The Ca$^{2+}$ Synapse Redo
A Matter of Location, Location, Location
Leighton T. Izu, C. William Balke

The past decade has witnessed the evolution of a new paradigm in thinking about the intimate interrelationship between cellular structure and physiological function in biological processes. It is not surprising that this evolution has, perhaps, its clearest history in the field of excitation-contraction (E-C) coupling, which has had to wrestle with the long-standing paradox of how the release of Ca$^{2+}$ from the sarcoplasmic reticulum (SR) can be graded by membrane potential$^1$ in the presence of regenerative Ca$^{2+}$ release (Ca$^{2+}$-induced Ca$^{2+}$ release$^{2–4}$). In retrospect, it is clear that the resolution of this paradox had to await both methodological and theoretical breakthroughs that allowed researchers to change the scale of our measurements and thinking. Until the early 1990s, measurements of intracellular Ca$^{2+}$ were limited to spatially averaged whole-cell Ca$^{2+}$ transients or Ca$^{2+}$ waves. The corresponding theoretical constructs assumed a spatially continuous distribution of SR Ca$^{2+}$ release, which successfully predicted the properties of Ca$^{2+}$ waves but precluded graded Ca$^{2+}$ transients—hence the paradox.

In 1992, Stern$^5$ made a key advance toward the resolution of this paradox by recognizing that all-or-nothing SR Ca$^{2+}$ release could be avoided by having discrete Ca$^{2+}$ release sites that were spatially segregated. This model was unique in that it included the intimate association or coupling of dihydropyridine receptors (DHPRs) to a discrete cluster of ryanodine receptors (RyRs), forming what he called a “Ca$^{2+}$ synapse.” In this model, each cluster of RyRs may release Ca$^{2+}$ in an all-or-nothing manner, but because of their physical separation, each cluster could act as an independent unit. Gradation of the magnitude of the whole-cell Ca$^{2+}$ transient is, then, determined by the number of activated Ca$^{2+}$ synapses, just as skeletal muscle contraction is graded by the number of firing motor neuron synapses. Stern’s important contribution was the recognition of the functional consequences of the cellular molecular architecture.

Methodological advances in laser scanning confocal microscopy and the development of a fluorescent Ca$^{2+}$ indicator with high quantum yield (fluo-3) provided new tools for measuring cytosolic Ca$^{2+}$ at an unprecedented submicron scale. The observation of Ca$^{2+}$ sparks by Cheng et al$^6$ and others$^7–10$ and the localization of Ca$^{2+}$ sparks to t-tubules$^{11}$ provided the experimental underpinnings for Stern’s concept of the Ca$^{2+}$ synapse.$^5$ These observations set the stage for the “local control” theory of E-C coupling, where SR Ca$^{2+}$ release is controlled by the L-type Ca$^{2+}$ current because independent, elementary events of SR Ca$^{2+}$ release are “recruited” by Ca$^{2+}$ flowing through single L-type Ca$^{2+}$ channels, and not by the average intracellular Ca$^{2+}$ concentration.$^{12}$ The molecular underpinnings for the local control theory were demonstrated by the colocalization of DHPRs and RyRs in several muscle types.$^{13,14}$ Clearly, this past work has been focused primarily on the role of molecular architecture as it pertains to the local control of SR Ca$^{2+}$ release. Recent work, as with the study by Yang et al$^{15}$ in this issue of Circulation Research, has expanded the notion of local control to include some of the cellular processes that are important for the regulation of Ca$^{2+}$ removal and/or uptake such as the sodium-calcium exchanger (NCX).

The sodium-calcium exchanger is a major route for Ca$^{2+}$ removal from the cytoplasm in cardiac muscle and might also contribute to the Ca$^{2+}$ trigger for SR Ca$^{2+}$ release. Accordingly, NCX would be predicted to be distributed in the vicinity of the Ca$^{2+}$ synapse machinery of DHPRs and RyRs at the t-tubule–junctional SR region. However, attempts at the molecular localization of the NCX have yielded disparate results with some studies showing NCX distributed primarily in the t-tubules$^{16,17}$ and other studies showing NCX distributed throughout all membranes in contact with the extracellular space.$^{18}$ Yang et al$^{15}$ provide functional information regarding the localization of NCX and Na$^+$ channels that goes a long way in resolving the controversies of the spatial localization of NCX. They exploited the ability to functionally inactivate t-tubules via osmotic shock (detubulate) in rat ventricular cells and they showed that in detubulated cells (1) the spatial pattern of SR Ca$^{2+}$ release resembled that observed in cardiac atrial cells that lack t-tubules, (2) the rate of decay of the whole-cell Ca$^{2+}$ transient (visualized as fluo-3 fluorescence) was markedly slowed compared with cells with t-tubules, (3) the magnitude of the Na$^+$ current declined in proportion to the decrease in membrane capacitance, and most interestingly (4) the NCX current was virtually abolished. These results emphasize the importance of the NCX in the t-tubules for Ca$^{2+}$ removal during E-C coupling and leave open the possibility that the NCX on the surface membrane may have an entirely different function. The functional results of Yang et al$^{15}$ complement nicely the immunocytochemistry experiments of Moore and coworkers,$^{17}$ which showed the localization of Na$^+$ channels and NCX in the t-tubules of rat ventricular cells. Importantly, however, the NCX and the Na$^+$

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channels were not found in the immediate vicinity of the Ca\(^{2+}\) synapse, but they were localized in the t-tubules.\(^\text{17}\)

The functional localization of NCX by Yang et al\(^\text{15}\) combined with the molecular localization studies of Scriven et al\(^\text{17}\) now enable us to expand the domain of Stern’s Ca\(^{2+}\) synapse\(^\text{5}\) to include the cellular processes that determine both SR Ca\(^{2+}\) release and cytoplasmic Ca\(^{2+}\) removal/uptake. Shown schematically in the Figure, the expanded version of the Ca\(^{2+}\) synapse depicts SR Ca\(^{2+}\) release via the intimate association of DHPRs and RyRs, which are in close proximity with one of the major processes influencing Ca\(^{2+}\) removal, namely NCX and also with Na\(^{+}\) channels. This model is also consistent with the putative role of the NCX to contribute, in part, to the Ca\(^{2+}\) trigger for SR Ca\(^{2+}\) release. As new information regarding the functional and molecular localization of additional ion channels, transporters, and regulatory molecules becomes available, this somewhat simple scheme will become undoubtedly richly populated with the full array of processes that are devoted to the singular task of transiently elevating Ca\(^{2+}\) for muscle contraction.

References


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