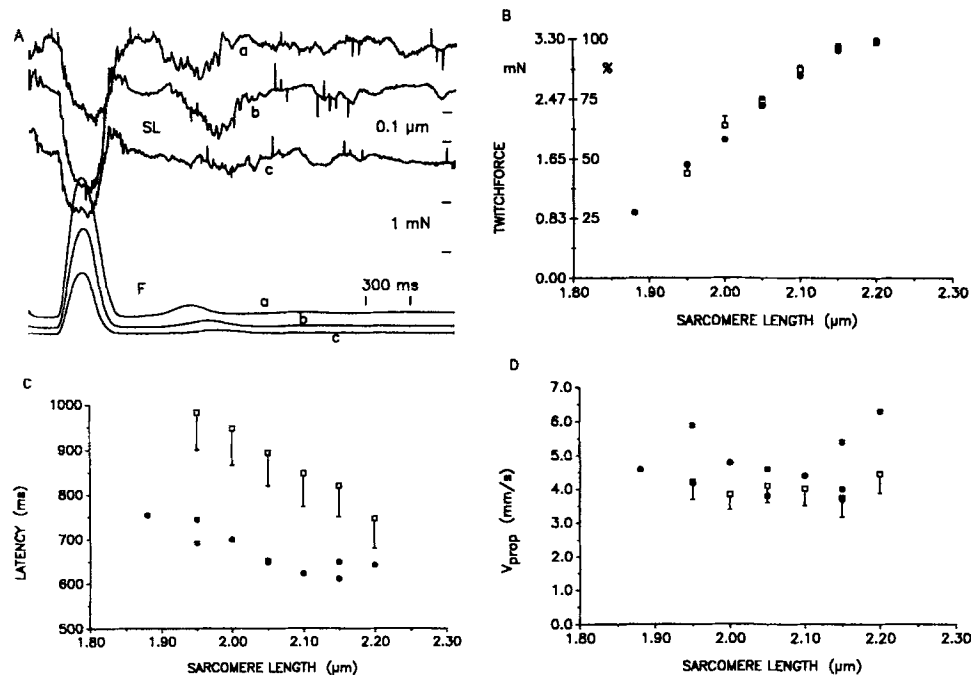


FIGURE 2. Effects of varied initial SL on TPCs. In A, F and SL tracings from a typical experiment at the same measuring site are shown. Initial SL was 2.05, 2.0, and 1.95 μm for a , b , and c , respectively. The SL tracings were artificially shifted, but the F tracings were superimposed, showing an increase in both active and passive force with increasing SL. For the same muscle the relationships between SL and F_t (B), latency (C), and V_{prop} (D) are given (filled circles). The Spearman rank correlation coefficient for the data points in D is 0.08 ($P > 0.50$). $[\text{Ca}^{++}]_0 = 4.0$ mM. The open squares represent the mean values \pm SEM for all muscles tested. In B this is expressed as the percentage of maximum force observed in a muscle. The changes of force and latency with SL were statistically significant ($P < 0.05$).

strates that the amplitude of the TPC always decreased, and became hardly detectable, when SL was lowered to or below slack length. Increasing SL (stretching the muscle) led to an increase in F_t (Fig. 2 B). This decreased latency progressively (Fig. 2 C), whereas V_{prop} remained constant (Fig. 2 D). All seven muscles studied behaved the same. A significant relation between SL and V_{prop} is unlikely: P values were >0.10 for two muscles, >0.20 for two other trabeculae, and >0.50 for three preparations (Spearman rank test; see Methods).

Effects of Changing Afterloads

Seven other muscles were allowed only to develop a preset force during all the triggering twitches, starting from constant SL. In three of these muscles additional stimulus strains were imposed of which only the last twitch was afterload-controlled. The results obtained with these two methods were the same (data not shown). Force

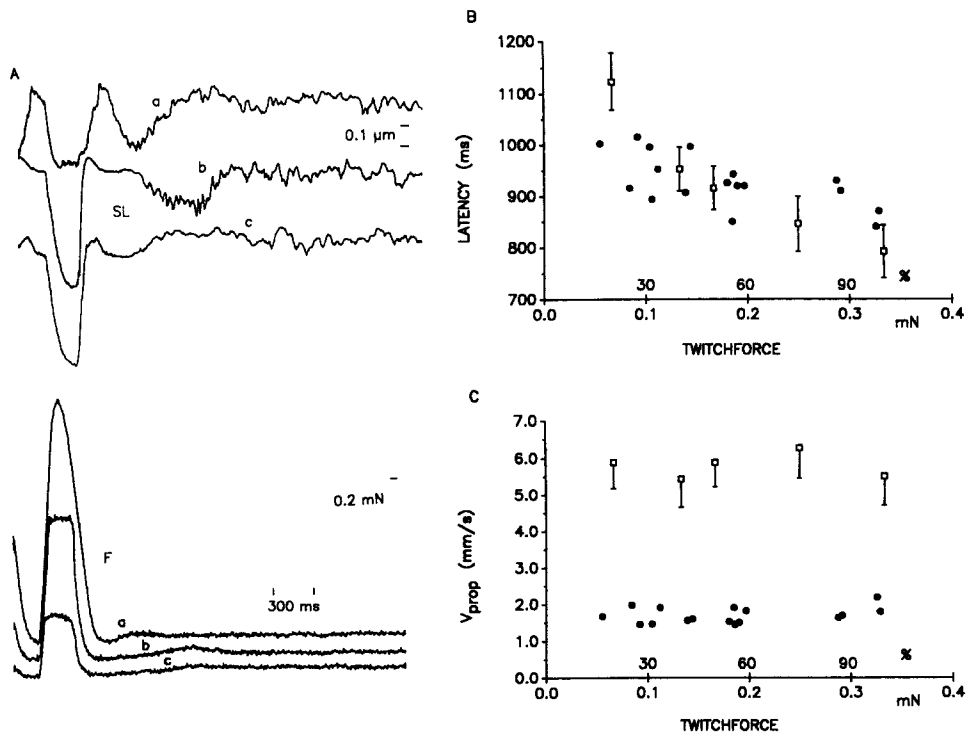


FIGURE 3. SL and F recordings of the last of the triggering twitches and a TPC at different afterloads (A, top and bottom panels, respectively). All recordings are from one site of the preparation. Both SL and F tracings were artificially shifted. With decreasing afterload the TPC is delayed (B, filled circles) and eventually becomes hardly detectable (tracing c in A). V_{prop} remained constant as long as the TPC was present (C, filled circles; Spearman rank correlation coefficient 0.10, $P > 0.50$). $[Ca^{++}]_o = 2.50$ mM. SL, 2.15 μ m. The open squares in B and C represent mean \pm SEM for all muscles tested; force is expressed as a percentage of maximum force observed in each muscle. The change of latency with force was statistically significant ($P < 0.05$).

and SL recordings of a typical experiment, in which all twitches of the train were afterloaded, are shown in Fig. 3. It can be seen that for this example and for the group of muscles, latency increased progressively with decreasing afterload (Fig. 3, A and B). TPCs virtually disappeared at low afterload in most preparations; in two muscles TPCs were still visible at the lowest afterload. In three additional experiments afterload was made zero by release of the muscle just before each stimulated twitch; this abolished the TPCs. In contrast, if a TPC was elicited, it propagated

along the trabeculae at a velocity that appeared to be independent of the level of afterload (Fig. 3 C). This was confirmed by Spearman rank analysis (P values >0.20 in two muscles and >0.50 in five preparations).

Effects of Changing $[Ca^{++}]_o$

Increasing $[Ca^{++}]_o$ increased the frequency of spontaneous sarcomere contractions in individual cells, as judged from microscopical observation. A typical response of F_i to varied $[Ca^{++}]_o$ is shown in Fig. 4 A. Fig. 4 also shows that increasing $[Ca^{++}]_o$ decreased latency progressively (Fig. 4 B); and increased V_{prop} (Fig. 4 C); V_{prop} for the four muscles studied varied between 1.25 and 16 mm/s. Spontaneous contractions that resembled the electrically stimulated twitches in their duration and fast upstroke of force and which were synchronous throughout the preparations (spon-

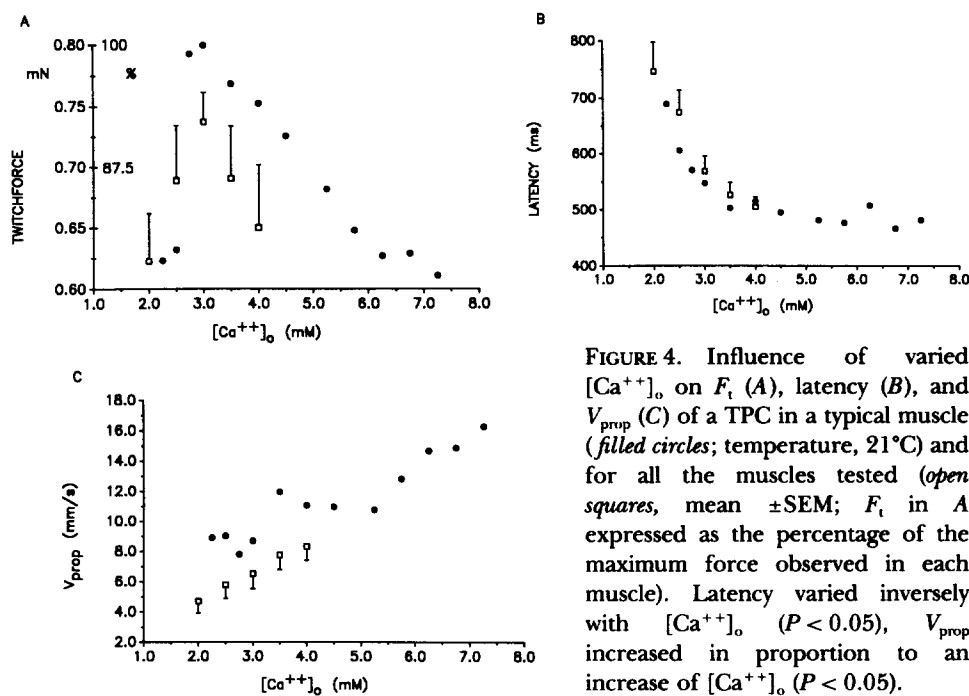


FIGURE 4. Influence of varied $[Ca^{++}]_o$ on F_i (A), latency (B), and V_{prop} (C) of a TPC in a typical muscle (filled circles; temperature, 21°C) and for all the muscles tested (open squares, mean \pm SEM; F_i in A expressed as the percentage of the maximum force observed in each muscle). Latency varied inversely with $[Ca^{++}]_o$ ($P < 0.05$), V_{prop} increased in proportion to an increase of $[Ca^{++}]_o$ ($P < 0.05$).

taneous twitches, Mulder et al., 1989) occurred above a critical $[Ca^{++}]_o$, the value of which was different for each muscle studied.

Effects of Changing the Number of Preceding Stimuli

The effects of varying n were similar to those of changing $[Ca^{++}]_o$. Contractile force of the electrically evoked twitches in eight muscles initially increased with increasing n and then decreased. Again, spontaneous twitches could be elicited by sufficiently increasing n . In three muscles F_i started to decrease at a high number of stimuli which also induced spontaneous twitches. In contrast to increasing $[Ca^{++}]_o$, spontaneous activity between stimuli, and at the end of the resting period, did not change with varied n . Fig. 5 A illustrates the effects of increasing n in a muscle that showed

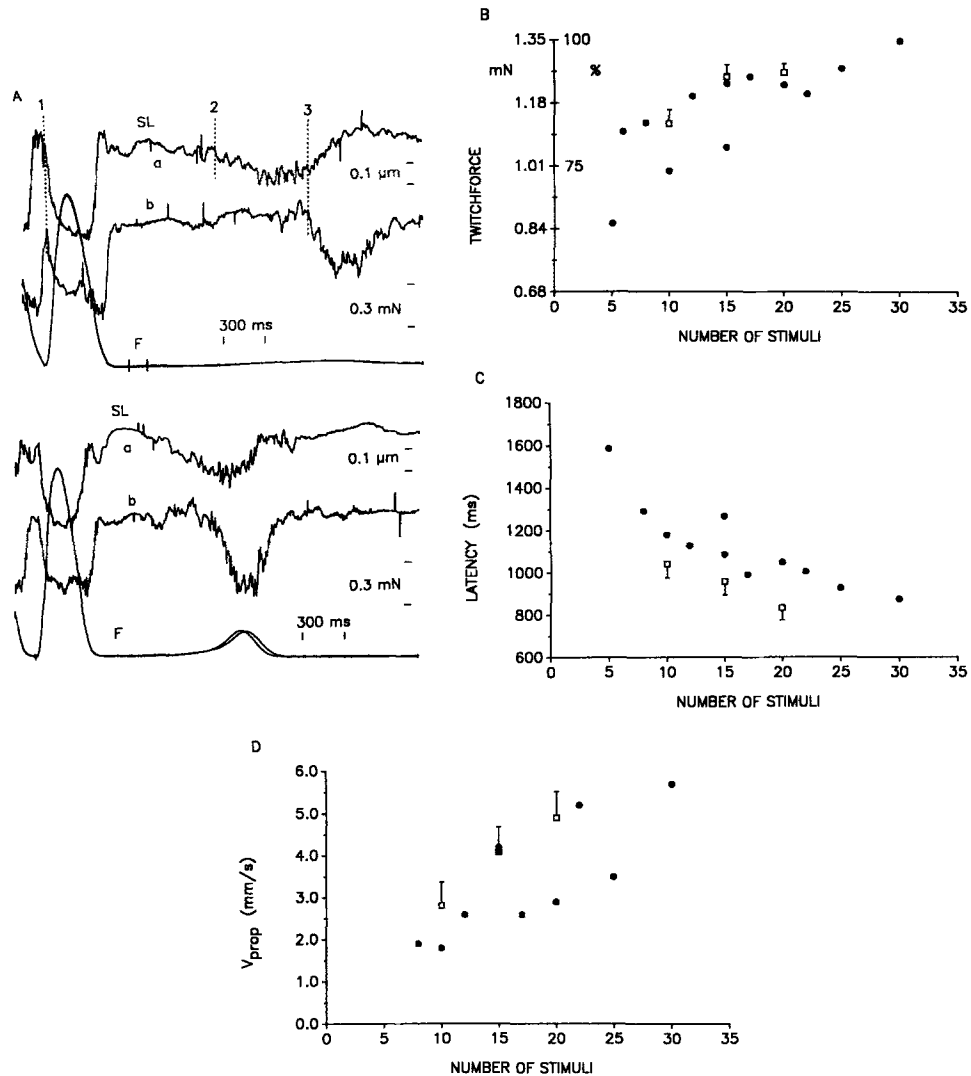


FIGURE 5. Effects of changing n on F_i (B), latency (C), and V_{prop} (D) in one preparation (filled circles; $[\text{Ca}^{++}]_o$ 1.75 mM; SL, 2.15 μm) and for all muscles tested (open squares, mean \pm SEM; F_i in *b* expressed as percentage of the maximum force observed in each muscle). The changes of latency and V_{prop} with the number of stimuli were significant ($P < 0.05$). In A, *F* and SL tracings are shown after 8 (*top*) and 25 (*bottom*) stimuli. Force tracings were superimposed, SL tracings were artificially shifted. The SL tracings are derived from a site close (A) to the origin of the TPC and from a site (B) at 1.23 mm from A. The interval 1-2 is the measured latency; V_{prop} was calculated from the interval 2-3. The vertical bars in the force tracing refer to the calculated times of origin of the TPC in the end region (610-740 ms after the last stimulus; see text for further explanation).

progressive increase of F_i (Fig. 5 B), decrease of latency (Fig. 5 C), and increase of V_{prop} with increase of the duration of the train; at 30 stimuli, spontaneous twitches developed. The results obtained for all preparations together are listed in the corresponding figures. The maximum V_{prop} observed in these muscles was 17 mm/s.

DISCUSSION

The results of this study show that two factors are essential to generation of propagated contractions. Firstly, propagated contractions occurred only if, during the preceding twitch, force was developed, indicating that the propagating contraction was actually triggered by an event during the twitch. We will term these contractions henceforth triggered propagated contractions (TPCs). Secondly, increase in the number of preceding twitches or in $[Ca^{++}]_i$ reduced the delay of TPCs irrespective of the magnitude and direction of the concomitant change of twitch force. The latter observation suggests that the occurrence of TPCs was facilitated by elevation of the intracellular calcium concentration.

It is well known that sarcomeres in the central region of cardiac muscle preparations in vitro shorten at the expense of a series elastic element during contractions in which muscle length is held constant (Krueger and Pollack, 1975; ter Keurs et al., 1980; Fig. 1). The series elastic element in our preparations is composed of valvular tissue attached to one end and damaged muscle (supposedly due to dissection and mounting) at both ends of the trabecula. It was observed that TPCs initiated in one of these end regions, at the transition from damaged to apparently healthy tissue. Although the TPC originated from the valvular side in most preparations, it did so, in this study, only if a remnant of ventricular tissue was present at this side (see also Mulder et al., 1989). The distance between the edge of the cut end of the remnant of the right ventricular free wall and the area of insertion of the trabecula was always smaller at the valvular side of the muscle than at the other side. It is likely, therefore, that damage due to dissection invades the trabecula to a larger extent at the valvular side. In conditions of clear damage to the area near the right ventricular free wall, TPCs started from this end region. Hence, a role of the valvular tissue in initiating a TPC is unlikely.

Although direct measurement of SL in the end region from which the TPCs started was not possible, we calculated the TPC to be triggered during or after the late phase of relaxation of the last stimulated twitch. This relates either to relaxation of undamaged sarcomeres or to release of damaged areas that were stretched during the twitch. To test the hypothesis that stretch and subsequent release of the damaged end regions is a main factor determining the initiation of TPCs we performed experiments aimed at controlling this stretch. The force developed by the preparation during a twitch is generally assumed to reflect the extent of stretch of the end regions (Brady, 1979). Therefore, interventions were used that alter the contractile force of stimulated twitches that precede a TPC. With decreasing SL or afterload, F_i decreased and time to onset of the TPC (latency) increased (Figs. 2 and 3). Concomitantly, sarcomeres in the central part of the muscle shortened to a greater extent (Figs. 2 A and 3 A). Below slack length or at low afterloads hardly any or no TPCs were observed (Figs. 2 A and 3 A). This indicates that a minimum F_i is

required to induce a TPC and suggests that sarcomere shortening and subsequent relaxation in undamaged regions does not trigger TPCs.

The "threshold" F_t required to elicit TPCs differed for each preparation; in the afterload-controlled experiments TPCs started to disappear below a F_t of 20% of maximum F_t , and were abolished with totally unloaded contractions in all preparations. The variability in "threshold" may be related to differences in intracellular calcium levels or to a varied extent of damage in the preparation. We could not objectively assess the latter. However, from the experiments in which $[Ca^{++}]_o$ was varied it can be derived that $[Ca^{++}]_i$ contributes to the "threshold" for generation of TPCs (see below).

It is well known that spontaneous activity of cardiac muscle is enhanced by interventions that increase $[Ca^{++}]_i$ (e.g., Fabiato and Fabiato, 1972; Rieser et al., 1979; Chiesi et al., 1981; Lakatta and Lappe, 1981; Kort and Lakatta, 1984; Capogrossi and Lakatta, 1985; Fabiato, 1985; Nieman and Eisner, 1985). This was confirmed in the present study; not only did spontaneous activity in the resting muscle increase, but increasing $[Ca^{++}]_o$ also facilitated initiation of TPCs, as is apparent from the decrease in latency (Fig. 4 B). Fig. 4 A illustrates that this is not the mere result of a modification of F_t : where F_t increased and subsequently declined with increasing $[Ca^{++}]_o$, latency decreased progressively. This phenomenon has been described before (Lakatta and Lappe, 1981; Allen et al., 1984) and has been attributed to the negative influence of increasing spontaneous activity on subsequent twitch force (Lakatta and Lappe, 1981; Capogrossi et al., 1988). Thus, at higher $[Ca^{++}]_o$, a smaller force induced a TPC with a shorter latency than a greater force at lower $[Ca^{++}]_o$ did. Because $[Ca^{++}]_o$ varied in our experiments this might, partly, explain the observed differences in the "threshold" F_t necessary to trigger a TPC. Increasing the number of stimuli had the same effects as increasing $[Ca^{++}]_o$, presumably because both interventions influence $[Ca^{++}]_i$ (Allen et al., 1984). Thus, latency is apparently inversely related to F_t at constant $[Ca^{++}]_i$ (Figs. 2 C and 3 B), and hence to the extent of stretch of damaged regions in the preparation, while $[Ca^{++}]_i$ modifies this relationship by facilitating the initiation of TPCs (Figs. 4 B and 5 C).

The cause for the dependence of the initiation of a TPC on the stretch of a damaged region in the muscle preparation is speculative. It seems attractive to suppose that stretch of damaged regions causes a synchronization of subsequent spontaneous release of calcium ions from the sarcoplasmic reticulum, perhaps by allowing more calcium to enter the damaged regions, which could serve as a basis for the start of a TPC through diffusion of calcium ions to adjacent areas. It is also possible that stretch inhibits calcium release, which then occurs during the subsequent release of the muscle, initiating the TPC. This is supported by our finding that TPCs always started after peak force of the last stimulated contraction. We favor the hypothesis argued that during relaxation, with rapid shortening of the damaged end, calcium ions dissociate from the myofilaments, causing $[Ca^{++}]_i$ to increase (Housmans et al., 1983), which itself would cause synchronized calcium release from the sarcoplasmic reticulum. In this model of calcium-induced calcium release, the time needed to reach threshold for calcium release would be reflected in the latency observed. Increased $[Ca^{++}]_i$ would facilitate the triggering of a TPC by lowering the threshold, while a stronger twitch would cause a larger release of calcium ions from

the myofilaments, reducing time to reach threshold. The latency might also result from the time needed for the SR to reach a state of calcium overload with subsequent calcium release (Fabiato and Fabiato, 1972). This hypothesis, however, is less attractive because it is difficult to see how stretch and release of the damaged end, necessary to induce a TPC, would influence this time-dependent process.

Our study clearly shows the contrast between the characteristics of latency and propagation velocity of the contraction which, once started, is independent of F_i , and thus of stretch (Figs. 2 D and 3 C). V_{prop} increased progressively only with increasing $[Ca^{++}]_o$ and number of stimuli (Figs. 4 C and 5 D), suggesting a dependency on $[Ca^{++}]_i$. V_{prop} in our experiments varied between 1.0 and 17.0 mm/s, depending on $[Ca^{++}]_o$ and number of stimuli. This is consistent with recent observations in trabeculae (Mulder et al., 1989), but the high velocity contrasts to the observations in unstimulated single cells (Fabiato and Fabiato, 1972; Rieser et al., 1979; Fabiato, 1985; Capogrossi et al., 1986; Wier et al., 1987) and papillary muscles (Kort et al., 1985), although a V_{prop} of 3 mm/s has been reported after repeated stimulation of single myocytes (Golovina et al., 1986).

The dependence of V_{prop} on $[Ca^{++}]_i$ is compatible with the hypothesis that calcium diffusion through the muscle causes calcium-induced calcium release from the sarcoplasmic reticulum, explaining propagation of the spontaneous contraction (Stern et al., 1983; Backx et al., 1989). V_{prop} was independent of sarcomere length; this is consistent with the observation that the transients of $[Ca^{++}]_i$, as reflected by aequorin transients, change relatively little during the first minutes after a length change (Allen and Kurihara, 1982).

Recent reports suggest that aftercontractions and concomitant depolarizations of the sarcolemma may play a role in the genesis of tachyarrhythmias in the whole heart, both in experimental animals (Thandroyen, 1982; Thandroyen et al., 1988; Kusuoka et al., 1988) and in patients (Bhandari et al., 1988). This reinforces the hypothesis that triggered and propagated contractions, which start in a region of damaged myocardium, as observed in this study, may play a role in disease of the human heart, in particular at elevated intracellular calcium levels.

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