Three Distinct Types of Ca\(^{2+}\) Waves in Langendorff-Perfused Rat Heart Revealed by Real-Time Confocal Microscopy

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Abstract—Although Ca\(^{2+}\) waves in cardiac myocytes are regarded as arrhythmogenic substrates, their properties in the heart in situ are poorly understood. On the hypothesis that Ca\(^{2+}\) waves in the heart behave diversely and some of them influence the cardiac function, we analyzed their incidence, propagation velocity, and intercellular propagation at the subepicardial myocardium of fluo 3–loaded rat whole hearts using real-time laser scanning confocal microscopy. We classified Ca\(^{2+}\) waves into 3 types. In intact regions showing homogeneous Ca\(^{2+}\) transients under sinus rhythm (2 mmol/L [Ca\(^{2+}\)]\(_{\text{i}}\)), Ca\(^{2+}\) waves did not occur. Under quiescence, the waves occurred sporadically (3.8 waves \(\cdot\) min\(^{-1}\) \cdot cell\(^{-1}\)), with a velocity of 84 \(\mu\)m/s, a decline half-time \((t_{1/2})\) of 0.16 seconds, and rare intercellular propagation (propagation ratio <0.06) (sporadic wave). In contrast, in presumably Ca\(^{2+}\)-overloaded regions showing higher fluorescent intensity (113% versus the intact regions), Ca\(^{2+}\)\(_{\text{i}}\) waves occurred at 28 waves \(\cdot\) min\(^{-1}\) \cdot cell\(^{-1}\) under quiescence with a higher velocity (116 \(\mu\)m/s), longer decline time \((t_{1/2}=0.41\) second), and occasional intercellular propagation (propagation ratio=0.23) (Ca\(^{2+}\)-overloaded wave). In regions with much higher fluorescent intensity (124% versus the intact region), Ca\(^{2+}\) waves occurred with a high incidence (133 waves \(\cdot\) min\(^{-1}\) \cdot cell\(^{-1}\)) and little intercellular propagation (agonal wave). We conclude that the spatiotemporal properties of Ca\(^{2+}\) waves in the heart are diverse and modulated by the Ca\(^{2+}\)-loading state. The sporadic waves would not affect cardiac function, but prevalent Ca\(^{2+}\)-overloaded and agonal waves may induce contractile failure and arrhythmias. (Circ Res. 2000;86:1093-1099.)

Key Words: Ca\(^{2+}\) wave ■ confocal microscopy ■ intercellular propagation ■ Langendorff-perfused heart

The elevation of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{\text{i}}\)) in cardiac contractile activities originates from elementary sarcoplasmic reticulum (SR) Ca\(^{2+}\)-release events, called Ca\(^{2+}\) sparks.\(^1\)–\(^3\) During membrane depolarization in the action potentials, simultaneous summation of Ca\(^{2+}\) sparks produces spatially uniform elevation of [Ca\(^{2+}\)]\(_{\text{i}}\), throughout the cells, ie, Ca\(^{2+}\) transients. Conversely, under unstimulated conditions, recruitment of Ca\(^{2+}\) sparks produces spontaneous localized elevation of [Ca\(^{2+}\)]\(_{\text{i}}\), which propagates within myocytes, especially under Ca\(^{2+}\) overload. This propagating [Ca\(^{2+}\)]\(_{\text{i}}\), elevation, originally observed as a wavelike motion of contractile bands in cardiac myocytes,\(^4\)–\(^6\) has been called the Ca\(^{2+}\) wave\(^7\)\(^8\) and is regarded as the origin of oscillatory membrane potentials leading to triggered arrhythmias.\(^9\) For the past decade, the properties of Ca\(^{2+}\) waves in cardiac myocytes have been studied precisely after the advent of fluorescent Ca\(^{2+}\) indicators\(^7\)–\(^13\) and laser scanning confocal microscopy.\(^11\)–\(^13\)

Despite ample information on Ca\(^{2+}\) waves,\(^7\)–\(^13\) the role of the waves in the heart in situ is poorly understood. This is because Ca\(^{2+}\) waves have been studied mostly in enzymatically isolated cells. Minamikawa et al\(^14\) first demonstrated that Ca\(^{2+}\) waves occur in the perfused rat whole heart. However, their detailed quantitative properties were not assessed because of the low temporal resolution of the X-Y images. We have developed a system for in situ imaging of [Ca\(^{2+}\)]\(_{\text{i}}\), equipped with a multipinhole-type confocal scanning device, which enabled us to visualize real-time X-Y images of Ca\(^{2+}\) waves.\(^15\)\(^16\) Using this system on Langendorff-perfused rat hearts with simultaneous recording of electrocardiograms, we found that Ca\(^{2+}\) waves were completely abolished by ventricular excitation, suggesting that the waves in the whole heart play little, if any, pathophysiological role.\(^15\) Nevertheless, it is possible that Ca\(^{2+}\) waves have some aggravating role on cardiac function if they occur frequently and propagate beyond individual cells on a large scale under certain Ca\(^{2+}\)-overloaded conditions. In this regard, quantitative analysis of Ca\(^{2+}\) waves in the working whole heart is essential to understand their functional significance.

We postulated that Ca\(^{2+}\) waves in the whole heart exhibit various distinct frequencies, propagation velocities, and intercellular propagations, all of which may depend on the

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degree of Ca\(^{2+}\) overload and electrical activities of the heart, ie, Ca\(^{2+}\) waves may occur more frequently and propagate more prevalently to the surrounding cells under highly Ca\(^{2+}\)-overloaded conditions. To examine these hypotheses, we used real-time confocal microscopy to conduct quantitative analyses of Ca\(^{2+}\) waves on the perfused rat whole hearts, focusing on spatiotemporal occurrence and intercellular propagation under both the intact and Ca\(^{2+}\)-overloaded conditions.

Materials and Methods
The preparation of the fluo 3-AM–loaded rat hearts and the confocal system were identical to those described previously.\(^\text{1,15}\) The experiments were conducted on 16 male Wistar rats (9 weeks old) at 23°C to 25°C under Langendorff perfusion (5 mL/min) with Tyrode’s solution ([Ca\(^{2+}\)]\(_0\)=2 mmol/L, unless otherwise specified). The confocal plane was optimized with a glass coverslip (170-μm thickness) placed gently on the heart. The motion artifact on the image was prevented by 20 mmol/L 2,3-butanedione monoxime (BDM), which was added to the perfusate. The ECG was recorded through silver wires (0.3-mm diameter) inserted into the apical region of the heart. Unless otherwise specified, Ca\(^{2+}\) waves were analyzed under quiescence via blockade of atrioventricular conduction produced by mechanical ablation of the atrioventricular junction. Some hearts were electrically stimulated through the silver wires. In some experiments, a glass microelectrode (tip diameter <10 μm) was inserted into localized regions to apply damage to the myocardium.

Analyses of images were conducted on digitized fluorescent signals (512×480 pixels) stored on a hard disk every 33 ms. Line-scan images were reconstructed by cumulatively layering a series of consecutive X-Y image frames scanned according to the line along the path of the waves. The propagation velocity was calculated from the slope of the line-scan image. The profile of Ca\(^{2+}\) waves was obtained by averaging the line-plot data of line-scan images from 5 to 7 adjoining scan lines at the middle of the cells along the long axis. The fluorescence intensity (FI) in a region of interest (ROI) (FI\(_{ROI}\)) was obtained by averaging the FI value of 250 pixels during diastole. With regions with homogeneous Ca\(^{2+}\) transients but no frequent Ca\(^{2+}\) waves regarded as intact, FI was estimated by use of a value obtained from FI\(_{ROI}\) relative to that in the neighboring intact region (FI\(_{INT}\)), ie, FI\(_{ROI}/FI_{INT}\). The incidence of Ca\(^{2+}\) waves and their intercellular propagation ratio (R\(_{prop}\)) were analyzed from continuously recorded video images. R\(_{prop}\) was defined as the ratio of the number of waves propagating to the adjacent cells over the total number of waves in an ROI for 5 minutes. The quantitative data (mean±SD) were statistically analyzed by ANOVA, and significance at P<0.05 was defined by Fisher’s test. An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results

Ca\(^{2+}\) Waves in the Intact Region
Figure 1A shows fluo 3-fluorescent images on a subepicardial region of the perfused heart at 2 mmol/L [Ca\(^{2+}\)]\(_0\). The Ca\(^{2+}\) transients occurred simultaneously with QRS waves, whereas no significant fluorescent elevation occurred during diastole. Similar findings were obtained in 12 other hearts with regular rhythm at 64±12 bpm. When the ventricular excitation rate was decreased (<5 bpm) by blockade of atrioventricular conduction (n=9), Ca\(^{2+}\) waves appeared (Figure 1B, 1 through 5) sporadically at a mean frequency of 3.8±2.4 (0.8 to 14.0) waves·min\(^{-1}\)·cell\(^{-1}\) (70 cells in 9 hearts) and were abolished by a spontaneous Ca\(^{2+}\) transient (6 through 10). Ca\(^{2+}\) transients evoked by electrical stimulation also abolished Ca\(^{2+}\) waves (not shown).

Ca\(^{2+}\) waves in the perfused hearts exhibited intracellular propagation similar to those in isolated myocytes studied previously.\(^\text{7–11}\) Sequential X-Y images at 2 mmol/L [Ca\(^{2+}\)]\(_0\) showed that the waves propagated along the longitudinal axis of the cells (Figure 1C). The corresponding line-scan images (Figure 1D) revealed that the waves proceeded at a constant velocity of ≈90 μm/s in 1 (a) or 2 (b) directions. In some cases, 2 waves collided within single cells and were subsequently annihilated (c). Ca\(^{2+}\) waves propagated longitudinally at 84±16 (52 to 129) μm/s, either unidirectionally or bidirectionally to the cell boundaries (n=73). The waves showed a monotonic decline in their fluorescent profile (Figure 1E) similar to that in isolated myocytes.\(^\text{17}\) After a quick rise, the waves declined with a decay half-time (τ\(_{1/2}\)) of 0.16±0.10 seconds (n=60). As for the intercellular propagation of Ca\(^{2+}\) waves, the waves in the perfused heart barely transmitted to the surrounding myocytes. Of 320 waves, 12 showed propagation to the adjacent myocyte with an R\(_{prop}\) of 0.034. Hereafter, we call these waves in the intact regions sporadic waves.

[Ca\(^{2+}\)]\(_0\) and Stimulus Dependence of the Sporadic Ca\(^{2+}\) Waves
The incidence of sporadic waves increased at higher [Ca\(^{2+}\)]\(_0\), (Figure 2A). At 4 or 6 mmol/L, 2 Ca\(^{2+}\) waves often collided and subsequently annihilated within single cells. The waves at higher [Ca\(^{2+}\)]\(_0\), showed a tendency to propagate with higher velocity (Figure 2B) and were also abolished by a Ca\(^{2+}\) transient. At such higher [Ca\(^{2+}\)]\(_0\), the waves occurred even between repetitive excitations at 1 Hz (not shown). Even at 6 mmol/L [Ca\(^{2+}\)]\(_0\), Ca\(^{2+}\) waves barely showed intercellular propagation (R\(_{prop}\)=0.057, n=35).

The sporadic waves occurred according to previous excitation. In one representative region (Figure 2C, data composed of ≈12 cells), the waves appeared after excitation by 30-second consecutive electrical stimulation. At 2 mmol/L [Ca\(^{2+}\)]\(_0\), they appeared within 30 seconds after 1-Hz stimuli (×) and occurred more frequently over time until the steady state ≈70 seconds after stimuli (left). As the stimulation frequency increased (∇ for 2 Hz, ○ for 3 Hz), the latency for the first appearance of the waves shortened and the waves occurred more frequently. The latency period also shortened when [Ca\(^{2+}\)]\(_0\) increased. At 4 mmol/L [Ca\(^{2+}\)]\(_0\), stimuli at ≈2 Hz produced a tremendous number of Ca\(^{2+}\) waves immediately after stimuli and a subsequent gradual decrease in incidence (right). Such robust Ca\(^{2+}\) waves barely propagated to the adjacent myocytes. Similar stimulation dependence of the waves was obtained in 5 other hearts.

Frequent Occurrence of Ca\(^{2+}\) Waves in Regions With Higher FI
In contrast to the sporadic waves, high-frequency Ca\(^{2+}\) waves were observed in some specific regions with higher FI (hereafter called Ca\(^{2+}\)-overloaded waves). Figure 3 shows sequential X-Y images (Figure 3A) and the corresponding line-scan images (Figure 3B) of Ca\(^{2+}\)-overloaded waves: failure of propagation (Figure 3B-a) and propagation with (Figure 3B-b) and without (Figure 3B-c) delay. These particular waves occurred at 10 waves·min\(^{-1}\)·cell\(^{-1}\). Adjacent to
the cell with the waves, there was a region with higher static FI (upper left). These Ca\textsuperscript{2+}-overloaded waves showed a longer fluorescent profile than the sporadic waves (Figure 3C). Of 60 Ca\textsuperscript{2+}-overloaded waves examined, the profile declined significantly slowly \((t_{1/2} = 0.41 \pm 0.20 \text{ seconds}, P < 0.01)\).

Ca\textsuperscript{2+}-overloaded waves were also observed in a region in which mechanical damage was caused by a glass microelectrode \((n=5)\). Figure 3D shows the waves in the damage-applied region. They occurred frequently, at 48 waves \cdot \text{min}^{-1} \cdot \text{cell}^{-1}. One Ca\textsuperscript{2+} wave at the center propagated to the adjacent cells transversely (4 to 7) and longitudinally (5 to 10). Similar patterns of intercellular wave propagation were observed in 31 regions of 7 hearts. The Ca\textsuperscript{2+}-overloaded waves occurred in regions with higher basal FI \((\text{FI}_{\text{ROI}} / \text{FI}_{\text{intact}} = 1.13 \pm 0.05, n=22, P < 0.01)\). They occurred at 28.1\pm8.3 waves \cdot \text{min}^{-1} \cdot \text{cell}^{-1} \((n=40, P < 0.01)\) with a velocity of 116.7\pm29.4 \text{ \mu m/s} \((n=63, P < 0.01)\), more frequently and more quickly than those for the sporadic waves at 6 mmol/L \([\text{Ca}^{2+}]_o\). The \(R_{\text{prop}}\) in such Ca\textsuperscript{2+}-overloaded regions was higher (0.23, \(n=467\)) than that in intact regions \((P < 0.01)\).

The third class of Ca\textsuperscript{2+} waves we examined were extremely high-frequency waves showing ripple-like wave fronts, which disappeared within 10 minutes, resulting in regions with high static FI and no response to electrical stimulation, indicating cell death (hereafter, agonal waves). In a typical example shown in Figure 4A, the waves occurred very frequently at 280 waves/min, as calculated from the line-scan image (C), and showed no propagation to the adjacent cells. Of 12 regions having isolated Ca\textsuperscript{2+} waves as in Figure 4A, 11 waves showed no intercellular propagation \((R_{\text{prop}} < 0.08)\). In the clusters composed of \(\geq 2\) waves, the waves barely propagated intercellularly \((R_{\text{prop}} < 0.09; n=93)\). In such specific regions, electrical stimulation failed to induce Ca\textsuperscript{2+} transients, and Ca\textsuperscript{2+} transients around the regions failed to abolish the waves.
Such high-frequency waves were also observed in regions damaged by microelectrodes (n=5). The agonal waves occurred in regions with higher FI (FI ROI/FI intact = 1.24±0.09, n=18, P<0.01), showing a frequency of 133.1±65.4 waves/min•cell•cell-1 (n=37) and a propagation velocity of 112±25.1 μm/s (n=37).

Figure 5 summarizes the 3 types of Ca2+ waves described above. The relative FI (FI ROI/FI intact) (Figure 5A) and the incidence of the waves (Figure 5B) showed clear differences among these regions. The propagation velocities of the Ca2+-overloaded and agonal waves were higher than those of the sporadic waves (Figure 5C). The Rprop was higher for the Ca2+-overloaded waves, whereas the sporadic and agonal waves showed poor intercellular propagation (Figure 5D).

Discussion

By focusing on incidence, we revealed the existence of 3 distinct types of Ca2+ waves in the subepicardial regions of the perfused heart. These 3 types of waves showed different propagation velocities, relative FIs (FI ROI/FI intact), and intercellular propagation ratios (Rprop). The incidence and velocity of Ca2+ waves are considered to reflect the difference in [Ca2+]i-loading states in the perfused heart, analogously to their [Ca2+]i dependence in isolated myocytes.5,7,12,13 The relative FI can also be regarded as a reliable indicator of the [Ca2+]i-loading state, for the following reasons: (1) the transcoronary perfusion of fluo 3-AM attained homogeneous fluo 3 loading at ROIs,14,15 (2) a motion artifact on the image was absent, and (3) there was no noticeable difference in size among the cells with different FI. Therefore, the 3 types of waves we identified are likely to originate from different [Ca2+]i-loading states. In other words, as the myocardial tissue becomes Ca2+-overloaded depending on the background conditions of myocytes (ie, functioning normally, Ca2+-overloaded and functional, and totally nonfunctional),

Figure 2. [Ca2+]-dependent incidence (A, *P<0.05) and velocity (B, *P<0.05 vs value at 1 mmol/L [Ca2+]o) of the sporadic waves from 4 hearts. The numbers of waves examined are shown in parentheses. C, Time course of sporadic wave incidence after electrical stimulation. Numbers of Ca2+ waves in an ROI (~12 cells) are plotted against time (every 10 seconds) after cessation of 30-second electrical stimuli at 2 mmol/L (left) and 4 mmol/L (right) [Ca2+]o. X, ▽, and • indicate numbers of waves for 10 seconds at 1, 2, and 3 Hz, respectively.

Figure 3. A, Intercellular propagation of Ca2+-overloaded waves (1 through 10) displayed every 100 ms with pseudocolors. Left, Location of waves and direction of propagation (arrows). B, Corresponding line-scan images (bottom) show failure of propagation (a) and propagation with (b) and without (c) delay. C, Comparison of fluorescent profiles of sporadic (dotted line) and Ca2+-overloaded (solid line) waves. D, Sequential X-Y images (every 100 ms) of Ca2+ waves in a region in which mechanical damage was applied via a microelectrode. Ca2+ wave initiated at center of frame shows transverse (4 through 7) and longitudinal (5 through 10) propagation to adjacent cells. Schematic representation beneath each image.
the properties of Ca\(^{2+}\) waves change progressively and successively.

We demonstrate here for the first time that Ca\(^{2+}\) waves did not occur in apparently intact regions during sinus rhythm, even at supraphysiological \([Ca^{2+}]_{o}\) (2 mmol/L) and room temperatures, according to real-time images of the waves (Figure 1A). Previously, Ca\(^{2+}\) waves were identified in intact multicellular ventricular preparations\(^{18,19}\) and perfused intact whole hearts in physiological \([Ca^{2+}]_{o}\) of 1 mmol/L.\(^{14}\) However, it has been unclear whether or not Ca\(^{2+}\) waves occur in the working heart under physiological conditions. This is because previous studies were conducted under arrested conditions.\(^{14,18,19}\) We further observed that the waves were abolished by Ca\(^{2+}\) transients (Figure 1B) and recurred with latency (Figure 2C). Therefore, it seems reasonable to consider that repetitive excitation of the myocardium prevents the hearts from producing sporadic Ca\(^{2+}\) waves.

In contrast to sporadic Ca\(^{2+}\) waves, Ca\(^{2+}\)-overloaded waves occurred frequently, even during repetitive excitation at 2 mmol/L \([Ca^{2+}]_{o}\). A higher basal FI (Figure 5A) and patchy distribution of cells with high static FI (Figures 3A and 3D) indicated that the regions were likely to be Ca\(^{2+}\)-overloaded.

Ca\(^{2+}\)-overloaded conditions in the regions were also suggested by the findings that the incidence and velocity of the Ca\(^{2+}\)-overloaded waves at 2 mmol/L \([Ca^{2+}]_{o}\), (Figure 5B and 5C) were much higher than those of the sporadic waves at 6 mmol/L \([Ca^{2+}]_{o}\) (Figure 2A and 2B). The Ca\(^{2+}\)-overloaded conditions were probably caused by some inevitable localized injury or damage, which occurred spontaneously but rarely during the preparation of Langendorff perfusion. Local damage by microelectrodes induced the same types of Ca\(^{2+}\) waves (Figure 3D), indicating that the waves occur under Ca\(^{2+}\) overload. The longer decline phase of the waves (Figure 3C) may also indicate Ca\(^{2+}\) overload, because previous reports demonstrated that the decline phase of Ca\(^{2+}\) transients was prolonged in damaged or failing myocytes\(^{20,21}\) and that Ca\(^{2+}\) waves and Ca\(^{2+}\) transients showed similar fluorescent profiles.\(^{17}\) Such prolongation of Ca\(^{2+}\) wave profiles may be attributed to some impairment of Ca\(^{2+}\) handling, especially reuptake of Ca\(^{2+}\) by SR, which is known to be impaired via alteration of phospholamban/SR Ca\(^{2+}\) (SERCA) pump activity in damaged or failing hearts.\(^{22,23}\) Direct evidence for the SERCA pump modulation of Ca\(^{2+}\) waves was provided in the phospholamban-deficient mouse heart, which lacks the inhibitory action of the SERCA pump, by demonstrating that Ca\(^{2+}\) waves declined faster.\(^{24}\)

We found that \(-20\% of myocytes with the Ca\(^{2+}\)-overloaded waves had the ability to propagate to adjacent myocytes. However, the waves showed no widespread propagation to the surrounding myocytes: at most, to 3 to 4 adjacent ones. These findings are in agreement with the results of Lamont et al.\(^{19}\) who reported that \(-13\% of waves propagated to the adjacent cells in the rat ventricular trabeculae. Two factors can be considered to be determinants for intercellular propagation: the inducibility of Ca\(^{2+}\) waves (ie, ability of regenerative propagation) and conductivity of the gap junctions. We consider that the limiting step for the observed intercellular wave propagation resides in the former rather than the latter. This is because the gap junctional conductance in the Ca\(^{2+}\)-overloaded regions is lower than that in the intact regions, according to its \([Ca^{2+}]_{o}\) dependence.\(^{25}\) The importance of the inducibility of Ca\(^{2+}\) waves in the adjacent (recipient) myocytes is supported by several previous reports. Lipp and Niggli\(^{13}\) proposed that the Ca\(^{2+}\)-loading state of SR determines the propagation distance of the wave by modulating the degree of positive feedback of SR Ca\(^{2+}\) release. Wier et al.\(^{18}\) also regarded the positive feedback of SR...
Ca\(^{2+}\) release as an important factor for wave propagation, because they observed occasional truncation of the waves in the middle of the cells of the intact rat ventricular trabeculae. Lamont et al\(^9\) reported that the waves in rat ventricular trabeculae occasionally propagated to the adjacent cells but aborted in the middle of the cells (called Ca\(^{2+}\) spritz), suggesting that the adjacent (recipient) cells are important to complete wave propagation. Taken together, Ca\(^{2+}\) waves from the adjacent cells would therefore propagate to the recipient cells only when the cells exert higher positive feedback of SR Ca\(^{2+}\) release to induce Ca\(^{2+}\) waves. The positive feedback of SR Ca\(^{2+}\) release does not proceed very far, even in the Ca\(^{2+}\)-overloaded regions.

The agonal waves occurred in regions with higher FI than the regions with the Ca\(^{2+}\)-overloaded waves, suggesting highly Ca\(^{2+}\)-overloaded conditions. Although the frequent and ripple-like fluctuations are often observed in single isolated myocytes just before they go into contracture (ie, an agonal state), we provide, for the first time, detailed information on the agonal waves in the whole heart. The waves showed little propagation to adjacent cells despite their highly frequent occurrence (ie, high positive feedback of SR Ca\(^{2+}\) release). This was due to collision of multifocal waves with subsequent refractoriness and possible closure of the gap junction by presumably higher [Ca\(^{2+}\)]\(_i\).\(^{25}\) A decrease in the gap junctional conductivity in the agonal regions was suggested by observations that electrical stimulation failed to induce Ca\(^{2+}\) transients and that the surrounding Ca\(^{2+}\) transients failed to abolish the waves. Another interesting finding regarding agonal waves was that the propagation velocities were not higher but rather almost equal to those of Ca\(^{2+}\)-overloaded waves (Figure 5C). Although we have no direct evidence to explain this finding, a refractoriness of Ca\(^{2+}\) release from SR in the extremely high-frequency waves may attenuate the increase in the propagation velocity by facilitating the use-dependent inactivation of ryanodine receptors\(^{26}\) at extremely high frequency.

We have to recognize several limitations in this study. First, Langendorff perfusion with a colloid-free solution can produce edema and minimal focal myocardial degeneration.\(^{27}\) Second, a relatively higher [Ca\(^{2+}\)]\(_i\) (2 mmol/L) at room temperature could render the heart Ca\(^{2+}\)-overloaded, even in an apparently “intact” region. Such conditions potentially augmented the occurrence of Ca\(^{2+}\) waves. The third limitation resulted from the use of BDM at a relatively higher concentration (>10 mmol/L), possibly precluding the occurrence of Ca\(^{2+}\) waves in the perfused heart through its direct effects on Ca\(^{2+}\) handling.\(^{28}\) Nevertheless, these limitations do not affect our conclusion that \(\geq3\) distinct types of Ca\(^{2+}\) waves occur in the perfused whole heart, depending on the Ca\(^{2+}\)-loading state.

Although direct evidence for the pathological significance of Ca\(^{2+}\) waves is still lacking, the following speculations can be made from our present whole-heart data. The Ca\(^{2+}\)-overloaded and agonal waves are likely to have pathological roles. Because Ca\(^{2+}\) waves produce arrhythmogenic depolarization in single myocytes,\(^8\) the frequent and prevalent Ca\(^{2+}\)-overloaded waves with intercellular propagation may provoke abnormal depolarization of cardiac tissue leading to contractile failure or triggered arrhythmia. The agonal regions may become origins of reentrant arrhythmias when they hamper electrical conduction. In turn, the loss of intercellular propagation of the agonal waves may serve as a protective mechanism against the spatial progression of myocardial damage. Although the pathogenesis of Ca\(^{2+}\) overload in the observed regions was not determined, the divergent properties of Ca\(^{2+}\) waves revealed in this study may represent certain aspects of the waves that occur under certain pathological conditions, such as myocardial ischemia/reperfusion injury and infarction. Delineation of the functional properties of Ca\(^{2+}\) waves under such specific pathological states is open for future research.

References


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