Stretch and quick release of rat cardiac trabeculae accelerates Ca$^{2+}$ waves and triggered propagated contractions

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Wakayama, Yuji, Masahito Miura, Yoshinao Sugai, Yutaka Kagaya, Jun Watanabe, Henk E. D. J. ter Keurs, and Kunio Shirato. Stretch and quick release of rat cardiac trabeculae accelerates Ca$^{2+}$ waves and triggered propagated contractions. Am J Physiol Heart Circ Physiol 281: H2133–H2142, 2001.—Rapid shortening of active cardiac muscle [quick release (QR)] dissociates Ca$^{2+}$ from myofilaments. We studied, using muscle stretches and QR, whether Ca$^{2+}$ dissociation affects triggered propagated contractions (TPCs) and Ca$^{2+}$ waves. The intracellular Ca$^{2+}$ concentration was measured by a SIT camera in right ventricular trabeculae dissected from rat hearts loaded with fura 2 salt, force was measured by a silicon strain gauge, and sarcomere length was measured by laser diffraction while a servomotor controlled muscle length. TPCs ($n = 27$) were induced at $28^\circ$C by stimulus trains (7.5 s at 2.65 ± 0.13 Hz) at an extracellular Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_o$) = 2.0 mM or with 10 μM Gd$^{3+}$ at $[\text{Ca}^{2+}]_o = 5.2 ± 0.73$ mM. QR during twitch relaxation after a 10% stretch for 100–200 ms reduced both the time between the last stimulus and the peak TPC (PeakTPC) and the time between the last stimulus and peak Ca$^{2+}$ wave (PeakCW) and increased PeakTPC and PeakCW ($n = 13$) as well as the propagation velocity ($V_{\text{prop}}$; $n = 8$). Active force during stretch also increased $V_{\text{prop}}$ ($r = 0.84, n = 12, P < 0.01$), but Gd$^{3+}$ had no effect ($n = 5$). These results suggest that Ca$^{2+}$ dissociation by QR during relaxation accelerates the initiation and propagation of Ca$^{2+}$ waves.

LIFE-THREATENING VENTRICULAR ARRHYTHMIAS are common in human hearts with ischemic disease or heart failure composed of normal as well as damaged myocardium with variations of both excitation-contraction (E-C) coupling and local mechanical properties that coexist with electrical nonuniformity. Electrical nonuniformity has been extensively discussed as a substrate for these arrhythmias. Less is known, however, about the role played by nonuniformities of E-C coupling and myocardial mechanics in these arrhythmias (39).

We have shown that propagation of local aftercontractions [denoted as triggered propagated contractions (TPCs)], which can be measured as waves of the sarcomere shortening after a twitch, are observed in both rat ventricular (14, 34, 43) and human atrial trabeculae (13). TPCs propagate at a constant velocity along trabeculae with a regional increase in intracellular Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_i$), which have been denoted as Ca$^{2+}$ waves. Such Ca$^{2+}$ waves propagate much faster than those observed in single myocytes (33, 39), and the propagation velocity ($V_{\text{prop}}$) ranges from 0.34 to 5.47 mm/s (31) depending on both [Ca$^{2+}$]; and sarcoplasmic reticulum (SR) Ca$^{2+}$ loading (32). Importantly, it has been reported that TPCs and Ca$^{2+}$ waves cause delayed afterdepolarizations (DADs) and triggered arrhythmias and that the triggered arrhythmias occur often when TPCs propagate rapidly (39).

Ca$^{2+}$ waves and TPCs invariably start from damaged regions within trabeculae, such as both ends of trabeculae or regions near cut side branches (39). The damaged cells and the border zone are weaker than those from the central regions of trabeculae during a twitch, that is, they exhibit nonuniform E-C coupling. Therefore, during a twitch, the central regions of the trabeculae stretch the damaged region, and, during relaxation, the damaged region shortens suddenly. Elimination of the stretch of the damaged region and border zone during the twitch and hence elimination of the quick release during the relaxation phase has been shown to prevent the induction of TPCs (14), suggesting that stretch or quick release of damaged ends of trabeculae during electrically stimulated twitch plays an important role in the triggering mechanism of TPCs. Therefore, we expect that the investigation of TPCs and Ca$^{2+}$ waves will contribute to knowledge of the role of nonuniform E-C coupling and myocardial mechanics played in the triggering of ventricular arrhythmias in the diseased heart (12, 34, 39).

Stretch of cardiac muscle is known to cause an increase in force without a change in $[\text{Ca}^{2+}]_i$, indicating that the increase in force is at least partially due to the length dependence of myofilament Ca$^{2+}$ sensitivity (1, 27), although an initial rapid increase in force is fol-
lowed by a slow increase with an increase in [Ca2+]i over a period of minutes (3, 4, 8, 9). Quick reduction of the muscle length after a transient stretch can dissociate Ca2+ from the myofilament Ca2+-binding proteins due to the change in myofilament Ca2+ sensitivity (3, 23, 26, 29). We hypothesized that dissociation of Ca2+ especially occurs near the damaged ends within trabeculae and triggers TPCs, because the damaged cells and the border zone may be more easily stretched by a transient increase of the muscle length (39). On the other hand, it has been reported that an increase in [Ca2+]i modulates the Ca2+-induced Ca2+ release (CICR) mechanism, which is known to be an important mechanism for the propagation of Ca2+ waves (6, 32). Therefore, we hypothesize that dissociated Ca2+ due to the reduction of myofilament Ca2+ sensitivity after a quick release may play a role in the initiation and propagation of TPCs and Ca2+ waves through the modulation of the CICR mechanism.

In the present study, focusing only on the rapid response to a transient change in muscle length, we first investigated the effect of a stretch (or release) pulse (~200 ms) during a twitch contraction on subsequent changes in [Ca2+]i during TPCs to evaluate whether Ca2+ dissociated from myofilaments plays a role in the initiation and propagation of TPCs and Ca2+ waves. We also tested whether Gd3+ can prevent the changes in [Ca2+]i caused by the transient stretch, because Gd3+ has been reported to block block mechanism-sensitive membrane channels [stretch-activated channels (SACs)] (24, 36, 40–42), which may conceivably play a role in the observed responses in this study.

MATERIALS AND METHODS

Dissection and mounting of rat ventricular trabeculae. The experiments were performed according to the guidelines for the care and use of laboratory animals of Tokoh University. Sprague-Dawley rats (250–300 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg). Hearts (n = 15) were excised, and the coronary arteries were immediately perfused via the aorta with a HEPES-buffered solution containing (in mM) 130.8 NaCl, 5 KCl, 1.2 MgCl2, 1.2 Na2SO4, 2.8 acetate, 10 HEPES, 10 glucose, and 0.7 CaCl2. The solutions were bubbled with 100% O2; pH was adjusted to 7.4 by addition of NaOH (42). After the heart was arrested by flushing with the same solution modified by addition of 15 mM KCl, trabeculae (n = 15, slack length, 1.45 ± 0.07 mm; slack width, 334 ± 27 μm; slack thickness, 114 ± 6 μm) were dissected from the right ventricle and mounted horizontally between a force transducer and a micromanipulator with a direct current torque motor (type 3123, No. 8-687, SAN-EL Instruments) in a perfusion bath located on the stage of an inverted microscope (Nikon) (31, 32). The trabeculae were equilibrated at 0.5 Hz with stimuli through parallel platinum electrodes in the bath with 5-ms pulses 50% above the threshold and superfused with HEPES-buffered solution [extracellular Ca2+ concentration ([Ca2+]o) = 0.7 mM] at room temperature (23–28°C).

Fura 2 loading and measurement of fluorescence. [Ca2+]i was measured as previously described (7, 31, 32). Briefly, fura 2 pentapotassium salt was microinjected electrophoretically into one cell and allowed to spread throughout the trabeculae via gap junctions. After the injection, the trabeculae were stimulated at 1 Hz for 30–60 min until the fura 2 had diffused uniformly throughout the preparation. The epifluorescence of fura 2 from the trabecula at excitation wavelengths of 340 and 380 nm was measured at 510 nm by a photomultiplier tube (PMT; E1341 with a C1556 socket, Hamamatsu). The signal from the PMT was stored (RD-130TE DAT Data Recorder, TEAC) for later analysis. Alternatively, the fluorescent image of the trabecula at excitation wavelengths of 360 and 380 nm was recorded by a SIT video camera (Hamamatsu C2400-8, Hamamatsu) through a 510-nm band-pass filter (25, 33). The images were recorded with a videocassette recorder (VCR) for off-line analysis.

Length changes and force measurements. Force was measured using a silicon semiconductor strain gauge and expressed as stress after normalization for the cross-sectional area of the muscle measured in slack conditions. Sarcomere length (SL) was measured using laser diffraction techniques (38). Changes in muscle length were introduced at the vascular end of trabeculae using a direct current torque motor driven by voltage commands from a personal computer via a 12-bit digital-to-analog converter (T-IFPC9 04120A-N, TSK). In this study, stretches were adjusted in the unstimulated muscle to cause 5 or 10% changes of SL. Thus we defined the length changes from 2.0 to 2.1 or 2.2 μm of resting SL as 5 or 10% stretches, respectively. After 10% stretch, peak active force increased by 68.0 ± 10.1% during electrically stimulated twitches at a 2-s stimulation interval ([Ca2+]o = 2.0 mM). Passive force was 2.5 ± 0.3% of the peak total force at a SL of 2.0 μm (no stretch) and 8.4 ± 1.0% of the peak total force at a SL of 2.2 μm (10% stretch). “Active force” was defined as total force (stress measured during a contraction) minus passive force (stress measured at a resting condition).

In addition to the stretch protocol described above, the muscle was shortened by 10% (10% release). In this release protocol, trabeculae were released from 2.0 μm to an almost slack SL (~1.8 μm).

Membrane potential measurements. Membrane potential was measured using ultracompact glass microelectrodes as described elsewhere (12, 17). Flexible stepped electrodes drawn with a glass microelectrode puller (Narishige) were filled with 3 M KCl and connected to a high-input impedance amplifier (MEZ-8201, Nihon Koden). The center of the trabecula was impaled. Long-term stable measurements were possible using these electrodes without causing local damage either during contraction or during changes of muscle length.

Analysis of the signal from the PMT: [Ca2+]i was determined using the following equation (19), as previously described (31, 32)

\[
[Ca^{2+}]_i = K_d \times \beta \times (R - R_{\text{min}})/(R_{\text{max}} - R)
\]

where \(K_d\) is the effective dissociation constant, \(R\) is the ratio of the fluorescence after subtraction of the autofluorescence of the muscle at 340-nm excitation to that at 380-nm excitation, \(R_{\text{min}}\) is \(R\) at zero [Ca2+], \(R_{\text{max}}\) is \(R\) at a saturating [Ca2+], and \(\beta\) is the ratio of the fluorescence value for Ca2+-free to the fluorescence value for Ca2+-bound dye at 380-nm excitation. Values for \(K_d\), \(R_{\text{min}}\), \(R_{\text{max}}\), and \(\beta\) were determined using in vitro calibrations, because we previously reported a good correlation between in vitro and in vivo calibrations when the free Mg2+ was 1 mM in the solutions mimicking the intracellular milieu (7). \(R_{\text{min}}\) was 0.48, \(R_{\text{max}}\) was 5.98, \(K_d\) was 382 nM, and \(\beta\) was 5.23. This \(K_d\) value is in agreement with previous data (31).

Analysis of fura 2 images. Fura 2 fluorescence images recorded at 30 frames/s on the VCR were analyzed as previously described (31, 32). Briefly, the fluorescence data of each video frame were digitized with an 8-bit analog-to-digital...
The measurement of \([\text{Ca}^{2+}]_o\) started when TPCs had reached a stable period for TPCs exceeded 30 min.

**RESULTS**

Figures 1 and 2 show that a transient change in muscle length during the last electrically stimulated twitch of the train can affect the latency of the TPC force and \([\text{Ca}^{2+}]_o\) waves. Figure 1 shows an example of force development and changes in \([\text{Ca}^{2+}]_o\), during the last two electrically stimulated twitches of a train at a stimulation interval of 280 ms. It is clear that the penultimate twitch was followed by a slow and small diastolic increase in force on which the last twitch was superimposed. The last twitch by itself was followed by a force transient with a time course similar to those that have been reported in the past as force transients accompanying TPCs (14, 34, 39). Indeed, we observed the occurrence of propagated sarcomere shortening as well as propagating \([\text{Ca}^{2+}]_o\) waves (data not shown) as have been shown previously during TPCs (31, 32, 39).

It was clear that TPCs could be elicited at 28°C by a suitable combination of stimulus conditions (stimulus rate, train length, and \([\text{Ca}^{2+}]_o\)) as chosen in this study. This confirms that TPCs can occur at a wide range of temperatures. The time over which the TPCs behave in a stable fashion decreased with increasing temperature (15). We observed in these experiments that the stable period for TPCs exceeded 30 min.
The time course of the TPCs appeared to reflect the time course of an underlying \([Ca^{2+}]_i\) transient (Fig. 1), as has been observed previously (31, 32). With a stretch pulse by 5 or 10\% for 150 ms during the last of the train, force increased, whereas \([Ca^{2+}]_i\) transients during the twitch showed no changes in their peaks but exhibited a faster decline and lower minimal \([Ca^{2+}]_i\).

With a release pulse for 150 ms, the force of the twitch decreased, and \([Ca^{2+}]_i\) transients during the twitch exhibited a slower decline with no changes in their peaks.

The stretch pulse accelerated and the release pulse suppressed the subsequent TPC force and underlying \([Ca^{2+}]_i\). Here, it should be noted that the acute return of muscle length, especially after the 10\% transient stretch, induced a small and rapid transient increase...
(denoted as “QR transient” in Fig. 1B) in [Ca\(^{2+}\)]\(_i\), followed by a larger and earlier increase in [Ca\(^{2+}\)]\(_i\) during the TPC.

To study the details of changes in the TPC force and the underlying increase in [Ca\(^{2+}\)]\(_i\), spatial changes in [Ca\(^{2+}\)]\(_i\) during comparable TPCs were determined directly after the recordings (such those shown in Fig. 1). Figure 2 shows spatial changes in [Ca\(^{2+}\)]\(_i\) during the last electrically stimulated twitches of a train at a stimulation interval of 280 ms and a Ca\(^{2+}\) wave. With transient stretch by 10\% during the last twitch, \(V_{prop}\) of the Ca\(^{2+}\) wave increased from 2.90 to 6.77 mm/s. With transient stretch by 5\% for 150 ms, \(V_{prop}\) of the Ca\(^{2+}\) wave increased from 2.90 to 4.29 mm/s (data not shown). We could not measure \(V_{prop}\) of the Ca\(^{2+}\) wave after a release pulse, because the Ca\(^{2+}\) wave was too small to detect the peak of [Ca\(^{2+}\)]\(_i\) at each position along the trabeculae.

In Fig. 3, the open circles show the effect of changes in the muscle length during the last twitch of the trains on TPC force and the underlying changes in [Ca\(^{2+}\)]\(_i\) obtained from nine trabeculae (\(n = 13\)). Transient stretch decreased \(T_{TPC}\) and \(T_{CW}\) and increased \(P_{TPC}\) and \(P_{CW}\), whereas the transient release increased \(T_{TPC}\) and \(T_{CW}\) and decreased \(P_{TPC}\) and \(P_{CW}\). These observations show that transient stretch can accelerate and the release pulse can suppress the subsequent TPC force and the underlying changes in [Ca\(^{2+}\)]\(_i\).

Figure 4 shows the changes of Ca\(^{2+}\) waves owing to transient stretch of the muscle during the last twitch of the trains. In Fig. 4A, we selected the peak of the Ca\(^{2+}\) transient at each pixel along the trabecula during the Ca\(^{2+}\) waves illustrated in Fig. 2 and superimposed the plots of the peak time against the peak position obtained from both Ca\(^{2+}\) waves to investigate the changes in the propagation of Ca\(^{2+}\) waves owing to the transient stretch. We noticed that, after the stretch pulse, Ca\(^{2+}\) waves started from closer to the end of the trabecula and propagated faster along the trabecula at a constant velocity. Figure 4B shows the effect of transient stretch by 5 or 10\% on \(V_{prop}\) of nine Ca\(^{2+}\) waves obtained from five trabeculae. The \(V_{prop}\) of Ca\(^{2+}\) waves increased significantly by the transient stretch. When we plotted the changes in \(V_{prop}\) of Ca\(^{2+}\) waves against those in \(T_{TPC}\) by comparable stretch (Fig. 4C), it appeared that the changes in \(V_{prop}\) correlated strongly (\(r = 0.84\)) with those in \(T_{TPC}\), i.e., changes in the amount of Ca\(^{2+}\) bound to the myofilament Ca\(^{2+}\)-binding proteins.

Figure 5A shows the effect of the stretch pulse during the last twitch of the trains on the membrane potentials. The resting membrane potential of this muscle was \(-70\) mV before the stimulus train, was depolarized during the stimulus train (\(-50\) mV), and then gradually returned to the basal level. We observed DADs after the cessation of electrical stimuli concurrent with the TPC as has been shown previously (12). With a transient stretch of muscle by 10\% for 200 ms, the DAD occurred earlier and appeared larger. Data (\(n = 9\)) obtained from three trabeculae showed that the resting membrane potential before the stimulus train was \(-69.7 \pm 3.5\) mV and that, with the transient stretch, \(T_{DAD}\) decreased by 7.0 \pm 1.9\% and \(P_{DAD}\) increased from \(-55.7 \pm 3.3\) mV to \(-54.7 \pm 3.3\) mV (\(P < 0.0005\)).

To eliminate TPCs and Ca\(^{2+}\) waves and clarify the Ca\(^{2+}\) dissociation from the myofilaments, we added the stretch or release pulse during the last stimulated twitch in the presence of 1 mM caffeine (Fig. 5B). In the presence of 1 mM caffeine, the amplitude of stimulated twitch in the presence of 1 mM caffeine was \(-70\) mV before the stimulus train, was depolarized during the stimulus train (\(-50\) mV), and then gradually returned to the basal level.
tively. In three trabeculae, we changed the muscle length during the last stimulated twitch in the presence of 1 mM caffeine and observed the same transient increase (or decrease) in [Ca\(^{2+}\)]\(_i\), at the quick return of the muscle length. We therefore assumed that the small and transient increase in [Ca\(^{2+}\)]\(_i\) was caused by Ca\(^{2+}\) dissociation from the myofilaments and occurs as the QR transient observed in the absence of caffeine.

It has been reported that \(V_{\text{prop}}\) of Ca\(^{2+}\) waves is determined by both the open probability of the SR Ca\(^{2+}\) release channels and cellular Ca\(^{2+}\) loading (32). To study the direct effect of the transient stretch pulse on the open probability of SR Ca\(^{2+}\) release channels, we stretched the muscle length during a TPC (Fig. 6A). The transient stretch during the TPC changed neither Time\(_{\text{TPC}}\), Time\(_{\text{CW}}\), nor Peak\(_{\text{CW}}\) but increased Peak\(_{\text{TPC}}\). Data obtained from nine trabeculae showed that transient stretch of the muscle during TPCs accelerated neither the TPCs nor the underlying increase in [Ca\(^{2+}\)]\(_i\) (data not shown).

To investigate whether SACs play a role in the acceleration of TPC forces and the underlying increase in [Ca\(^{2+}\)]\(_i\), we used Gd\(^{3+}\), as shown in Figs. 3 and 6B. Figure 6B shows an example of force developments and changes in [Ca\(^{2+}\)]\(_i\), during the last two electrically stimulated twitches of a train at a stimulation interval of 320 ms in the presence of 10 \(\mu\text{M}\) Gd\(^{3+}\). With a transient stretch of muscle by 5 or 10% for 180 ms during the last stimulated twitch of the train, the developed force and Ca\(^{2+}\) transients during the last twitch were affected to the same extent as those in the absence of Gd\(^{3+}\). In addition, proceeding from a small QR transient of [Ca\(^{2+}\)]\(_i\) (see above), the acceleration of the TPC force and the underlying increase in [Ca\(^{2+}\)]\(_i\) owing to the stretch were also observed. In Fig. 3, the open squares show the effect of the transient stretch pulse on TPC forces and the underlying increase in [Ca\(^{2+}\)]\(_i\), from five trabeculae in the presence of 10 \(\mu\text{M}\) Gd\(^{3+}\). After the rapid stretch, Time\(_{\text{TPC}}\) and Time\(_{\text{CW}}\) still decreased, and Peak\(_{\text{TPC}}\) and Peak\(_{\text{CW}}\) increased \((n = 5)\). In addition, these changes in the presence of Gd\(^{3+}\) were not significantly different from those in the absence of Gd\(^{3+}\). Thus 10 \(\mu\text{M}\) Gd\(^{3+}\) did not suppress the acceleration of the TPC force and the underlying increase in [Ca\(^{2+}\)]\(_i\), induced by the transient stretch.

Figure 7 shows that the transient length changes can be arrhythmogenic (18, 21). The TPC force and the underlying increase in [Ca\(^{2+}\)]\(_i\) were accelerated by the transient stretch pulse during the last stimulated twitch of trains with stimulation intervals of 350 ms. It was striking that, after the transient stretch of the muscle length by 10% for 200 ms, a Ca\(^{2+}\) transient and force transient similar to those induced by electrical stimulation occurred without electrical stimuli, preceded by enhanced TPC force and an increase of the [Ca\(^{2+}\)]\(_i\) transient. In addition, \(V_{\text{prop}}\) of the Ca\(^{2+}\) wave calculated in this trabecula was 8.82 mm/s without stretch and increased to 9.74 mm/s after a 5% transient stretch. With the 10% stretch pulse, the Ca\(^{2+}\) wave propagated too fast to be calculated from video frames.
obtained at 30 frames/s. Moreover, [Ca\(^{2+}\)]\(_i\) showed synchronous changes throughout the trabecula during the extra twitch (data not shown).

**DISCUSSION**

This is the first study to characterize the effect of Ca\(^{2+}\) dissociated from myofilaments into the cytosol on TPCs and Ca\(^{2+}\) waves by direct measurement of the spatial and temporal properties of Ca\(^{2+}\) changes in a cardiac multicellular preparation. Our observations suggest that 1) a transient stretch (or release) pulse of the muscle length during the last twitch of electrical trains can accelerate (or suppress) the TPC force and the underlying increases in [Ca\(^{2+}\)]\(_i\) through changes in \(V_{\text{prop}}\) of Ca\(^{2+}\) waves and 2) the changes in \(V_{\text{prop}}\) of Ca\(^{2+}\) waves can be explained by Ca\(^{2+}\) dissociated from myofilaments into the cytosol after rapid shortening but by neither a direct increase in the open probability of the

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**Fig. 5.** A: effect of a transient 10% stretch of the muscle for 200 ms during the last twitch of electrical trains on force and delayed afterdepolarizations (DADs). Thick line, 10% stretch; thin line, no stretch. Top, force; top middle and bottom middle, changes in membrane potential and the expansion of the recording in the ordinate recorded by an ultracompliant glass microelectrode during the last two electrically stimulated twitches (stimulation interval of 300 ms) and subsequent TPC; bottom, muscle length. Peak\(_{\text{DAD}}\), peak DAD; Time\(_{\text{DAD}}\), time from the last stimulus to Peak\(_{\text{DAD}}\). Time\(_{\text{DAD}}\) decreased from 350 to 298 ms, and Peak\(_{\text{DAD}}\) increased by 8.9 to 57.7 mV as a result of transient 10% stretch (thick line) of the muscle. S, moment of electrical stimulation (indicated by arrow). Temperature, 27.4°C; Exp. 000511. B: effect of transient changes in the muscle length for 300 ms during the last twitch of electrical trains (stimulation interval of 350 ms) on force and [Ca\(^{2+}\)]\(_i\) in the presence of 1 mM caffeine. Top, top middle, bottom middle, and bottom are the same as those shown in A. Red line, 10% stretch; black line, no stretch; blue line, 10% release. Red arrow, increase in [Ca\(^{2+}\)]\(_i\) at the moment of quick return of muscle length after the stretch pulse. Temperature, 28.1°C; Exp. 990902.

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**Fig. 6.** A: effect of a transient 10% stretch of the muscle during TPCs on force and [Ca\(^{2+}\)]\(_i\). Thick line, 10% stretch; thin line, no stretch. Top, force; middle, [Ca\(^{2+}\)]\(_i\); bottom, muscle length during the last two electrically stimulated twitches (stimulation interval of 400 ms) and subsequent TPC. Time\(_{\text{TPC}}\), Time\(_{\text{CW}}\), and Peak\(_{\text{CW}}\) showed no changes, whereas Peak\(_{\text{TPC}}\) increased as a result of the transient 10% stretch (thick line) for 500 ms during TPCs. S, moment of electrical stimulation (indicated by arrow). Temperature, 28.4°C; Exp. 990531. B: effect of a transient stretch of the muscle during the last twitch of electrical trains on force and [Ca\(^{2+}\)]\(_i\) in the presence of 10 μM Gd\(^{3+}\). Thick line, 10% stretch; thin line, 5% stretch; intermediate line, no stretch. Top, force; middle, [Ca\(^{2+}\)]\(_i\); bottom, muscle length during the last two electrically stimulated twitches (stimulation interval of 320 ms) and a subsequent TPC. Time\(_{\text{TPC}}\) and Time\(_{\text{CW}}\) shortened, whereas Peak\(_{\text{TPC}}\) and Peak\(_{\text{CW}}\) increased as a result of transient stretch of the muscle for 180 ms. Inset, arrowhead showing the small QR transient of [Ca\(^{2+}\)]\(_i\). Temperature, 27.7°C; extracellular Ca\(^{2+}\) concentration, 5.0 mM; Exp. 990708.


Figure 7. Effect of a transient stretch of the muscle on the induction of arrhythmias. Thick line, 10% stretch; thin line, 5% stretch; intermediate line, no stretch. Top, force; middle, \([\text{Ca}^{2+}]_i\); bottom, muscle length during the last two electrically stimulated twitches (stimulation interval of 350 ms) and subsequent TPC. Note that a twitch contraction was induced (arrowheads) as a result of transient 10% stretch of the muscle for 200 ms. S, moment of electrical stimulation (indicated by arrow). Temperature, 28.1°C; Exp. 991004.

SR \(\text{Ca}^{2+}\) release channel, SACs, nor voltage-gated channels, as will be discussed below.

Effect of stretches on stimulated twitches. It has been observed that a rapid response in force to a change in the muscle length occurs without a change in \([\text{Ca}^{2+}]_i\) using rat ventricular muscles (3, 5, 27), cat papillary muscles (3), and ferret ventricular muscles (29), and it has been reported that most of the change in force by stretch is due to the length dependence of myofilament \(\text{Ca}^{2+}\) sensitivity (1, 3, 27). Thus the change in twitch force during a stretch (or release) pulse of the muscle length observed in this study (Fig. 1) was at least partially due to the change in myofilament \(\text{Ca}^{2+}\) sensitivity. The faster (or slower) decline rate of \(\text{Ca}^{2+}\) transients during the stretch (or release) pulse was also due to the change in myofilament \(\text{Ca}^{2+}\) sensitivity (11, 28, 30).

Effect of transient stretch (or release) pulses on TPCs and \(\text{Ca}^{2+}\) waves. When \(\text{Ca}^{2+}\)-activated cardiac muscle is allowed to shorten, the amount of \(\text{Ca}^{2+}\) dissociated from myofilaments depends on the changes in force or the number of force-generating cross-bridges rather than on changes in muscle length (2, 35). Dissociated \(\text{Ca}^{2+}\) may change cellular \(\text{Ca}^{2+}\) loading, which is one of the determinants of \(V_{\text{prop}}\) of \(\text{Ca}^{2+}\) waves (6, 14, 32). Thus the observation that the changes in the active force (\(F_p\)) by the transient stretch pulse correlated strongly with those in \(V_{\text{prop}}\) (Fig. 4C) suggests that the amount of \(\text{Ca}^{2+}\) dissociated from myofilaments can determine \(V_{\text{prop}}\) of \(\text{Ca}^{2+}\) waves.

One possible explanation is that rapid shortening of the muscle causes a dissociation of \(\text{Ca}^{2+}\) from the myofilaments throughout the trabecula (3, 23, 26, 29) and therefore leads to a rapid increase in \([\text{Ca}^{2+}]_i\) (QR transient in Figs. 1B and 6B). The response of the myofilaments to dissociate \(\text{Ca}^{2+}\) after a quick release is fast enough to account for the QR transient. Moreover, the dissociated \(\text{Ca}^{2+}\) from myofilaments is expected to be a potent source of intracellular \(\text{Ca}^{2+}\) (3, 23, 26, 29). The time course of decline of the QR transient would be dictated by \(\text{Ca}^{2+}\) removal mechanisms and is thus expected to be similar to that of \(\text{Ca}^{2+}\) transients by electrical stimuli. Even the \(\text{Ca}^{2+}\) transients of twitches decline from 90 to 10% of their peaks within ~100 ms at this temperature (Fig. 1). On the other hand, the time required for the passage of the \(\text{Ca}^{2+}\) waves through the ROI used for image analysis (~0.62 mm) is ~100 ms when \(V_{\text{prop}}\) was 6.77 mm/s (Figs. 2 and 4A). Hence, during the passage of the \(\text{Ca}^{2+}\) waves, the \([\text{Ca}^{2+}]_i\) level due to the QR transient is expected to decline substantially throughout the trabecula. In this case, \(V_{\text{prop}}\) of \(\text{Ca}^{2+}\) waves should decrease gradually with the decrease in the QR transient during the propagation and return to the level of \(V_{\text{prop}}\) in a manner similar to that without the stretch pulse. However, \(\text{Ca}^{2+}\) waves propagated faster along the trabecula at a constant velocity after the release of the muscle at the end of a transient stretch (Fig. 4A), suggesting that an initial potential for the acceleration of \(\text{Ca}^{2+}\) waves triggered by the QR transient is maintained throughout the propagation.

An alternative explanation for the acceleration of \(\text{Ca}^{2+}\) waves is that the QR transient occurs not uniformly but regionally by the quick reduction of muscle length in the border zone near a damaged region (3, 23, 29) and then induces \(\text{Ca}^{2+}\) release from the adjacent SR of the border zone as the initiating trigger for \(\text{Ca}^{2+}\) waves. The magnitude of the trigger dictates the amount of \(\text{Ca}^{2+}\) released from the SR during \(\text{Ca}^{2+}\) waves. The amount of \(\text{Ca}^{2+}\) released from the SR during the \(\text{Ca}^{2+}\) wave determines the amount of \(\text{Ca}^{2+}\) released in adjacent sites and, hence, dictates \(V_{\text{prop}}\) via modulation of the CICR mechanism (32). This explanation would be feasible if the border zone is mainly stretched by the twitch contraction of the central regions as well as by the stretch pulse and then shortens during the rapid relaxation of the central region (39). Indeed, elimination of the afterload to the trabecula prevents the induction of TPCs (14), and the transient release pulse of the muscle length did suppress the TPC force and underlying \([\text{Ca}^{2+}]_i\) (Figs. 1 and 3).
Although it is still not proven that Ca\textsuperscript{2+} dissociated from myofilaments affects the initiating process or the propagating process of TPCs (or both) as described above, there is ample evidence to suggest an interaction between myofilaments and Ca\textsuperscript{2+} plays an important role in E-C coupling and is a logical candidate for an important mechanism that accelerates TPCs and underlying Ca\textsuperscript{2+} waves.

No effect of SACs on the acceleration of Ca\textsuperscript{2+} waves. Several reports (24, 36, 41) have suggested that SACs are active in the heart because Gd\textsuperscript{3+} has been found to block stretch-induced phenomena in many cardiac systems. SACs (20, 24, 41) in the heart are cation selective, and the majority of them are permeable to Ca\textsuperscript{2+} (24, 36). In this study, however, SACs are unlikely to play an important role in the increase of [Ca\textsuperscript{2+}]\textsubscript{i} through the muscle for the following reasons: 1) the stimulated Ca\textsuperscript{2+} transient (Fig. 1) as well as [Ca\textsuperscript{2+}]\textsubscript{i} during TPCs (Fig. 6A) showed no increase during a stretch pulse; 2) the duration of the action potential did not become longer by the stretch pulse (Fig. 5A); and 3) 10 \( \mu \text{M} \) Gd\textsuperscript{3+} did not block the acceleration of the TPC force and the underlying changes in [Ca\textsuperscript{2+}]\textsubscript{i} induced by the transient stretch of the muscle (Figs. 3 and 6B). The latter result was consistent with our previous observation that Gd\textsuperscript{3+} sensitive SACs are involved in neither the triggering nor propagation processes of TPCs (42). In this study, we tested the full Gd\textsuperscript{3+} dose-response curve and observed that the effect of Gd\textsuperscript{3+} at 10 \( \mu \text{M} \) was 90% of the maximal effect. However, the effect of lowering [Ca\textsuperscript{2+}]\textsubscript{o} was identical to that of Gd\textsuperscript{3+} at all concentrations used, suggesting the absence of a specific effect due to SAC inhibition (42). In the present series of experiments, we corrected for the decrease of twitch force and the corresponding [Ca\textsuperscript{2+}]\textsubscript{i} transient by raising [Ca\textsuperscript{2+}]\textsubscript{o} to 5.2 mM to compensate (to within 15%) for the nonspecific inhibitory effect of Gd\textsuperscript{3+}, especially on L-type Ca\textsuperscript{2+} currents.

Other possible mechanisms for the acceleration of Ca\textsuperscript{2+} waves. First, stretch of the muscle may directly modulate the open probability of SR Ca\textsuperscript{2+} release channels, because it has been suggested that decreasing the SL below optimum (2.2 \( \mu \text{m} \)) results in a partial inhibition of CICR from the SR in cardiac muscle (16, 35). The transient stretch during the TPC, however, did not change [Ca\textsuperscript{2+}]\textsubscript{i} during TPCs (Fig. 6A), suggesting that the stretch pulse cannot modulate directly the open probability of SR Ca\textsuperscript{2+} release channels.

Second, voltage-gated currents may influence the acceleration of Ca\textsuperscript{2+} waves. It has been reported that delayed K\textsuperscript{+} current is increased by hypotonic cell swelling but not by axial stretch; other K\textsuperscript{+} currents and Ca\textsuperscript{2+} currents showed almost no change with axial stretch or swelling (10, 22). These reports suggest that the voltage-gated currents are unlikely to play a role in the changes in \( V_{\text{prop}} \) of Ca\textsuperscript{2+} waves after the application of the axial transient stretch pulse observed in this study.

TPCs, DADs, and clinical relevance. Figure 5A confirms that TPCs are accompanied by DADs (12). It has been reported that the increase in [Ca\textsuperscript{2+}]\textsubscript{i} underlying a TPC is accompanied by a DAD via electrogenic Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange and activation of Ca\textsuperscript{2+}-sensitive nonselective channels in the sarcolemma (37, 39). The amplitude of the DAD has been shown to correlate tightly with the amplitude of TPC. In turn, DADs may trigger action potentials (12, 37). Thus the rapid increase in [Ca\textsuperscript{2+}]\textsubscript{i} upon the reduction of the muscle length after the stretch may accelerate TPCs and the underlying increase in [Ca\textsuperscript{2+}]\textsubscript{i} and, thereby, induce arrhythmias, as we have shown here (Fig. 7).

This interrelation between TPCs and arrhythmias provides an attractive model of damage-induced arrhythmias, as has been shown previously (39). In diseased hearts, there must be variations of mechanical stresses and strains between weak and normal regions, i.e., nonuniform E-C coupling and mechanics. Little is known about the role played by nonuniform E-C coupling in the mechanisms underlying the premature beats that initiate an arrhythmia in clinical scenarios. An especially important clinical scenario in which arrhythmias occur is that of ischemic damage of myocardium. The situation after acute damage due to ischemia with impaired (stretched) cells adjacent to normal (shortening) cells resembles our model in which TPCs and Ca\textsuperscript{2+} waves were observed. Although arrhythmias in vivo are not likely to result from mechanical damage as was the case in our model, we suggest that our model of arrhythmias caused by TPCs and Ca\textsuperscript{2+} waves in the trabecula contributes to the understanding of the clinical mechanisms underlying damage-induced arrhythmias.

Limitations of the study. We observed a small QR transient in [Ca\textsuperscript{2+}]\textsubscript{i}, using PMT, which may have been caused by Ca\textsuperscript{2+} dissociated from myofilaments into the cytosol, whereas we could not find comparable changes in Ca\textsuperscript{2+} waves using the SIT camera. This difference between the recordings may have occurred for the following reasons. First, the field of view using the PMT is larger (diameter of \( \sim 1.3 \text{ mm} \)) than that using the SIT camera (see MATERIALS AND METHODS) such that the initiating event of Ca\textsuperscript{2+} waves, which occurred usually at the ends of the trabecula, was out of view with the SIT camera but “visible” to the PMT. Second, for the analysis of the image, we defined the ROI (615 \( \times \) 41 \( \mu \text{m} \)) horizontally along the long axis of the trabecula. If the initiating events of Ca\textsuperscript{2+} waves occurred outside of the ROI, we would not be able to detect these events. Third, the rising phase of the small QR transient of [Ca\textsuperscript{2+}]\textsubscript{i} lasted for \( \sim 20 \text{ ms} \), which was too short to be detected from video frames obtained at 30 frames/s. Thus, to observe directly how Ca\textsuperscript{2+} dissociated from myofilaments into the cytosol modulates Ca\textsuperscript{2+} waves, we must await exploration using probes with a higher spatial and temporal resolution.

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