Normal contraction of cardiac muscle requires a coordinated cellular mechanical response to the electric pacemaker signal that begins in the sinoatrial node and spreads through the conduction system and ultimately across the ventricular myocardium via gap junctions. In ventricular myocytes, depolarization by the action potential causes clusters of L-type calcium channels (LCCs) to open, allowing a small amount of Ca to enter the diadic cleft space between the sarcolemma and the sarcoplasmic reticulum (SR). When the Ca concentration in this space becomes sufficiently high to gate ryanodine receptors (RyRs), they open and release SR Ca into the cytoplasm, so that it can bind to and activate the LCCs on the sarcolemma, its opposing cluster of RyRs across the 10- to 12-nm diameter diadic cleft space, and associated SR regulatory proteins is a couplon. According to the local control theory of cardiac excitation-contraction (EC) coupling, couplons are activated independently. Independent activation of couplons depends on the extent of LCC activation, providing a mechanism for regulation of contractile force by stochastic recruitment of couplons. In systolic heart failure, numerous cellular defects in EC coupling and Ca regulation have been identified that contribute to contractile dysfunction (and ventricular arrhythmias), although there is a host of other major abnormalities leading to loss of force development, including fibrosis, defective energy metabolism, pH changes, heterogeneou conductance across connexin hemichannels, and defects in sarcromeric proteins. Thus, it is important to keep in mind that no single defect can explain the contractile dysfunction of systolic failure.

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**Loss of Intracellular Synchrony**

Recent studies of defective EC coupling in heart failure have addressed not only reductions in the extent and kinetics of Ca released in response to the action potential (ie, reduced recruitment of couplons and reduced SR content), but also changes in the synchronization of intracellular Ca release. In remodeled ventricular myocytes isolated from the peri-infarct zone of rabbit hearts and studied with confocal microscopy, Litwin et al showed that during electrical stimulation, the local Ca release events (ie, Ca sparks) produced by couplons no longer activate in a synchronous fashion. This results in reduced Ca flux with the expectation of reduced contractility at the cellular level. Loss of subcellular synchrony has a number of causes. One of these, reduction in couplon size due to attrition of LCCs during metabolic stress, is clearly an important factor contributing to the loss of synchrony as we have shown previously. The effect of losing LCCs on Ca release can be explained as follows. If, for the sake of simplicity, we assume that all RyRs in a couplon can be activated by a single LCC, then the Ca spark’s latency will reflect the first latency of opening of this LCC. The fewer the number of LCCs in the couplon, the lower the chances of finding an LCC that opens with a short first latency. Thus, the mean first latency will increase as LCCs are lost. LCC first latencies exhibit an exponential distribution. Such distributions exhibit a mean, which is equal to the SD of the distribution. As the mean of the first latency increases, the standard deviation and hence the variance of first latency also increases. Because the first latency of LCCs underlies the first latency of sparks, these too will display a greater variance (ie, asynchrony) as we see in single cells from heart failure models.

Alternatively, RyR clusters that form part of the couplon may become isolated during the remodeling process that removes t-tubules,12,13 If these RyR clusters are in fact activated during depolarization, it must be by a common pool mechanism, which due to its inherent positive feedback will prevent regulation of the Ca transient by stochastic recruitment of individual couplons. Furthermore, these couplons will no longer reflect the activity and first latency of LCCs because RyRs will be physically separated. The net effect is a reduction in the synchronization of Ca release, which we will discuss in detail later.

**Loss of Intercellular Synchrony**

In this issue of *Circulation: Heart Failure*, Wasserstrom et al draw attention to another phenomenon contributing to contractile dysfunction that single-cell studies, by their inherent nature, fail to reveal: loss of intercellular synchrony. The authors of this article use confocal microscopy to study Ca release in the epicardial layer of ventricular cells in intact hearts from failing spontaneously hypertensive rats. Although the spatial and temporal resolution of this technique does not allow detailed study of couplon activation, the authors nevertheless demonstrate that in failing hearts Ca release is not uniform across all ventricular cells. Indeed, they identify...
several different Ca release types: (1) triggered release with reduced amplitude and kinetics compared with normal rats; (2) spontaneous release either before or after the electrical trigger that is not present in normal rats; and (3) triggered release of slow intracellular waves that is not present in normal rats. Each of these phenomena can be identified in isolated cell studies, but until now have not been demonstrated occurring together in intact tissue. The reduced amplitude and kinetics of the Ca transient in some cells are likely the result of loss of LCCs or reduced SR Ca load caused by reduced sarcoendoplasmic reticulum calcium ATPase (SERCA) activity, whereas spontaneous release in other cells is probably caused by SR Ca overload and possibly leaky RyRs. The slow wavelike diffusion of Ca in some cells in response to electrical stimulation is suggestive of clusters of RyRs not activated directly by closely opposed LCCs in a coupon. There are at least 2 possible explanations for these waves. One, suggested by the authors, is that “orphaned” RyRs in their failure model are activated by diffusion of Ca through the cytoplasm from neighboring Ca release sites instead of the customary triggering of intact couplons by the electrical signal. Presumably if this occurs, the activation of nonjunctional RyR clusters that are separated from coupons requires Ca release from intact couplons to spread to these nonjunctional RyRs. Thus, we may expect that the activation of these RyR clusters is delayed. Because RyRs are largely confined to the z-disk, a transverse scan is likely to display an inhomogeneous rise in Ca because couplons will be activated before nonjunctional RyR clusters. Transients of this nature are apparent in Figure 8A of the article. Thus, not only can the production of sparks be temporally asynchronous, but also they can be spatially nonuniform. This seems to be consistent with the findings of the authors.

It is also possible that the spread and shape of the action potential is abnormal in many of these cells because of faulty intercellular conduction due to abnormal Na channels, connexin hemichannels and gap junctions, fibrosis, or refractoriness associated with abnormal K currents. All these changes have been implicated in arrhythmogenesis, but could also lead to heterogeneous electric activation of ventricular myocytes, which would be expected to trigger Ca release in a heterogeneous fashion. This is certainly an area that deserves further study, and which could be addressed by applying voltage sensitive dyes to the author’s multicellular preparation and then correlating changes in voltage with abnormal Ca release.

**Mechanical Consequences**

We would expect inhomogeneities at the cellular level to impair contractility. For example, if sarcomeres that are in series are activated at different times because of temporally inhomogeneous Ca release, the sarcomere that is activated first will stretch the compliance of the sarcomere with which it is in series before this neighboring sarcomere is activated. Thus, the neighboring sarcomere will produce less force or shortening, and the contraction of these 2 sarcomeres together will be inhomogeneous. This will have the effect of reducing contractility within the cell. Sufficient serial elements in the cell activated in this fashion could greatly reduce the efficacy with which contraction occurs above and beyond that expected from reduction of the amplitude and kinetics of Ca transients alone. The observation by Wasserstrom et al. that Ca transients are both temporally and spatially inhomogeneous between cells, suggests an extension of this idea. If I myocyte tugs on its neighbor before the neighbor has been activated, this will have the effect of significantly reducing contractility for the reasons already mentioned. Thus, we can expect contractility to be grossly impaired at both the cellular and the multicellular levels.

It is now understood that t-tubules enter the cell at the z-disk. If a significant number of RyRs are orphaned in the z-disk by virtue of t-tubule loss, this means that the spread of Ca across the z-disk is likely to be significantly inhomogeneous, with parallel sarcomeres quite possibly activated at different extents and at different times. The mechanical consequences of this are undisclosed; however, it is unlikely to improve contractility.

**Implications for the Study of EC Coupling in Heart Failure**

This work exposes the limitations of single-cell studies of EC coupling in heart failure and likely other cardiac pathologies. It also underscores the importance of simultaneously observing the behavior of collections of cells, which in the case of heart failure display a wide variety of failure modes. This leads to insights and conclusions that could not possibly be drawn from the study of single cells, for example the realization that multiple failure modes within a single-diseased heart defy a single solution. This has implications for designing therapies based on cellular mechanisms contributing to systolic dysfunction. The results also suggest that the asynchronous behavior of Ca release within single cells and between adjacent cells may have a common origin. However, the precise consequences of this remain to be disclosed by rigorous experimentation.

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**Disclosures**

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Joshua I. Goldhaber and John H.B. Bridge

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