of local potential changes can be calculated and correlated with changes in other mitochondrial activities. An obvious example is respiratory activity, another is membrane permeability. Large-conductance, voltage-gated ion channels have been detected in the inner mitochondrial membrane by patch-clamping (e.g., Sorgato and Moran (1993)). While available evidence suggests that these channels are usually closed, might transient openings correlate with membrane potential fluctuations? These questions and others relating to the distribution and regulation of mitochondrial membrane potentials should begin to be answered as 3D fluorescence microscopy continues to advance.

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$[Ca^{2+}]_i$ Waves in Heart Cells: More Than a Passing Fancy

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Images of calcium ion concentration ($[Ca^{2+}]_i$) in living cells take a starting number of forms. In heart cells, such images reveal that regions of elevated $[Ca^{2+}]_i$ can propagate as "waves" (at constant velocity) or, as in the elegant images from Lipp and Niggli (1993) in this issue, as waves that spiral about subcellular cores. These author's use of high spatial and temporal resolution confocal microscopy enabled them to observe details of $[Ca^{2+}]_i$ waves in three dimensions that had escaped notice previously with lower resolution techniques. They saw for the first time that spiral waves may initiate "linear" waves and that when such waves collide, they annihilate each other. Their results indicate unequivocally that $[Ca^{2+}]_i$ waves in heart cells are produced by a process with high positive feedback. As strongly suspected already, that process is certainly Ca^{2+}-induced release of Ca^{2+} from internal stores (sarcoplasmic reticulum or SR). The new idea compelled by their observations is that (in their own words) there is a "variability of positive feedback even on the subcellular level" and that "this notion implies the existence of functionally separate SR elements exhibiting differeces in gain." As I explain below, this fits in well in a larger picture of control of $[Ca^{2+}]_i$ in heart cells, in which certain paradoxes concerning $[Ca^{2+}]$-induced release of Ca^{2+} and its role in both $[Ca^{2+}]_i$ waves and in normal excitation-contraction (E-C) coupling may finally be resolved.

The $[Ca^{2+}]_i$ transients (waves) observed by Lipp and Niggli (1993) and others appear to be "uncontrolled" in the sense that they may develop spontaneously and may propagate without change until an obstacle (nucleus), another wave, or the end of the cell is reached. Under other circumstances however, $[Ca^{2+}]_i$ transients appear (in the available images, at least) to be uniform throughout the cell, and to be controlled, in the sense that transmembrane Ca^{2+} current is required to initiate them, and that stopping the Ca^{2+} current stops the $[Ca^{2+}]_i$ transient. Under yet other circumstances, imaging reveals that $[Ca^{2+}]_i$ transients may be localized in subcellular regions, and that the elevated $[Ca^{2+}]_i$ may fail to propagate out of such a region (Valdeolmillos et al., 1989). At first, all this might seem to indicate that the control of $[Ca^{2+}]_i$ is highly diverse; surely such a diversity of types of changes in $[Ca^{2+}]_i$ implies a diversity of control mechanisms. In particular, it has always been difficult for researchers in this area to understand how the autocatalytic process of Ca^{2+}-induced release of Ca^{2+} from SR, which can easily be imagined to underlie spontaneous regenerative, propagating $[Ca^{2+}]_i$ waves, might be "harnessed", under other circumstances, to produce controlled changes in $[Ca^{2+}]_i$. Indeed, Fabiato (1985) postulated, in his original description, that "spontaneous cyclic Ca^{2+} release and Ca^{2+}-induced release of Ca^{2+} do not occur through the same mechanism." Nevertheless, the evidence, from many types of experiments, is that all these types of changes in $[Ca^{2+}]_i$ involve exactly the same cellular structures and molecules (e.g., ryanodine receptors or SR Ca^{2+} release channels). There is little doubt now that, however it might work, Ca^{2+}-induced release of Ca^{2+} from SR underlies both $[Ca^{2+}]_i$ waves and the $[Ca^{2+}]_i$ transients of normal E-C coupling. Thus, the major challenge to those attempting to understand the roles of $[Ca^{2+}]_i$ in heart cells is to unify these diverse, even seemingly contradictory phenomena, all of which seem to involve Ca^{2+}-induced release of Ca^{2+}. As noted by Lipp and Niggli (1993), the explanation of spiral $[Ca^{2+}]_i$ waves may lie in the fact that Ca^{2+}-activated SR Ca^{2+}-release channels are not homogeneously arranged throughout the cell but are arranged, functionally, at least, in "clusters." The notion of functional clusters of SR release channels was introduced in a seminal article by Stern in recently *Biophysical Journal* (Stern, 1992), and this notion has since been invoked to explain both controlled and uncontrolled changes in $[Ca^{2+}]_i$ in heart cells. Indeed the "new and notable" idea is that Ca^{2+}-induced release of Ca^{2+} at functional clusters of SR channels may be able to explain how

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spatio-temporal changes in $[Ca^{2+}]_i$ can range from regenerative spiral waves, as observed by Lipp and Niggli, to rapid, spatially uniform, tightly controlled $[Ca^{2+}]_i$ transients (as elicited by depolarization during normal E-C coupling). While the "functional cluster" may correspond to the group of SR Ca^{2+} release channels in junctional regions of SR (where the terminal cisterns of the SR are apposed closely to the transverse tubules), it should be recognized that these is little direct evidence at all for independent, functional clusters of SR Ca^{2+} release channels; the existence of such clusters was postulated (Stern, 1992) entirely as a device to explain certain phenomena of E-C coupling, and to acknowledge the fact that, once SR Ca^{2+} release starts, the activation of nearby SR Ca^{2+}-release channels (i.e., within the same cluster) may be unavoidable. (The clusters would be independent because they are supposed to be spatially isolated from each other, so that a cluster may fail to be activated, even if a neighboring cluster is activated).

The concept of "gain" of SR Ca^{2+} release is fundamental to understanding the problem of how Ca^{2+}-induced release of Ca^{2+} can be either controlled (E-C coupling) or uncontrolled (waves). "Gain" is a term that has been used loosely in the past to indicate how much SR Ca^{2+}-release is induced by a change in cytoplasmic $[Ca^{2+}]$. Of course, high gain could be the result of the positive feedback on SR Ca^{2+} release that should occur as released Ca^{2+} continues to activate its own further release. For normal E-C coupling, Stern (1992) defined gain as the ratio of the amount of Ca^{2+} entering via L-type Ca^{2+} channels to that released from the SR. There is general agreement that, under different conditions, release of Ca^{2+} from the SR of mammalian cardiac cells exhibits both "high gain" and "low gain." The low gain case is that in which SR Ca^{2+}-release is controlled tightly by the entering Ca^{2+} current, as is normally the case during excitation-contraction coupling. The high gain case is that of uncontrolled or spontaneous SR Ca^{2+} release, as evident in propagation of regions of elevated $[Ca^{2+}]_i$ (viz. "waves"). Recently, gain, (defined as the ratio of peak SR Ca^{2+} release flux to peak rate of Ca^{2+}-influx through L-type Ca^{2+} channels) was measured experimentally for the first time in voltage-clamped rat heart cells (Wier et al., in press). An important result was that gain decreased sharply as clamp pulses were given to more positive membrane potentials. Thus, Ca^{2+}-induced release of Ca^{2+} seems not to be characterized by one particular gain. According to Stern (1992) this drop in gain could occur as independent functional clusters of SR Ca^{2+} release channels fail to be activated by entering Ca^{2+}, as the Ca^{2+} current through L-type Ca^{2+} channels falls as the equilibrium potential for Ca^{2+} is approached. A similar phenomenon has been noted for the dependence of transmitter release on whole-cell Ca^{2+} current and other "anomalous" Ca^{2+}-dependent phenomena (Ched and Eckert, 1984). In those cases also, the variation (voltage dependence) of gain was explained on the basis of local "domains" of $[Ca^{2+}]_i$, different than spatial average $[Ca^{2+}]_i$, beneath L-type Ca^{2+}-channels, with the $[Ca^{2+}]_i$ domain depending on the amplitude of Ca^{2+} current through single Ca^{2+} channels.

But what might account for the high gain and the spatial variation in positive feedback of Ca^{2+}-induced Ca^{2+} release as observed by Lipp and Niggli? In their experiments, voltage-dependent entry of Ca^{2+} through L-type Ca^{2+} channels was not involved. The high-gain behavior (i.e., uncontrolled Ca^{2+}-induced release of Ca^{2+} propagating as a wave of elevated $[Ca^{2+}]_i$) was induced by "overloading the cell with Ca^{2+}." A possible explanation is that, when the SR is highly loaded with Ca^{2+}, the putative clusters of SR Ca^{2+} channels are no longer functionally independent. So much Ca^{2+} may be released that neighboring clusters become activated, and the result is a propagating wave of elevated $[Ca^{2+}]_i$. A spiral wave could result from anistropy in this process. Localized regions of elevated $[Ca^{2+}]_i$ may fail to propagate (Valdeolmillos et al., 1989) when this process just fails to occur. A second factor that may be involved is that the sensitivity of SR Ca^{2+} release to cytoplasmic Ca^{2+} may be increased when the SR is highly loaded with Ca^{2+}. In these ways gain might depend on the loading of the SR with Ca^{2+}.

All of this taken together indicates that some sort of model which involves $[Ca^{2+}]$ domains beneath L-type Ca^{2+} channels, clusters of Ca^{2+}-activated SR Ca^{2+} release channels, and variability in the amount of Ca^{2+} available to be released, may explain phenomena as apparently diverse as spiral Ca^{2+} waves and tight control of $[Ca^{2+}]_i$ transients by Ca^{2+} influx during normal E-C coupling. This would constitute, on one level at least, a resolution of a paradox that has puzzled cardiac physiologists for more than 20 years. Perhaps the greatest significance of the paper by Lipp and Niggli (1993) is that it underscores the fact for biophysicists that the understanding of Ca^{2+}-dependent phenomena may lie in spatial realms that are still difficult to probe, and that we must constantly seek to extend the resolution of our measurements. The "easy" measurements, which are made on whole cells (whole-cell currents, whole-cell $[Ca^{2+}]_i$ transients, low spatial resolution images) will certainly not be sufficient for understanding fully the control of $[Ca^{2+}]_i$ and Ca^{2+} signalling.

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