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Spatial Nonuniformity of Excitation–Contraction Coupling Causes Arrhythmogenic Ca\textsuperscript{2+} Waves in Rat Cardiac Muscle

Yuji Wakayama, Masahito Miura, Bruno D. Stuyvers, Penelope A. Boyden, Henk E.D.J. ter Keurs

Abstract—Ca\textsuperscript{2+} waves underlying triggered propagated contractions (TPCs) are initiated in damaged regions in cardiac muscle and cause arrhythmias. We studied Ca\textsuperscript{2+} waves underlying TPCs in rat cardiac trabeculae under experimental conditions that simulate the functional nonuniformity caused by local mechanical or ischemic local damage of myocardium. A mechanical discontinuity along the trabeculae was created by exposing the preparation to a small jet of solution with a composition that reduces excitation–contraction coupling (ECC) in myocytes within that segment. The jet solution contained either caffeine (5 mmol/L), 2,3-butanedione monoxime (BDM; 20 mmol/L), or low Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]; 0.2 mmol/L). Force was measured with a silicon strain gauge and sarcomere length with laser diffraction techniques in 15 trabeculae. Simultaneously, [Ca\textsuperscript{2+}]\textsubscript{i} was measured locally using epifluorescence of Fura-2. The jet of solution was applied perpendicularly to a small muscle region (200 to 300 \(\mu\)m) at constant flow. When the jet contained caffeine, BDM, or low [Ca\textsuperscript{2+}], during the stimulated twitch, muscle-twitch force decreased and the sarcomeres in the exposed segment were stretched by shortening normal regions outside the jet. Typical protocols for TPC induction (7.5 s-2.5 Hz stimulus trains at 23°C; [Ca\textsuperscript{2+}]; 2.0 mmol/L) reproducibly generated Ca\textsuperscript{2+} waves that arose from the border between shortening and stretched regions. Such Ca\textsuperscript{2+} waves started during force-relaxation of the last stimulated twitch of the train and propagated (0.2 to 2.8 mm/sec) into segments both inside and outside of the jet. Arrhythmias, in the form of nondriven rhythmic activity, were induced when the amplitude of the Ca\textsuperscript{2+}-wave was increased by raising [Ca\textsuperscript{2+}]. Arrhythmias disappeared rapidly when uniformity of ECC throughout the muscle was restored by turning the jet off. These results show, for the first time, that nonuniform ECC can cause Ca\textsuperscript{2+} waves underlying TPCs and suggest that Ca\textsuperscript{2+} dissociated from myofilaments plays an important role in the initiation of Ca\textsuperscript{2+} waves. (Circ Res. 2005;96:1266-1273.)

Key Words: rat trabeculae ■ nonuniformity ■ troponin C ■ Ca\textsuperscript{2+} waves ■ arrhythmias

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scemic and failing hearts are both prone to ventricular arrhythmias and commonly show regional differences in contractile strength caused by heterogeneous impairment of excitation–contraction coupling (ECC). It is generally accepted that lethal arrhythmias are frequently associated with alterations of the excitation step of ECC.\textsuperscript{1} It is less well known what role nonuniform ECC\textsuperscript{2} plays in initiating arrhythmias.\textsuperscript{3,4}

We have previously investigated the triggered propagated contractions (TPCs) phenomenon in rat cardiac trabeculae. TPCs probably result from local damage and the ensuing nonuniform ECC.\textsuperscript{4} TPCs consist of local sarcomere shortening\textsuperscript{5-7} associated with a [Ca\textsuperscript{2+}]\textsubscript{i} transient that propagates in a wave-like manner along the muscle.\textsuperscript{8-10} Ca\textsuperscript{2+} waves underlying TPCs cause delayed after-depolarizations (DADs) and triggered arrhythmias.\textsuperscript{6,7,10} In the model of damaged muscle, TPCs and underlying Ca\textsuperscript{2+} waves started invariably in regions located near the dissected end of the muscle or near cut branches.\textsuperscript{4,5} The regions bordering the damaged areas exhibit elevated cytosolic and sarcoplasmic reticulum (SR)-Ca\textsuperscript{2+} and constitute a source of nonuniformity in ECC.\textsuperscript{6} However, a detailed study of the role of these regions in the initiation of arrhythmogenic Ca\textsuperscript{2+} waves is hampered by the difficulty in controlling the extent and severity of damage, and as such neither sarcomere length (SL) nor [Ca\textsuperscript{2+}]\textsubscript{i} can be measured reliably.

Here, we developed a novel model of controlled nonuniformity in rat trabeculae. Using this model, we show that controlled initiation of Ca\textsuperscript{2+} waves underlying TPCs can trigger nondriven regular spontaneous contractions in cardiac muscle. The initiation of arrhythmogenic Ca\textsuperscript{2+} waves can be explained by nonuniform ECC and Ca\textsuperscript{2+}-dissociation from the contractile filaments occurring during relaxation of nonuniform cardiac muscle.

Materials and Methods
Measurements of Force, SL, and [Ca\textsuperscript{2+}], in Rat Trabeculae

Trabeculae (n=15; length: 2.30±0.09 mm, width: 262±25 \(\mu\)m, thickness: 103±4 \(\mu\)m in slack conditions) were dissected from the...
right ventricle of Lewis Brown Norway rats5-14 (Charles River Canada, Saint Constant, QC, Canada) and mounted between a motor arm and force (F) transducer in a bath perfused by HEPES solution on an inverted microscope. SL was measured by laser diffraction techniques12 (Figure 1A).

Measurement of [Ca\textsuperscript{2+}] has been described previously5-11 Briefly, Fura-2 salt was microinjected iontophoretically into the trabecula11 Excitation light of 340, 360, or 380 nm was used and fluorescence was collected using an image intensified CCD camera (IIC) at 30 frames/s to assess local [Ca\textsuperscript{2+}] (Figure 1A).11 We calculated [Ca\textsuperscript{2+}]i in a region of interest along the trabecula from the calibrated ratio of F_{340}/F_{370} (see Miura et al for details5 and Figure 1A, available online at http://circres.ahajournals.org).

Reduction of Local Contraction
To produce nonuniform ECC, a restricted region was exposed to a small jet of solution (~0.06 mL/min) that had been directed perpendicularly to a small muscle segment (300 µm; Figure 1) using a syringe pump connected to a glass pipette (~100 µm diameter) (Figure 1A and 1B; see also supplemental Movie 1 in the online data supplement). The jet was positioned with respect to the muscle using a neutral colorant (Figure 1B) or fluorescein (~0.01 mg/mL). To reduce contraction in the exposed region by modified ECC, the jet solution was composed of standard HEPES solution containing either: (1) Coffee (CF; 5 mmol/L); (2) 2,3-butanedione monoxime (BDM; 20 mmol/L); or (3) low [Ca\textsuperscript{2+}] (low [Ca\textsuperscript{2+}]o) solution had distinct effects on [Ca\textsuperscript{2+}]i (Figure 1A). We have described previously5-11

During exposure to the jet, Ca\textsuperscript{2+} waves underlying TPCs were induced by stimulation of the muscle at 2.5 Hz for 7.5 s every 15 s at [Ca\textsuperscript{2+}]o of 2 mmol/L (caffeine, BDM) or 2.7±0.2 mmol/L (low [Ca\textsuperscript{2+}]o) at 23.7±2°C4,7,10,13,14 Measurement of [Ca\textsuperscript{2+}]i commenced when the amplitude of stimulated twitches, TPCs, and underlying Ca\textsuperscript{2+} waves were constant (within 10 minutes).

Data Analysis
Data were expressed as mean±SEM. Statistical analysis was performed using ANOVA followed by a Post-hoc test. Differences were considered significant when P<0.05 (see online data supplement).

Results
Nonuniformity and Sarcomere Mechanics
The jet reached one short muscle segment (~300 µm) (Figure 1A and 1B). Figure 1C shows that the fluid flow from the pipette using a solution with composition similar to the bath solution (HEPES) had no effect on F or SL by itself.

When a jet containing either BDM (Figures 1C and 2A), caffeine (Figure 2A), or low [Ca\textsuperscript{2+}] (Figure 2A) was applied to the stimulated trabeculae, sarcomere stretch rapidly replaced the normal active shortening in the exposed segment (Figure 2B), whereas peak force (F/F\textsubscript{max}) decreased (~11±3% with low [Ca\textsuperscript{2+}]o (n=5), -28±5% with caffeine (n=6), and -36±7% with BDM (n=5) (Figure 2C; Table). Low [Ca\textsuperscript{2+}]o solution did not affect resting SL (SL\textsubscript{o}), whereas caffeine and BDM slightly decreased (~2.2±0.7%) and increased (+3.1±0.6%) SL\textsubscript{o}, respectively. All effects were rapidly reversible (Figure 1C). Sarcomere dynamics along muscles exposed to a jet revealed 3 distinct regions during the twitch (Figure 2B): (1) a region located >200 µm from the jet where sarcomeres exhibited typical shortening (see [1] in Figure 2B); (2) the segment exposed to the jet where sarcomeres were stretched (see [2] in Figure 2B); (3) in a region between [1] and [2] sarcomeres shortened early during the twitch and then were stretched although less than in segment [2]; we denoted this region [3] the Border Zone (BZ). The BZ extended 1 to 2 cell lengths (100 to 200 µm) beyond the jet-exposed region (Figure 2B). The diffraction pattern of sarcomeres in BZ illuminated by a ~150 µm diameter laser beam showed a clear single peak during both shortening and lengthening; similar changes in regional sarcomere dynamics were observed in caffeine and low [Ca\textsuperscript{2+}]o experiments (data not shown).

Nonuniformity and [Ca\textsuperscript{2+}] Transients
In contrast to the similarity of the effects of the various jet solutions on sarcomere dynamics, jets of caffeine, BDM, or low [Ca\textsuperscript{2+}]o solution had distinct effects on [Ca\textsuperscript{2+}]i (Figure 3A, 3B, and 3C respectively). Robust electrically driven...
[Ca\textsuperscript{2+}]\textsubscript{i}-transients occurred in regions ([1]) outside the jet independent of the composition of the jet. The caffeine-jet decreased the peak of the stimulated [Ca\textsuperscript{2+}]\textsubscript{i}-transient (C\textsubscript{T}) and increased diastolic [Ca\textsuperscript{2+}]\textsubscript{i} ([Ca\textsuperscript{2+}]\textsubscript{diast} or C\textsubscript{D}) in the jet-region (Figure 3A), whereas BDM decreased the [Ca\textsuperscript{2+}]\textsubscript{i}-transient only slightly (Figure 3B). Low [Ca\textsuperscript{2+}]\textsubscript{i}-transient and [Ca\textsuperscript{2+}]\textsubscript{diast} (Figure 3C). Average [Ca\textsuperscript{2+}]\textsubscript{i}-transients decreased by $-36 \pm 6$, $-17 \pm 3$, and $-37 \pm 7\%$, and [Ca\textsuperscript{2+}]\textsubscript{diast} changed by $+82 \pm 28$, $+48 \pm 13$, $-44 \pm 14\%$ respectively in segments exposed to caffeine (n=9), BDM (n=9), and low [Ca\textsuperscript{2+}]\textsubscript{i} (n=9), compared with [Ca\textsuperscript{2+}]\textsubscript{i}-transients and [Ca\textsuperscript{2+}]\textsubscript{diast} outside the jet (see Table and online data supplement). The [Ca\textsuperscript{2+}]\textsubscript{i}-changes were smaller in BZ, consistent with a gradient between regions caused by diffusion of the contents of the jet. Ca\textsuperscript{2+} waves (caffeine: n=9; BDM: n=9; and low [Ca\textsuperscript{2+}]\textsubscript{i}: n=9 [15 muscles]) started systematically in the BZ after the decline of the last stimulated Ca\textsuperscript{2+} transient. These waves propagated into the regions outside and, in the cases of BDM and low [Ca\textsuperscript{2+}]\textsubscript{i}, inside the jet exposed region (Figure 3; supplemental Movie 2 in the online data supplement). Figure 3B (BDM jet) clearly shows 2 initiation sites of Ca\textsuperscript{2+} waves in the BZ and symmetric propagation into regions outside and inside the jet.

Lowering [Ca\textsuperscript{2+}] to 0.2 mmol/L in the jet also triggered Ca\textsuperscript{2+} waves if [Ca\textsuperscript{2+}]\textsubscript{i} in the main solution was slightly increased (from 2 to 2.5 mmol/L). These waves started in the BZ and propagated inside and outside the jet exposed region at different velocities, with waves in the jet region being the slowest (Figure 3C).

### Initiation of Ca\textsuperscript{2+} Waves

Figure 4 shows initiation of Ca\textsuperscript{2+} waves in the BZ of a BDM exposed trabecula. All muscles responded reproducibly to increasing [Ca\textsuperscript{2+}]\textsubscript{i}; at [Ca\textsuperscript{2+}]\textsubscript{i}=1 mmol/L (Figure 4A), only a localized transient in [Ca\textsuperscript{2+}]\textsubscript{i}, ($\approx$300 mmol/L), denoted as initial Ca\textsuperscript{2+} surge (see arrow), occurred along $\approx$100 to 150 $\mu$m of the BZs without apparent propagation of Ca\textsuperscript{2+} waves. The initiating Ca\textsuperscript{2+} surge took place $\approx$325 ms after stimulation; ie, late during twitch relaxation, when F had declined by 70% to 80% (Figure 4B). Increasing [Ca\textsuperscript{2+}]\textsubscript{i}, (Figure 4B and 4C) accelerated, increased the Ca\textsuperscript{2+} surge (Figure 4A), and induced bidirectional propagating Ca\textsuperscript{2+} waves. The initial Ca\textsuperscript{2+} surge always occurred in the BZs late during relaxation of the last stimulated Ca\textsuperscript{2+} transient (see arrows). Increasing [Ca\textsuperscript{2+}]\textsubscript{i}, also further accelerated propagation of the Ca\textsuperscript{2+} waves (Figure 4).

Similar observations were made in low [Ca\textsuperscript{2+}]\textsubscript{i}-exposed muscles. In caffeine-exposed muscles, Ca\textsuperscript{2+} waves did not propagate into the jet region (Figure 3A). This precluded determination of the site of origin of Ca\textsuperscript{2+} waves, but the earliest Ca\textsuperscript{2+} surge was again observed in the BZ. These observations suggest strongly that the initial surge in [Ca\textsuperscript{2+}]\textsubscript{i}, in the BZ initiated Ca\textsuperscript{2+} waves.

Ca\textsuperscript{2+} waves induced by exposure to either BDM, caffeine, or low [Ca\textsuperscript{2+}]\textsubscript{i} started late during relaxation, ie, 40±5 ms after the maximal rate of sarcomere shortening in the stretched segment and 35±15 ms after twitch force had decline below 30% of peak force (F\textsubscript{p} in Figure 4C; Table). The delay between peak of the last stimulated Ca\textsuperscript{2+} transient and the start of the propagating Ca\textsuperscript{2+} transient in BZ decreased inversely with the amplitude of the initial Ca\textsuperscript{2+}-surge (C\textsubscript{w}/C\textsubscript{T}; r=0.61, P<0.001; see supplemental Figure IIs) in all jet exposures.

### Propagation of Ca\textsuperscript{2+} Waves

Propagation velocity of the Ca\textsuperscript{2+} waves (V\textsubscript{prop}) (Figure 5), outside (n=27) and inside (n=13) the jet region, ranged from 0.2 to 2.8 mm/s, ie, comparable to Ca\textsuperscript{2+} waves observed in our damaged muscle studies (0.34 to 5.47 mm/s),\textsuperscript{8-10} V\textsubscript{prop} correlated with the [Ca\textsuperscript{2+}]\textsubscript{i}, increase seen in the BZ during the initial Ca\textsuperscript{2+} surge ($\Delta$C\textsubscript{w} (r=0.66, P<0.0001; n=40; Figure 5C). Furthermore, V\textsubscript{prop} correlated with the amplitude of the waves both inside and outside the jet (data not shown). These
Nonuniformity and Arrhythmias

Nonuniformity of ECC created by the jet induced nondriven rhythmic activity. The arrhythmia consisted of spontaneous switches at regular intervals starting after an after-contraction that followed the last stimulated contraction. The arrhythmia continued until the next stimulus train (7.5 s; Figure 6 [muscle exposed to BDM]). The intervals between nondriven contractions were usually slightly longer than those of the preceding stimulus train. As shown in Figure 6, these arrhythmias terminated abruptly when the jet was turned off and the uniformity of ECC restored.

Discussion

The Model of Nonuniform ECC

We have used in this study a novel model of nonuniform ECC in cardiac muscle to study arrhythmogenic Ca2+ waves underlying TPCs in cardiac muscle. We created nonuniformity in ECC by exposing a small segment of the muscle to caffeine, BDM, or low [Ca2+]o. We expected that (1) low [Ca2+]o would reduce Ca2+ current and the Ca2+ transient attributable to ECC19 despite increased SR-Ca2+ content,16 (2) caffeine would open SR-Ca2+ release channels and thereby deplete the SR,15,17,18 and (3) BDM would modestly affect Ca2+ transients attributable to ECC19,20 because of a reduced SR-Ca2+ content21,22 and potentiation of RyR23 and inhibited crossbridge cycling.20 Consistent with these expectations, the amplitude of stimulated Ca2+ transients decreased dramatically in regions exposed to caffeine and low [Ca2+]o but only slightly with BDM (Figure 3).

Each of these perturbations reduced muscle force because of creation of a muscle segment which developed less twitch force than the normal cells remote from the jet, as is witnessed by stretch of the weakened sarcomeres in the jet by the fully activated sarcomeres outside the exposed region (Figure 2C). These regions were connected mechanically by a border zone of 1 to 2 cells, where the sarcomeres first contracted and, then, were stretched (Figure 2B, region [3]). The diffraction pattern of sarcomeres in the BZ illuminated by an ~150 μm diameter laser beam (ie, 1.5 cell lengths) showed a clear single peak during both shortening and lengthening, strongly suggesting that sarcomere contraction in BZ was also partially suppressed, probably owing to diffusion of the contents of each jet solution.

These observations confirm that this method causes nonuniform ECC along the muscle and affects specifically a
The observation that the effect of force was common to all 3 interventions whereas the effect on [Ca\(^{2+}\)\], and on the Ca\(^{2+}\) transient was dramatically different between the interventions makes it reasonable to assume that Ca\(^{2+}\) waves are initiated as a result of nonuniformity of sarcomere force generation and the resultant sequence of stretch and release of sarcomeres in the BZ by contraction of normal cells in regions remote from the jet.

By varying [Ca\(^{2+}\)]\(_i\) we detected a small localized [Ca\(^{2+}\)], surge in the BZ, which developed into a propagating wave when [Ca\(^{2+}\)], was increased (Figure 4A). Once initiated, these Ca\(^{2+}\) waves traveled from the region with the localized [Ca\(^{2+}\)], rise proving that this region constitutes the initiation site for Ca\(^{2+}\) waves.

The initiating Ca\(^{2+}\)-surge took place late during twitch relaxation when both F and free Ca\(^{2+}\) in the cytosol had decayed by 70% to 80% (Figure 4B). By this time the SR-Ca\(^{2+}\) channels have partially recovered\(^{15}\) and are able to support Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) and Ca\(^{2+}\) wave generation.\(^{15}\) However, the delay between the stimulus-moment and Ca\(^{2+}\)-surge makes it highly unlikely that Ca\(^{2+}\) entry via \(\alpha\)-type Ca\(^{2+}\)-channels causes CICR from the SR and the initial Ca\(^{2+}\) surge. Furthermore, Ca\(^{2+}\) waves never started in jet-exposed regions where sarcomeres were maximally stretched even if the amplitude of the stimulated Ca\(^{2+}\) transients witnessed a robust SR-Ca\(^{2+}\) content.\(^{15}\) Ca\(^{2+}\) waves never started simultaneously with the peak of stretch (Figure 4C) making it unlikely that a stretch-related mechanism such as activation of Gd\(^{3+}\)-sensitive stretch-activated channels\(^{10–14}\) is involved in the initial Ca\(^{2+}\)-surge.

It has been shown that caffeine\(^{25}\) and BDM increase the open probability of SR-Ca\(^{2+}\) release channels (RyR),\(^{21,23}\) whereas low [Ca\(^{2+}\)]\(_i\) could theoretically do so by increasing the SR-Ca\(^{2+}\) load.\(^{18}\) However, several observations make it unlikely that potentiation of RyR caused spontaneous Ca\(^{2+}\) release in the BZ: (1) the same interventions cause no spontaneous Ca\(^{2+}\) release in uniform muscle\(^{26}\) or myocytes\(^{21}\); (2) the effect of these interventions must have been maximal in the jet-exposed region, whereas Ca\(^{2+}\) waves never started in this region.

A Novel Mechanism Underlying Arrhythmias

We suggest that Ca\(^{2+}\) that is bound to Troponin C (TnC) during this phase of twitch underlies the Ca\(^{2+}\)-surge that initiates Ca\(^{2+}\) waves.\(^{27}\) It is well known that a quick release of Ca\(^{2+}\)-activated cardiac muscle induces a surge of Ca\(^{2+}\) ions dissociating from myofilaments\(^{11,28}\) because of rapid reduction of the TnC affinity for Ca\(^{2+}\) owing to a reduction in the number of Ca\(^{2+}\)-activated cross-bridges.\(^{29}\) The concept of quick-release–induced Ca\(^{2+}\) dissociation from TnC, demonstrated in uniform cardiac muscle, is applicable to the chain of cells in the nonuniform muscle exposed to the jet. Rapid sarcomere shortening during the force decline occurred both in the jet region and in the BZ, but led only to a Ca\(^{2+}\) surge and Ca\(^{2+}\) wave initiation in the BZ (Figure 4B), making it probable that quick-release–induced Ca\(^{2+}\) dissociation from TnC caused by the decline of force in the shortening BZ sarcomeres led to the local Ca\(^{2+}\) surge.\(^{11,28–32}\) The region inside the jet, where ECC was all but abolished, probably

selected region of the trabeculae, which results in regional decrease of contractile force of the sarcomeres.

Nonuniform ECC and Initiation of Ca\(^{2+}\) Waves

Ca\(^{2+}\) waves and TPCs have been closely related to Ca\(^{2+}\) overload in damaged regions and the resultant nonuniformity of muscle contraction.\(^4\) However, in that model it is difficult to investigate underlying mechanisms because damage is difficult to control. This study shows clearly that Ca\(^{2+}\) waves are reversibly initiated in regions without damage and, more specifically, from the BZ, in which contraction is partially suppressed. The common effect of the 3 protocols was to suppress contraction and reduce sarcomere force (Figure 2); the latter occurred with caffeine and low [Ca\(^{2+}\)]\(_i\) or without change of the Ca\(^{2+}\) transient (BDM), and with (caffeine) or without change of diastolic [Ca\(^{2+}\)], (BDM).

Figure 3. Three-Dimensional (left) and corresponding 2-Dimensional (right) spatio-temporal representations of [Ca\(^{2+}\)]\(_i\) during induction of TPGs whereas a small segment of the trabecula is exposed to caffeine (CF; A), BDM (B), or low [Ca\(^{2+}\)]\(_i\) (LC; C) solutions. Images show the 2 last stimulated Ca\(^{2+}\) transients (see arrowheads for moments of electrical stimulation) and Ca\(^{2+}\) events occurring subsequently inside (indicated by the dashed lines) and outside the jet-exposed segment. X-axis: time; Y-axis: position along the long axis of the trabecula (Figure 1A in online supplement); Z-axis or color bar. [Ca\(^{2+}\)], [Ca\(^{2+}\)]\(_o\), and [Ca\(^{2+}\)]\(_i\) were 2.0 mmol/L except when low [Ca\(^{2+}\)]\(_i\) was used, [Ca\(^{2+}\)]\(_i\)=0.2 mmol/L was used, [Ca\(^{2+}\)], was 2.5 mmol/L; bath temperature: 23.8 (CF), 23.1 (BDM), and 23.3°C (LC). A, Exp000809cf5-2; B, 000703BDM2(1)1; C, 01051672LC1.
contained either little TnC-Ca\(^{2+}\) (caffeine or low [Ca\(^{2+}\)]\(_o\)) or only few Ca\(^{2+}\)-activated force generating cross-bridges (BDM), which would render a quick release of this region unable to generate a Ca\(^{2+}\) surge and Ca\(^{2+}\) wave. The BZ, on the other hand, could generate a Ca\(^{2+}\) surge that is large enough to induce local CICR and thus a Ca\(^{2+}\) wave even if only a fraction of TnC\(^{33,34}\) were occupied with Ca\(^{2+}\). A quantitative analysis of the relation between quick release dynamics and onset of the Ca\(^{2+}\) surge or waves may shed further light on this mechanism of initiation of Ca\(^{2+}\) waves. Our present study allows for an estimate of the latency (\(\approx 40\ ms\)) between rapid sarcomere shortening and the initial rise of [Ca\(^{2+}\)]\(_i\) during the Ca\(^{2+}\) surge in a small BZ region \(\approx 100 \mu m\) (Figure 4A), although the precision of this estimate

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Figure 4. A, Initiating events of Ca\(^{2+}\) waves induced by local BDM exposure at [Ca\(^{2+}\)]\(_o\)=1, 2, and 4 mmol/L. At low [Ca\(^{2+}\)] in the bath (1 mmol/L, top) only a local Ca\(^{2+}\) surge (starting 360 ms) after stimulation is observed. Increasing [Ca\(^{2+}\)] \(2\) and 4 mmol/L; middle and bottom) led to the initiation of bi-directional Ca\(^{2+}\) waves, which propagate into the segment inside the jet and into the normal muscle. Both amplitude of the initial and propagating transient as well as propagation velocity increased with increase of [Ca\(^{2+}\)], whereas the latency of onset of the Ca\(^{2+}\) transient decreased (300 ms). Arrows indicate initiation sites of propagating waves. B, Ca\(^{2+}\) waves in a region exposed to BDM with [Ca\(^{2+}\)]\(_o\)=2 mmol/L; white arrow in the upper figure indicates termination of 2 opposite waves after they collide. C, Comparison between [Ca\(^{2+}\)] and F and SL in the BZ detected in the BDM experiment of panel B; [Ca\(^{2+}\)] traces results from the average of profiles indicated by the square bracket along the top. The onset (see arrow) of initial [Ca\(^{2+}\)] rise (defined as the moment of the nadir between the last stimulated Ca\(^{2+}\) transient and [Ca\(^{2+}\)] rise) corresponded with the time at which the twitch had relaxed to 10% \(F_{onset}\). The times of peak force \(F_{peak}\), \(-dF/dt_{max}\), and \(F_{30}\) at which the sarcomere shortening rate is maximal are indicated. [Ca\(^{2+}\)]\(_o\)=2.0 mmol/L; temperature was 23.1 °C (A), 23.0 °C (B).

Figure 5. A, Low [Ca\(^{2+}\)]\(_o\)-induced Ca\(^{2+}\) waves and corresponding traces of F and of regional [Ca\(^{2+}\)]\(_i\) (averaged from regions indicated by the brackets) inside (green) and outside (blue) the low [Ca\(^{2+}\)]\(_o\) jet and in the BZ (pink). Propagation velocities \(V_{prop}\) were calculated using a linear regression through peak values (yellow circle) of the Ca\(^{2+}\) wave (a: 2.84, b: 0.22, and c: 0.16 mm/s). The outward wave started (see arrow) at the moment of 21% twitch force. \(C_T\) indicates peak of the last Ca\(^{2+}\)-transient; \(C_{on}\) peak of the initial [Ca\(^{2+}\)] rise in the BZ; \(C_D\) reflects diastolic [Ca\(^{2+}\)] and corresponds to the minimum [Ca\(^{2+}\)] between \(C_T\) and \(C_{on}\); \(t(C_T−C_{on})\) is the latency between \(C_T\) and \(C_{on}\). B, Peak [Ca\(^{2+}\)]\(_i\) of the low [Ca\(^{2+}\)]\(_o\)-induced Ca\(^{2+}\) waves (a, b, and c) were plotted as a function of position along the trabecula. [Ca\(^{2+}\)]\(_o\)=2.5 mmol/L; temperature, 23.3 °C. Exp010516T2LC1.
Ca$^{2+}$ waves propagated in this model at slightly lower velocity (0.2 to 2.8 mm/s) than those of previous studies of regionally damaged muscles.\textsuperscript{9,10,35} In this study the amplification of the Ca$^{2+}$ signal by SR-Ca$^{2+}$ release required for propagation may have been lower in the absence of Ca$^{2+}$ loading of the muscle by damaged areas.\textsuperscript{4–6,8,13,34,36–38} A lower cellular Ca$^{2+}$ load would explain why Ca$^{2+}$ waves did not propagate through regions exposed to caffeine (Figure 3A), which is consistent with the effect of caffeine to deplete SR-Ca$^{2+}$ required for wave propagation.\textsuperscript{8}

**Figure 6.** Nonuniform ECC, Ca$^{2+}$ Waves, and Arrhythmias

One striking finding of this study is the arrhythmogenic nature of mechanically nonuniform myocardium independent of any damage, which is clearly caused by the induction of Ca$^{2+}$ waves (Figure 6). Diastolic [Ca$^{2+}$], transients are known to cause transient depolarizations caused by electrogenic Na$^+$–Ca$^{2+}$ exchange and Ca$^{2+}$-sensitive inward currents\textsuperscript{7,10,39–41} and alter action potential configuration.\textsuperscript{32} Several compartments which normally only release Ca$^{2+}$ during the cardiac cycle in response to the action potential can release Ca$^{2+}$ spontaneously during diastole. Such compartments include the SR in which abnormal Ca$^{2+}$ storage may be arrhythmogenic.\textsuperscript{42–44} In addition, increased open probability of the SR-Ca$^{2+}$ release channels\textsuperscript{45} owing to channel gene mutation\textsuperscript{40,42,46} or to posttranslational channel changes in heart failure may cause arrhythmias.\textsuperscript{43}

In this study of controlled nonuniformity of muscle contraction, we identify nonuniform ECC in cardiac muscle for the first time as a possible arrhythmogenic mechanism. Whether this mechanism plays a role in the wall of the ventricles remains to be proven, although the arrangement of the cardiac wall in muscle fascicles, which transmit force longitudinally and therefore are subject to comparable constraints as trabeculae in this study, makes this possibility highly likely. This mechanism may contribute to arrhythmogenesis in diseased heart where nonuniform segmental wall motion\textsuperscript{46} may result from ischemia, nonuniform electrical activation, or nonuniform adrenergic activation.\textsuperscript{46}

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