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# Spatial non-uniformity of excitation–contraction coupling can enhance arrhythmogenic-delayed afterdepolarizations in rat cardiac muscle

**Masahito Miura[1]*, Yuji Wakayama[2], Hideaki Endoh[2], Makoto Nakano[2], Yoshinao Sugai[2], Masanori Hirose[2], Henk EDJ ter Keurs[3], and Hiroaki Shimokawa[2]**

[1]*Department of Clinical Physiology, Health Science, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan;* [2]*Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan; and* [3]*Department of Medicine, University of Calgary, Calgary, Canada T2N 4M1*

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**Aims** We examined whether non-uniform muscle contraction affects delayed afterdepolarizations (DADs) by dissociating $Ca^{2+}$ from myofilaments within the border zone (BZ) between contracting and stretched regions.

**Methods and results** Force, sarcomere length (SL), membrane potential, and $[Ca^{2+}]_i$ dynamics were measured in 31 ventricular trabeculae from rat hearts. Non-uniform muscle contraction was produced by exposing a restricted region of muscle to a jet of solution containing 20 mmol/L 2,3-butanedione monoxime (BDM). DADs were induced by 7.5 s-2 Hz stimulus trains at an SL of 2.0 μm (24°C, $[Ca^{2+}]_o$ 2.0 mmol/L). The BDM jet enhanced DADs ($n = 6$, $P < 0.05$) and aftercontractions ($n = 6$, $P < 0.05$) with or without 100 μmol/L streptomycin and occasionally elicited an action potential. A stretch pulse from an SL of 2.0 μm to 2.1 or 2.2 μm during the last stimulated twitch of the trains accelerated $Ca^{2+}$ waves in proportion to the increment of force by the stretch ($P < 0.01$) with or without streptomycin. In the presence of 1 mmol/L caffeine, rapid shortening of the muscle after the stretch pulse increased $[Ca^{2+}]_i$ within the BZ, whose amplitude correlated with the increment of force by the stretch ($n = 15$, $P < 0.01$).

**Conclusion** These results suggest that non-uniform muscle contraction can enhance DADs by dissociating $Ca^{2+}$ from myofilaments within the BZ and thereby cause triggered arrhythmias.

## 1. Introduction

Life-threatening ventricular arrhythmias are likely to occur in diseased hearts,[1,2] where non-uniform segmental wall motion commonly occurs[3,4] as a result of ischaemia,[5] heterogeneous adrenergic activation,[6] heterogeneous protein expression,[7] or heterogeneous electrical activation.[8,9] Improving the mechanical uniformity by resynchronization pacing can reduce the occurrence of sudden death in patients with heart failure,[10] indicating a possible arrhythmogenic nature of the mechanically non-uniform muscle contraction.[11] It remains unknown, however, how non-uniformity of muscle contraction in such diseased hearts by itself would affect the development of delayed afterdepolarizations (DADs), which may play a critical role in the incidence of ventricular arrhythmias.[12,13]

Exposing a trabecula to a small jet of solution with a composition that reversibly reduces excitation–contraction coupling (ECC) within the segment can create a non-uniform ECC model of a trabecula,[14] which is amenable to the investigation of the role of non-uniform muscle contraction in arrhythmogenesis. In this model, a surge of $Ca^{2+}$ emerges within the border zone (BZ) between contracting and stretched regions[15–18] and propagates in a wave-like manner along the muscle, triggering arrhythmias.[14] This $Ca^{2+}$ surge always emerges during the relaxation phase,[14,19] whose maximum rate is important for the determination of the amplitude of the $Ca^{2+}$ surge and the propagation velocity of $Ca^{2+}$ waves.[20] In addition, the reduction of sarcomere shortening within the BZ during the relaxation phase can decrease the amplitude of aftercontractions.[20] From these observations, dissociation of $Ca^{2+}$ from the myofilaments within the BZ during relaxation has been proposed as a possible mechanism underlying the emergence of the $Ca^{2+}$ surge.[14,19] It is still unclear, however, how mechanically non-uniform muscle contraction can affect the DADs with the emergence of the $Ca^{2+}$ surge and induce a triggered action potential.

* Corresponding author. Tel/fax: +81 22 717 7920.
*E-mail address:* mmiura@cardio.med.tohoku.ac.jp

Thus, using the model of trabeculae with non-uniform ECC produced by the regional application of 2,3-butanedione monoxime (BDM) jet to trabeculae, we measured the membrane potential to determine how the regional application of the BDM jet affects the membrane potential with the emergence of the $Ca^{2+}$ surge. We also investigated which source of $Ca^{2+}$ is responsible for the formation of the $Ca^{2+}$ surge, using a transient stretch of the muscle length in the presence or absence of a stretch-activated channel blocker. Results of the present study indicate that non-uniform muscle contraction can enhance DADs by inducing a $Ca^{2+}$ surge within the BZ without a further global increment of intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) and that the $Ca^{2+}$ surge consists of the $Ca^{2+}$ dissociated from the myofilaments within the BZ, but not the $Ca^{2+}$ through a stretch-activated channel.

## 2. Methods

### 2.1 Measurements of force, membrane potential, $[Ca^{2+}]_i$, and sarcomere length in rat trabeculae

All animal procedures were performed according to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Briefly, trabeculae ($n = 31$, length $2.0 \pm 0.4$ mm, width $225 \pm 37$ μm, thickness $89 \pm 4$ μm in a slack condition) were dissected from the right ventricle of rats and mounted between a force transducer and a micromanipulator with a direct current torque motor, which was controlled by a personal computer, in a bath superfused with HEPES solution on an inverted microscope.[21,22] Sarcomere length (SL) was determined from the first order of the He–Ne laser light diffraction band, and membrane potential was measured using ultracompliant glass microelectrodes, as described previously.[23]

$[Ca^{2+}]_i$ was measured as described previously.[21,22] Briefly, fura-2 pentapotassium salt was microinjected electrophoretically into a trabecula. Excitation light of 340, 360, or 380 nm was used, and the fluorescence was collected using a photomultiplier tube or an image-intensified CCD camera at 30 frames/s to assess local $[Ca^{2+}]_i$. We calculated $[Ca^{2+}]_i$ from the calibrated ratio of $F_{360}/F_{380}$ in the region of interest along the trabecula.

### 2.2 Reduction of local contraction

A non-uniform ECC model was produced as described previously.[14] Briefly, a restricted region of a trabecula was exposed to a small jet of solution (~0.06 mL/min) directed perpendicular to a small muscle segment (~300 μm) using a syringe pump connected to a glass pipette (~100 μm in diameter). To reduce contraction in the exposed region, the jet solution was composed of standard HEPES solution containing 20 mmol/L BDM. The $Ca^{2+}$ concentration in the jet solution was set at 1 mmol/L in all measurements.

### 2.3 Experimental protocol

Trains of electrical stimuli at intervals of 500 ms for 7.5 s were repeated every 15 s ($[Ca^{2+}]_o$ 2.0 mmol/L; SL 2.0 μm; temperature 24°C). First, force, membrane potential, and $[Ca^{2+}]_i$ were measured when the BDM jet was turned off or on. To determine the roles of stretch-activated channels, 100 μmol/L streptomycin, a stretch-activated channel blocker,[24,25] was added both to the superfusing HEPES solution and to the jet solution. Second, to augment the non-uniform muscle contraction produced by the BDM jet, a 5 or 10% stretch pulse was imposed for 240 ms during the last stimulated twitch contraction of the trains[23] in the presence or absence of 100 μmol/L streptomycin, and force and spatio-temporal changes in $[Ca^{2+}]_i$ were then measured. Finally, to detect the $Ca^{2+}$ dissociated from myofilaments, changes in $[Ca^{2+}]_i$ within the BZ created by the BDM jet were measured in the presence of 1 mmol/L caffeine with or without the stretch pulse for 400 ms. $\Delta[Ca^{2+}]_i$ was calculated from the maximal difference between $[Ca^{2+}]_i$ without the stretch pulse and that with the stretch pulse.

### 2.4 Statistics

All measurements were expressed as mean ± SEM. Statistical analysis was performed using ANOVA and linear regression analysis as appropriate. Values of $P < 0.05$ were considered to be significant.

## 3. Results

Regional application of 20 mmol/L BDM to trabeculae reduced electrically stimulated twitch forces by $41 \pm 6\%$ ($n = 6$), but inversely enhanced aftercontractions and DADs by inducing a $Ca^{2+}$ surge within the BZ (*Figure 1A*). As shown in *Figure 1B*, this regional application increased the amplitudes of aftercontractions and DADs and shortened the intervals between the last stimulus of the trains and their peaks, although it did not change the peak values of the electrically stimulated $Ca^{2+}$ transients outside the exposed region (*Figure 2*). Moreover, the regional application of the BDM jet enhanced DADs sufficiently to induce a triggered action potential when $[Ca^{2+}]_o$ was further increased (*Figure 3A*), showing the arrhythmogenic nature of the mechanically non-uniform muscle contraction. Even in the presence of 100 μmol/L streptomycin, a stretch-activated channel blocker,[24,25] the regional application of the BDM jet increased the amplitudes of aftercontractions and DADs and shortened the intervals between the last stimulus of the trains and their peaks (*Figure 3B*).

The regional application of the BDM jet to trabeculae initiated a $Ca^{2+}$ wave from the BZ with aftercontractions, as reported previously.[14] When a 240 ms stretch pulse was imposed during the last twitch of the electrical trains to augment sarcomere shortening within the BZ during the relaxation phase (*Figure 4A*), the stretch increased the twitch force, and the quick return of muscle length following the stretch induced a larger $Ca^{2+}$ surge and initiated a faster $Ca^{2+}$ wave (*Figure 4B*). The changes in propagation velocity of $Ca^{2+}$ wave following a 240 ms stretch pulse correlated significantly with changes in twitch force during the stretch pulse in the presence or absence of streptomycin (*Figure 5A*). In addition, the augmented sarcomere shortening following the stretch pulse increased the amplitude of aftercontractions and shortened the interval between the last stimulus of trains and their peaks, even in the presence or absence of streptomycin (*Figure 5B*). Note that the changes in the presence of streptomycin were not significantly different from those in the absence of streptomycin.

To examine the roles of $Ca^{2+}$ dissociated from myofilaments within the BZ in initiating $Ca^{2+}$ waves, the changes in $[Ca^{2+}]_i$ following a stretch pulse ($\Delta[Ca^{2+}]_i$), which could have been responsible for the $Ca^{2+}$ surge in *Figure 4B*, were measured within the BZ in the presence of 1 mmol/L caffeine (*Figure 6A*). When the amplitude of $\Delta[Ca^{2+}]_i$ within the BZ was plotted against changes in twitch force by transient stretch, they were significantly correlated (*Figure 6B*), suggesting that the $Ca^{2+}$ dissociated from myofilaments owing to the fall in the affinity of troponin C for $Ca^{2+}$ can enhance the $Ca^{2+}$ surge within the BZ, thereby accelerating the $Ca^{2+}$ waves.

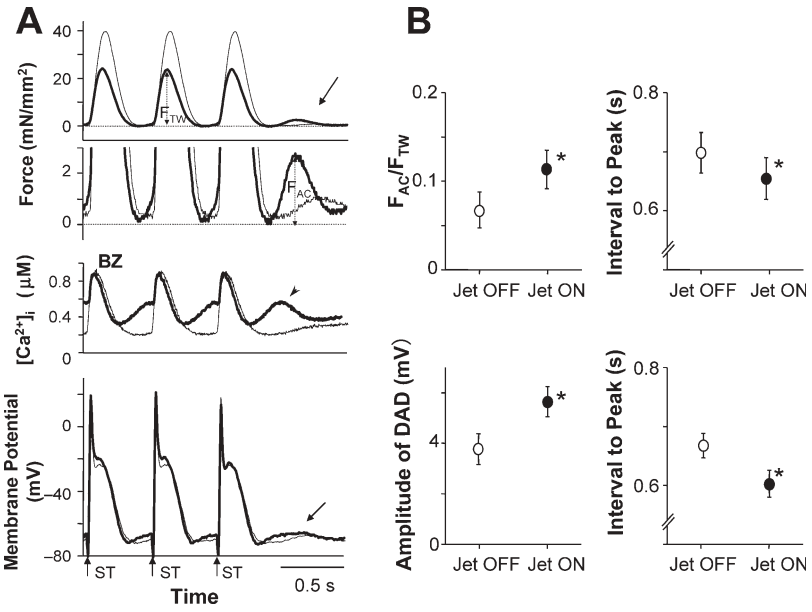


**Figure 1** Effect of regional jet exposure on aftercontractions and delayed afterdepolarizations (DADs). (*A*) Representative recordings of force, intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$), and membrane potential when a 20 mmol/L 2,3-butanedione monoxime jet was turned OFF (thin lines) or ON (thick lines). The upper panels show force recordings and the expansion of the recordings in the ordinate, the middle panel shows $[Ca^{2+}]_i$ within the border zone (BZ), and the lower panel shows membrane potential during the last three electrical stimuli (500 ms stimulation interval). When the 2,3-butanedione monoxime jet was turned ON, the amplitude of electrically stimulated twitches ($F_{TW}$) decreased, but the amplitudes of a subsequent aftercontraction increased ($F_{AC}$, arrow in the upper panel) and a delayed afterdepolarization increased (arrow in the lower panel) with the emergence of the $Ca^{2+}$ surge (arrowhead in the middle panel). Arrows with ST indicate the moments of electrical stimulation ($[Ca^{2+}]_o$ 2 mmol/L, temperature 24.0°C, Experiment Number 060623). (*B*) Aftercontractions and delayed afterdepolarizations when a 20 mmol/L 2,3-butanedione monoxime jet was turned OFF (open circles, $n = 6$) or ON (closed circles, $n = 6$). The upper panels show ratios of $F_{AC}$ to $F_{TW}$ and the intervals between the last stimulus of a train and the peaks of aftercontractions. The lower panels show the amplitude of delayed afterdepolarizations and the intervals between the last stimulus of a train and the peaks of delayed afterdepolarizations. *$P < 0.05$ vs. Jet OFF.

## 4. Discussion

The present study characterized the roles of non-uniform muscle contraction in the development of DADs and the emergence of a $Ca^{2+}$ surge, using a non-uniform ECC model of trabeculae. To the best of our knowledge, the present study shows for the first time that non-uniform muscle contraction can enhance DADs by dissociating $Ca^{2+}$ from myofilaments within the BZ and work as a possible arrhythmogenic mechanism without a further global increment of $[Ca^{2+}]_i$, as will be discussed in what follows.

### 4.1 Enhancement of delayed afterdepolarizations by non-uniform excitation–contraction coupling

Interventions, which increase the $Ca^{2+}$ load of cardiac muscle, such as high $[Ca^{2+}]_o$,[21,26] digitalis glycosides,[27,28] and β-adrenergic agonists,[29] are known to induce spontaneous $Ca^{2+}$ release from the sarcoplasmic reticulum (SR)[30] and generate DADs through electrogenic $Na^+$–$Ca^{2+}$ exchanger[31,32] and $Ca^{2+}$-activated $Cl^-$ channels.[33,34] In contrast, the present study showed for the first time that non-uniform muscle contraction enhanced DADs (*Figure 1*) without requiring a further global increase in $[Ca^{2+}]_i$ outside the exposed region of trabeculae (*Figure 2*) and occasionally elicited action potentials (*Figure 3A*). Regarding the mechanism of this arrhythmogenesis, it has been reported that, whenever a $Ca^{2+}$ wave occurs, it is accompanied by a depolarization similar to a DAD.[35–37] Without regional exposure to the BDM jet, $Ca^{2+}$ waves start from both ends of trabeculae with DADs because in this preparation both ends of trabeculae are fixed and can be stretched during twitch contraction.[21,22] When the BDM jet is turned on, this

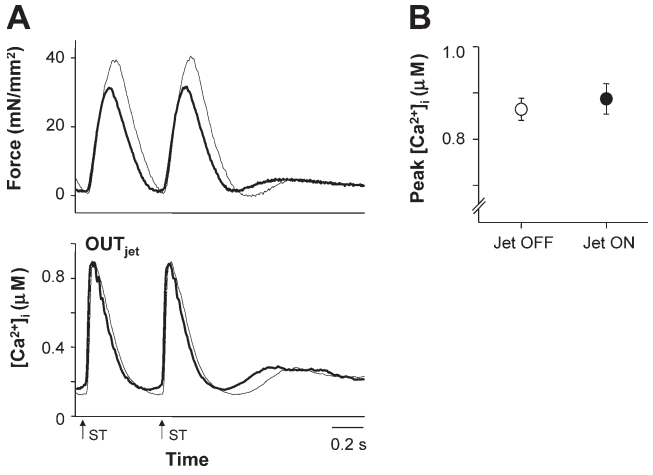


**Figure 2** Effect of regional jet exposure on electrically stimulated $Ca^{2+}$ transients. (*A*) Representative recordings of force and intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) outside the exposed region ($OUT_{jet}$) when a 20 mmol/L 2,3-butanedione monoxime jet was turned OFF (thin lines) or ON (thick lines). The upper panel shows force recordings and the lower panel shows $[Ca^{2+}]_i$ transients within $OUT_{jet}$ during the last two electrical stimuli (500 ms stimulation interval). When the 2,3-butanedione monoxime jet was turned ON, the amplitude of electrically stimulated twitches decreased, but the peaks of the stimulated $[Ca^{2+}]_i$ transients showed no change. Arrows with ST indicate the moments of electrical stimulation ($[Ca^{2+}]_o$ 2 mmol/L, temperature 23.9°C, Experiment Number 050210). (*B*) Peak values of the stimulated $[Ca^{2+}]_i$ transients within $OUT_{jet}$ when a 20 mmol/L 2,3-butanedione monoxime jet was turned OFF (open circles, $n = 8$) or ON (closed circles, $n = 8$). There was no significant change.

regional exposure can induce a $Ca^{2+}$ surge within the BZ (*Figure 1A*) and initiate new $Ca^{2+}$ waves from the BZ[14] with additional depolarization of the membrane, suggesting that

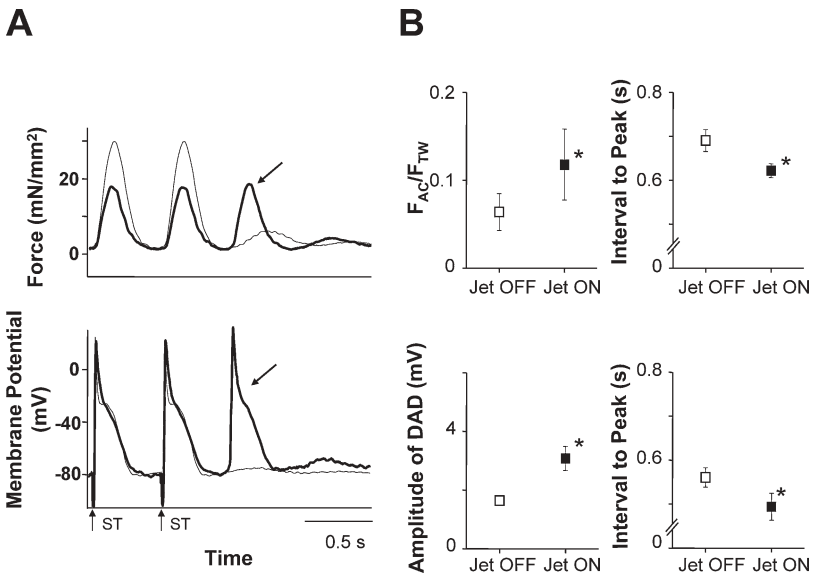


**Figure 3** (*A*) Induction of an action potential by regional jet exposure. Force and membrane potential at higher $[Ca^{2+}]_o$ when a 20 mmol/L 2,3-butanedione monoxime jet was turned OFF (thin lines) or ON (thick lines). The upper panel shows force recordings and the lower panel shows membrane potential during the last two electrical stimuli (500 ms stimulation interval). When the 2,3-butanedione monoxime jet was turned ON, a twitch contraction (arrow) in the upper panel and an action potential (arrow) in the lower panel were induced. Arrows with ST indicate the moments of electrical stimulation ($[Ca^{2+}]_o$ 3 mmol/L, temperature 24.2°C, Experiment Number 060809). (*B*) Effect of regional jet exposure on aftercontractions and delayed afterdepolarizations (DADs) in the presence of 100 μmol/L streptomycin when a 20 mmol/L 2,3-butanedione monoxime jet was turned OFF (open squares, $n = 6$) or ON (closed squares, $n = 6$). The upper panels show ratios of the amplitude of aftercontractions ($F_{AC}$) to the amplitude of electrically stimulated twitches ($F_{TW}$) and the intervals between the last stimulus of a train and the peaks of aftercontractions. The lower panels show the amplitude of delayed afterdepolarizations and the intervals between the last stimulus of a train and the peaks of delayed afterdepolarizations. $^*P < 0.05$ vs. Jet OFF.

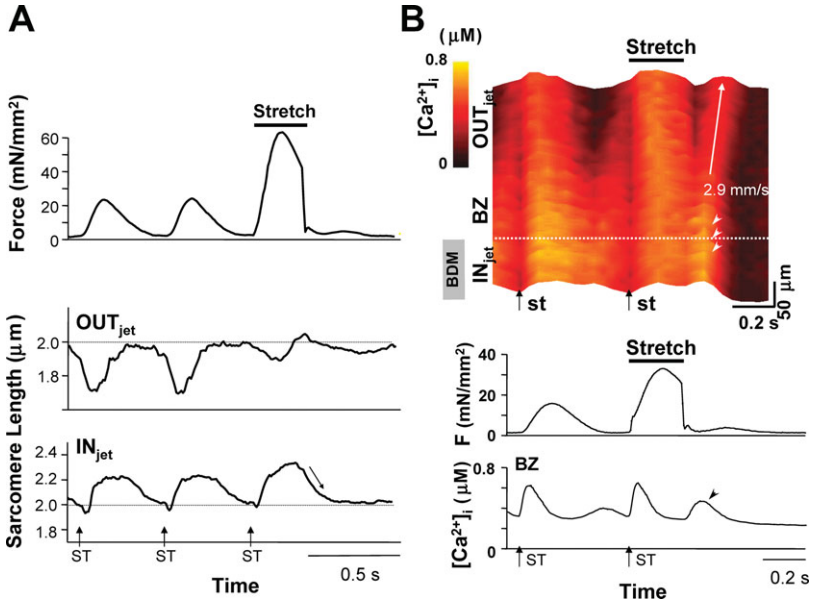


**Figure 4** Effect of a 240 ms stretch pulse during the last stimulated twitch of the electrical trains on force, sarcomere length, and intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) when a 20 mmol/L 2,3-butanedione monoxime (BDM) jet was turned ON. $IN_{jet}$ indicates the region exposed to the 2,3-butanedione monoxime jet, $OUT_{jet}$ indicates the region outside the jet, and BZ indicates the border zone between $IN_{jet}$ and $OUT_{jet}$. Arrows with ST indicate the moments of electrical stimulation. (*A*) The upper panel shows a force recording and the lower panels show the recordings of sarcomere length within the $OUT_{jet}$ and $IN_{jet}$ during the last three stimulated twitches (500 ms stimulation interval). During the stimulated twitches, sarcomere length showed normal active shortening within the $OUT_{jet}$ and passive stretch within the $IN_{jet}$. Note that the 240 ms stretch pulse augmented the sarcomere shortening within the $IN_{jet}$ during the relaxation phase (arrow) ($[Ca^{2+}]_o$ 2 mmol/L, temperature 23.6°C, Experiment Number 051026). (*B*) The upper panel shows three-dimensional spatio-temporal representations of $[Ca^{2+}]_i$ during the last two electrical stimuli (500 ms stimulation interval). The abscissa is time, the ordinate is $[Ca^{2+}]_i$, and the *Z*-axis is the position along the long axis of the trabecula. The middle panel shows force (F) and the lower panel shows $[Ca^{2+}]_i$ within the border zone. The quick return of muscle length following the stretch induced a larger $Ca^{2+}$ surge (arrowheads in the upper panel and the lower panel) and increased the velocity of the $Ca^{2+}$ wave from 1.6 to 2.9 mm/s (arrow) ($[Ca^{2+}]_o$ 2 mmol/L, temperature 24.2°C, Experiment Number 051005).

these new $Ca^{2+}$ waves from the BZ can cause the enhancement of the DADs. Here, it is noteworthy that BDM is unlikely to involve the enhancement of DADs by its direct effect on the SR because BDM modestly reduces SR–$Ca^{2+}$content[38,39] to below the threshold for $Ca^{2+}$ waves[40] owing to the regulation of the ryanodine receptors.[41] Rather, the main action of BDM

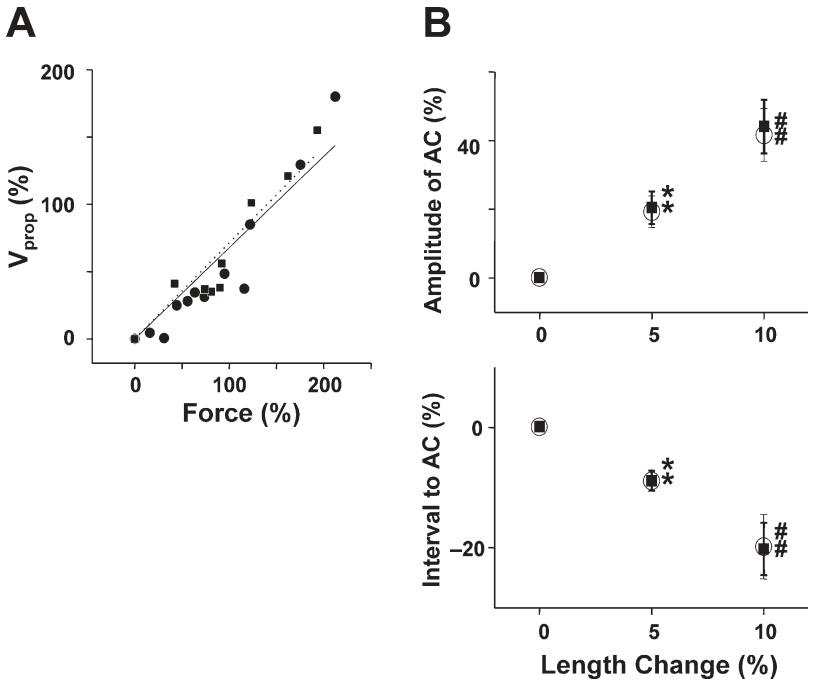


**Figure 5** Effect of a 240 ms stretch pulse during the last stimulated twitch of an electrical train (500 ms stimulation interval) on the velocity of $Ca^{2+}$ waves ($V_{prop}$) and aftercontractions (ACs) when a 20 mmol/L 2,3-butanedione monoxime jet was turned ON. (*A*) Changes in $V_{prop}$ following a 240 ms stretch pulse are plotted as a function of changes in the last stimulated twitch force caused by the transient stretch in the presence (closed squares, $n = 9$) or absence of 100 μmol/L streptomycin (closed circles, $n = 12$). They are linearly correlated in the presence ($r = 0.84$, $P < 0.01$, broken line) or absence of streptomycin ($r = 0.82$, $P < 0.01$, solid line). (*B*) Changes in the amplitude of aftercontractions (upper panel) and those in intervals between the last stimulus and the peaks of aftercontractions (lower panel) are plotted against the changes in muscle length caused by the stretch pulse in the presence (closed squares, $n = 6$) or absence of 100 μmol/L streptomycin (open circles, $n = 6$). Changes in the presence of streptomycin were not significantly different from those in the absence of streptomycin. *$P < 0.05$ vs. stretch (-), #$P < 0.01$ vs. stretch (-).

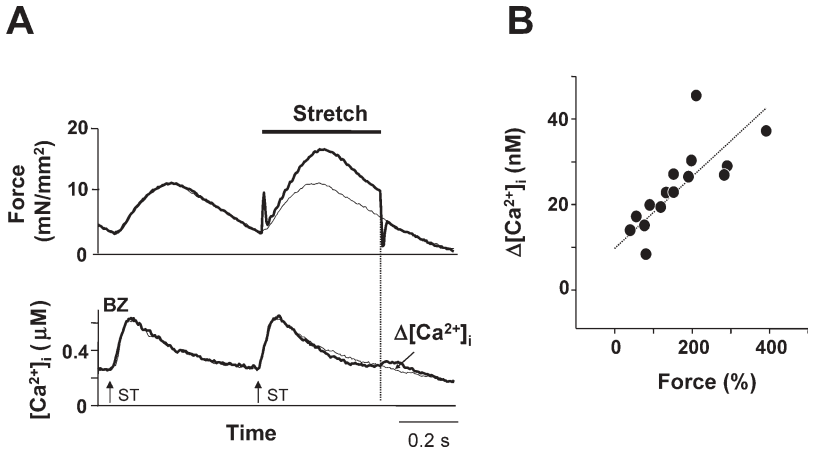


**Figure 6** Changes in $[Ca^{2+}]_i$ by a stretch pulse in the presence of 1 mmol/L caffeine when a 20 mmol/L 2,3-butanedione monoxime jet was turned ON. (*A*) Effect of a 400 ms stretch pulse during the last stimulated twitch of an electrical train (500 ms stimulation interval) on force and $[Ca^{2+}]_i$ in the presence of 1 mmol/L caffeine with regional exposure to the jet. The upper panel shows force recordings and the lower panel shows $[Ca^{2+}]_i$ transients within the border zone during the last two electrical stimuli (500 ms stimulation interval). Thin tracings indicate recordings without a stretch and thick tracings indicate those with a 5% stretch pulse. The arrow indicates a $Ca^{2+}$ surge at the moment of quick return of muscle length after the stretch pulse ($\Delta[Ca^{2+}]_i$). Note that the $Ca^{2+}$ surge emerged not during the stretch but upon shortening after the stretch pulse ($[Ca^{2+}]_o$ 2 mmol/L, temperature 24.4°C, Experiment Number 060308). (*B*) $\Delta[Ca^{2+}]_i$ within the border zone is plotted as a function of changes in the last stimulated twitch force caused by the transient stretch ($n = 15$). They are linearly correlated ($r = 0.80$, $P < 0.01$).

in the present study was to inhibit cross-bridge cycling within cardiac muscle.[42]

## 4.2 Role of $Ca^{2+}$ dissociated from myofilaments

We have reported previously that the non-uniform muscle contraction produced by regional application of caffeine, BDM, or low $[Ca^{2+}]_o$ can induce a $Ca^{2+}$ surge within the BZ during the relaxation phase and initiate a $Ca^{2+}$ wave from the BZ.[14] Indeed, in the present study, the regional application of the BDM jet induced a $Ca^{2+}$ surge within the BZ (*Figure 1A*) and initiated a $Ca^{2+}$ wave. This $Ca^{2+}$ surge emerged not during the stretch but upon shortening after a stretch pulse in the presence of 1 mmol/L caffeine[40,43] (*Figure 6A*), suggesting that $Ca^{2+}$ through a stretch-activated channel[44] and $Ca^{2+}$ released from the SR play

only minor roles in the generation of the $Ca^{2+}$ surge, because 1 mmol/L caffeine is a concentration sufficient to suppress $Ca^{2+}$ waves.[21] Actually, non-uniform muscle contraction enhanced DADs even in the presence of 100 μM streptomycin[24,25] (*Figure 3B*).

On the other hand, we have reported previously that the maximum rate of force relaxation can determine the amplitude of the $Ca^{2+}$ surge[20] and that the reduction of sarcomere shortening within the BZ during relaxation can decrease the amplitude of aftercontractions.[20] In addition, in the present study, augmentation of sarcomere shortening during relaxation resulted in a higher $Ca^{2+}$ surge (*Figure 4*) and accelerated $Ca^{2+}$ waves from the BZ (*Figure 5A*) with larger aftercontractions even in the presence of streptomycin (*Figure 5B*), depending on the changes in twitch force by the muscle stretch. These observations suggest that the more $Ca^{2+}$ is dissociated from myofilaments owing to shortening of sarcomeres in the BZ,[15–18] the higher the emergent $Ca^{2+}$ surges, resulting in faster $Ca^{2+}$ waves with higher aftercontractions.[45] Taken together, these results support the hypothesis that $Ca^{2+}$ dissociated from myofilaments by rapid sarcomere shortening in the BZ during the decline of force initiates $Ca^{2+}$ waves[19] and enhances DADs of sufficient amplitude to trigger an action potential.[33,35]

### 4.3 Clinical implications

$Ca^{2+}$ overload within cardiac muscle, especially in the SR, has been regarded as the basal mechanism involved in the occurrence of DADs.[26–29] The present study shows, however, that non-uniformity of muscle contraction can enhance DADs without a further global increase in $[Ca^{2+}]_i$ and occasionally generate triggered arrhythmias. Because the arrangement in the cardiac wall of muscle fascicles transmits force longitudinally and causes corresponding constraints in ventricles, it is reasonable to assume that regional differences in contractile strength in diseased hearts can enhance DADs and cause arrhythmias in the same manner as shown in trabeculae. This may explain, at least in part, why cardiac resynchronization therapy has reduced the occurrence of sudden death as well as death due to worsening heart failure.[10] Therefore, the mechanical non-uniformity of muscle contraction may work by itself as a possible arrhythmogenic mechanism in the diseased hearts.

**Conflict of interest:** none declared.

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