Calcium Waves

Physiological Relevance in Cardiac Function

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Modulation of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) constitutes a fundamental mechanism of signal transduction in excitable cells. Generally, this phenomenon is triggered by an excitation process of the external membrane and subsequently leads to activation of functional proteins specific to the cell type in which the increase of $[Ca^{2+}]_i$ has occurred. However, in addition to the normal excitation-activation coupling providing Ca^{2+} influx, fluctuations in $[Ca^{2+}]_i$ can occur spontaneously, without external electrical or hormonal stimulation. A spontaneous increase in Ca^{2+}concentration can occur at a single focus or multiple foci within a cell and can lead to propagation of an elevation of $[Ca^{2+}]_i$ throughout the cytosol in a wave-like pattern. Although these Ca^{2+} waves have been observed widely in both excitable and inexcitable cells,[1] their role in the cardiac cell function remains unclear.

In the last 2 decades, a substantial body of literature documenting Ca^{2+} waves in cardiac tissues has been created. Studies on waves in cardiac cells provided fundamental information about the role of the sarcoplasmic reticulum (SR) in excitation-contraction coupling.[2] Local contractions were reported in cells with disrupted sarcolemma,[2] clearly demonstrating that Ca^{2+} waves were not related to depolarization of the membrane. Interest in cardiac Ca^{2+} waves was further stimulated by a study that suggested a role for them in the generation of abnormal, nondriven electrical activity of the heart.[3] Initially, Ca^{2+} waves in cardiac muscle were assessed indirectly from observations of local sarcomere contraction and waves of propagating sarcomere shortening,[4,5] and direct evidence of spontaneous fluctuations in $[Ca^{2+}]_i$ was obtained from analysis of aequorin photoluminescence.[6,7] Progress in the development of digital-imaging techniques and fluorescent Ca^{2+}-sensitive dyes[8] enabled investigators to provide a more precise, quantitative spatiotemporal analysis of the dynamics and propagation of Ca^{2+} waves in and between cardiac myocytes.[9–12] More recently, laser confocal microscopy has brought this analysis to the submicrometer scale, revealing that like the stimulated action potential–elicited Ca^{2+} transient, spontaneous Ca^{2+} waves result from Ca^{2+} sparks.[13] It has been shown that Ca^{2+} waves are based on the localized Ca^{2+} release occurring during a spark that couples to release by adjacent SR sites by diffusion of the released Ca^{2+} ions.[13]

Early consideration of a role for Ca^{2+} waves in cardiac electrical activity arose from studies showing that spontaneous increase in $[Ca^{2+}]_i$ could activate transient inward currents.[14] The transient inward current is generally assumed to be arrhythmogenic[15–17] and is likely carried by the Na^+-Ca^{2+} exchanger and other Ca^{2+}-activated channels.[18] Furthermore, Capogrossi et al[3] showed that when spontaneous Ca^{2+} release occurred simultaneously at several subcellular loci, membrane depolarization occurred and under some circumstances could induce an action potential. Subsequently, several investigators, using different methods, have studied the spatiotemporal aspects of spontaneous increases in $[Ca^{2+}]_i$, $[Ca^{2+}]_i$ propagation, and resulting membrane depolarizations. For example, using ouabain or potassium-free solutions in single myocytes, Miura et al[19] showed the concomitant occurrence of propagating Ca^{2+} waves, cell shortening, and membrane depolarization, which is referred to as a delayed afterdepolarization.

These studies together have shown convincingly that spontaneous release of Ca^{2+} in a myocyte constitutes a potential arrhythmogenic process in the heart. Importantly, additional evidence showed that Ca^{2+} waves with similar characteristics are present in the working heart in situ. A recent study by Wier et al[20] revealed that cardiac multicellular preparations such as trabeculae exhibit similar properties of intracellular Ca^{2+} cycling compared with isolated single myocytes. The challenge of applying our knowledge of Ca^{2+} waves from cell studies to the more integrated system is now being addressed in intact heart studies, as presented by Kaneko et al[21] in this issue of *Circulation Research.* Kaneko et al[21] confirm an earlier study by Minamikawa et al[22] and extend their findings by providing a descriptive and quantitative analysis of 3 different types of Ca^{2+}waves in the intact heart using a Langendorff perfusion system. Importantly, their results are clearly consistent with data obtained from single myocytes, aggregates of myocytes, or isolated trabeculae. No spontaneous fluctuations of $[Ca^{2+}]_i$ could be detected in regions that had no apparent damage when the healthy heart was paced at a physiological rate, similar to earlier reports in stimulated myocytes. When pacing was terminated, Ca^{2+} waves appeared sporadically, each having limited intercellular propagation. The observations by Kaneko et al[21] are also consistent with the confocal studies of cardiac trabeculae, in which Ca^{2+} waves were shown to propagate at low velocity ($\approx$30 μm/s) and over a limited distance throughout the muscle.[20] It is

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generally assumed that under physiological conditions, the probability of spontaneous local Ca^{2+} release is low. Hence, the probability that Ca^{2+} waves propagate to adjacent cells is likely to be small.[23] Kaneko et al[21] found that in regions where higher basal levels of Ca^{2+} were suspected, Ca^{2+} waves occurred more frequently and propagated at a higher velocity (116 μm/s). Again, this finding is consistent with observations in Ca^{2+}-loaded myocytes.[19] Finally, Kaneko et al[21] report the presence of a third pattern of spontaneous Ca^{2+} waves (agonal waves) in regions of the intact heart that had been overtly damaged by microelectrode impalement. Agonal waves occur at high frequency over a small extent throughout cells. As described by Kaneko et al,[21] agonal waves are similar to waves observed in Ca^{2+}-overloaded myocytes during the hypercontraction that precedes cell death.[14] As concluded by the authors and consistent with data obtained in isolated myocytes, the occurrence of each type of Ca^{2+} wave depends on the degree of cellular Ca^{2+} load, ie, SR load and cytosolic Ca^{2+} load.[24] This dependence, as well as the fundamental properties of Ca^{2+} waves observed in isolated myocytes, has been reproduced in the study by Kaneko et al[21] on the intact heart. Thus, the study establishes an important link between isolated cells and the working heart. Therefore, the assumption that the fundamental mechanisms of Ca^{2+} cycling in myocytes constitute a potential substrate for certain types of arrhythmias is applicable to the myocardium in situ.

Nevertheless, some aspects of the conclusions formulated in this study should be considered with caution and merit additional exploration. The authors claim that "the spatiotemporal properties of Ca^{2+} waves in the heart are diverse and modulated by the Ca^{2+}-loading state" (page 1093). It is important to consider the extent of the damage inherent to the experimental model used by the authors. First, as mentioned by the authors, manipulation of the heart during the mounting in the Langendorff apparatus and positioning of the electrodes are significant mechanical (and thus electrical and biochemical) perturbations that may damage the preparation. Hence, one may suspect the presence of Ca^{2+}-overloaded cells in the epicardial area because this is the region directly exposed to this type of injury. Second, the isolated hearts were perfused through coronary arteries with a protein-free solution. In the absence of plasma proteins, exudation of water into the interstitial space occurs simultaneously with an increase of the geometrical resistance of the coronary bed.[25] These latter factors may affect supply of O_2, nutrients, and Ca^{2+} of the myocytes. More importantly, edema is bound to occur during perfusion in the absence of colloid osmotic particles. Edema leads unavoidably to the stretching of intercellular structures, including the collagen meshwork attached to the surface of the cardiac myocytes. Strain of the myocyte membrane is therefore likely and may contribute to increased Ca^{2+} entry into the cells in this model. Ca^{2+}overload of cardiac myocytes is accelerated in metabolically deficient regions.[26] It is possible that the 3 distinctive patterns in Ca^{2+} waves described by Kaneko et al[21] reflect the response to incremental Ca^{2+} overload of the myocytes in this preparation. The velocity of propagation and the incidence of Ca^{2+} waves may reveal an increased extent of damage. It would be reasonable to assume that in the heart in vivo, the probability of occurrence of Ca^{2+} waves is near the lower limits of the observations by Kaneko et al.[21]

Furthermore, questions about quantitative analysis of the dynamics of intracellular Ca^{2+} transients may arise from other technical limitations of the Langendorff method of perfusion of the heart. Analysis of the effects of dynamic changes in extracellular ion concentrations, such as quantitative analysis of velocity or frequency of waves in response to changes in the extracellular milieu, will be hampered by the presence of edema. Another limitation of the study is the lack of information about uniformity of the loading of myocytes with fluorescent dye. This problem gains critical importance when the fluorescence of a nonratiometric dye such as fluo 3 is used in the estimate of Ca^{2+} load of the cells, as was used in the present study. The same problem leads to questions about the extent of propagation of Ca^{2+} waves in the multicellular preparation. When dye is not uniformly distributed among cells, it is difficult to determine whether the disappearance of a wave is due to termination of propagation or to the low concentration of dye in the sampling area. An additional concern, applying to all observations of processes that extend in 3 dimensions under the confocal microscope, is that Ca^{2+} waves may seem to be limited in extent in the plane of observation simply because they have disappeared from that confocal plane. Ca^{2+} waves are 3-dimensional dynamic events. Thus, full evaluation of their properties requires 3-dimensional spatial as well as temporal analyses.

In summary, Kaneko et al[21] have presented challenging data suggesting that intracellular Ca^{2+} waves do occur in the intact heart and thereby confirm concepts proposed from studies of structures at a simpler level of organization. The incidence of Ca^{2+} waves may be at the lower limits of this study in the heart in vivo, but this study suggests that the mechanisms of Ca^{2+} cycling derived from work on isolated cells are present in the intact organ and may underlie arrhythmogenicity. This notion may be specifically relevant to the heart with acute damage, and it warrants additional exploration of the effects of damage shown in isolated muscle preparations in which Ca^{2+} waves are initiated near identified damaged regions of the tissue.[27] The results of Kaneko et al[21] are consistent with the model underlying damage-induced Ca^{2+} waves in which cellular-Ca^{2+} load is one of the main determinants of the velocity of propagation of Ca^{2+} waves in cardiac muscle.[24]

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