New and Notable

Caged Calcium and the Ryanodine Receptor

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In cardiac muscle excitation-contraction coupling, surface membrane depolarization is considered to activate L-type Ca^{2+} channels, resulting in the entry of Ca^{2+} into the muscle cell. This elevation in myoplasmic Ca^{2+} is now generally accepted to induce the release of Ca^{2+} from the sarcoplasmic reticulum by a process termed Ca^{2+}-induced Ca^{2+} release. A recent report by Györke and Fill (1993) using a novel experimental approach provides additional support for this Ca^{2+}-induced Ca^{2+} release mechanism in cardiac muscle. In the first work of this kind on the sarcoplasmic reticulum Ca^{2+} release channel (ryanodine receptor), Györke and Fill adapted a planar lipid bilayer apparatus so that Ca^{2+} could be liberated photolytically from DM-nitrophen (caged Ca^{2+}) in a very restricted region of the medium near the bilayer. After insertion of the ryanodine receptor into the lipid bilayer, Ca^{2+} was elevated on the myoplasmic side of this channel protein by an ultraviolet flash. The liberation of Ca^{2+} in the microenvironment of the channel triggered a transient burst of channel activity; the original conditions could be reestablished by just stirring the medium. In this manner, ensemble currents could be generated by summing single channel sweeps derived from successive ultraviolet flashes. The time constants for activation of the cardiac ryanodine receptor channels determined from such ensemble currents ($\tau = 1.2$ ms) are consistent with the initial rate of activation of Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum in vivo. The most unexpected result, however, was that after the photolytic increase in $[Ca^{2+}]$ and ryanodine receptor Ca^{2+} channel activation, the probability of channel opening peaked and then spontaneously decayed; in contrast, the $[Ca^{2+}]$ in the channel vicinity remained constant. The rate of decay in channel activity closely correlates with the rate of Ca^{2+}-dependent inactivation of Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum in permeabilized cardiac myocytes (Fabiato, 1985), indicating the physiological relevance of this decay. Furthermore, the channel's sensitivity to Ca^{2+} generated rapidly by photolysis was approximately 10 times greater than the channel's sensitivity to Ca^{2+} under steady-state conditions. Thus, the Ca^{2+} sensitivity of ryanodine receptor activation decreases during prolonged exposure to Ca^{2+}, supporting the hypothesis of ryanodine receptor Ca^{2+} adaptation.

The new report by Györke et al. (1994) now documents the methodology used to activate photolytically the caged Ca^{2+} compound in these intricate experiments. The authors adopt an ingenious approach in which an intense UV flash from a Nd:yttrium-aluminum-garnet laser is directed at the solution directly in front of the bilayer through a 450 μm diameter, micropositioned, single fused silica optic fiber. The potential failing of such a technique is that the fiber ensures that only a very small portion of the medium is exposed to the UV flash, so that Ca^{2+} is only liberated from caged-Ca^{2+} in that very small region of the medium. If the flash should not be focused directly on the hole in the Delrin partition of the bilayer apparatus, then Ca^{2+} will not be elevated in the environment of the bilayer. This would confound interpretation of experiments in which activation of the channel in the bilayer by flash-photolyzed caged Ca^{2+} is to be examined. The authors take a novel approach in addressing this issue. In control experiments, they converted the bilayer chamber into a Ca^{2+} electrode by filling the bilayer aperture with a Ca^{2+} ionophore resin. They then showed that photolytic Ca^{2+} stimuli were essentially steps in Ca^{2+} concentration, because diffusion of Ca^{2+} in the vicinity of the bilayer was physically restricted and rather slow. This Delrin partition containing the resin could then be carefully removed and replaced with the Delrin partition that was to be used for recording single channel activity of ryanodine receptor Ca^{2+} channels.

Lamb et al. (1994) have recently criticized this caged Ca^{2+} approach claiming that Györke and Fill may have generated an extremely large transient increase in the $[Ca^{2+}]$, which itself might have been responsible for the behavior of the channels. However, Györke and Fill (1994) challenge this conclusion because it is far from certain that fast $[Ca^{2+}]$ spikes actually occur when using this caged Ca^{2+} compound. Furthermore, under conditions set to maximize the amplitude of such fast spikes in Ca^{2+} concentration, there was no triggering of channel activity. Conversely, when conditions were set to minimize the amplitude of such spikes, photolysis induced fast activation followed by the slow decay of channel activity.

The latest report by Györke et al. (1994) also contains an initial description of the analysis of the skeletal muscle ryanodine receptor by the caged Ca^{2+} technique and provides more confounding data for those struggling to understand the mechanism of excitation-contraction coupling in skeletal muscle. Several lines of evidence have led to the general agreement that the Ca^{2+}-induced Ca^{2+} release mechanism does not operate in skeletal muscle excitation-contraction coupling. However, Györke et al. (1994) now demonstrate, in a very convincing fashion, that the rate constants for Ca^{2+} activation of both the cardiac and skeletal muscle ryanodine receptor Ca^{2+} channels are essentially identical, i.e., the activation of the skeletal ryanodine receptor by Ca^{2+} is fast enough for Ca^{2+}-induced Ca^{2+} release to be the mechanism of excitation-contraction

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coupling in skeletal muscle. The apparent identity of the kinetics of activation of the skeletal and cardiac ryanodine receptor Ca^{2+} channels to photolyzed Ca^{2+} indicates that the difference in excitation-contraction coupling mechanisms in these two types of muscle are unlikely caused solely by differences in ryanodine receptor subtypes.

To reconcile their data with the existing literature on excitation-contraction coupling in skeletal muscle Györke et al. (1994) suggest that the Ca^{2+} activation site of the skeletal muscle ryanodine receptor may be morphologically restricted in situ, or that physiologically important ligands may be omitted in the planar lipid bilayer experiments. Such ligands may either stimulate or inhibit the ryanodine receptor Ca^{2+} channel and they may be specific for the skeletal or cardiac isoform of this channel protein. In support of this latter proposal, Fruen et al. (1994) have recently shown that a number of inorganic anions activate the skeletal, but not the cardiac, muscle ryanodine receptor Ca^{2+} channel. Thus, other yet undiscovered ligands that specifically regulate the skeletal muscle ryanodine receptor Ca^{2+} release channel may ensure that the activation of these two ryanodine receptor isoforms are very different in situ. What other treats are in store for us using this novel adaptation of the flash photolysis procedure are awaited with great interest by those studying excitation-contraction coupling in muscle.

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Toward Unified and Consistent Views of Protein Dynamics

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The physical and functional consequences of internal protein dynamics have long been of interest (see Frauenfelder et al. (1990) and references therein). In many respects, however, the various time scales and corresponding manifolds of dynamics have been artificially isolated by the techniques used to probe and understand them. Thus, for example, there has been little reconciliation historically between the long time scale dynamical behavior seen in computer simulations with comprehensive experimental observations. Indeed, this pattern of isolated investigation is seen even among experimentalists concerned with biopolymer dynamics on the same time scale. A classic example might be the combined application of fluorescence and NMR techniques to characterize the subnanosecond motions of proteins. In a paper in this issue of *Biophysical Journal* that, one would hope, will initiate a trend in these kinds of studies, Kemple and co-workers use the dynamical information inherent in both fluorescence and NMR spectroscopy to characterize tryptophan side-chain motions in the native and two single tryptophan mutant forms of *E. coli* thioredoxin.

This paper is timely in many significant respects. The methodologies of both NMR and fluorescence spectroscopy have recently emerged as mature, sophisticated, and powerful technologies. This is especially true for NMR, where tremendous strides have been made in the methodologies employed in the solution of the resonance assignment problem (Bax and Grzesiek, 1993), the determination of the solution structures of proteins (Clore and Gronenborn, 1991) and, most recently, in the characterization of the internal dynamics of proteins by use of NMR relaxation phenomena.

After a rough start where concern was raised about the experimental and analytical strategies of early approaches (Boyd et al., 1990; Peng et al., 1991; Dellwo and Wand, 1991), the application of NMR relaxation techniques to the exploration of the fast internal dynamics of proteins has resulted in the study of two dozen or more systems in the past few years. Most have involved characterization of main-chain dynamics by use of ^{15}N relaxation. Kemple and co-workers move the dynamic issue to the more interesting realm of internally buried side chains and employ ^{13}C relaxation phenomena. The combined use of both NMR and fluorescence phenomena allow Kemple and co-workers to explore with some confidence a number of issues that have plagued isolated studies. These have to do with origins of artifacts and inaccuracies such as complete accommodation of chemical shift anisotropy, the functional form of the spectral density, and the nature of global tumbling, which must enter into consideration explicitly. The functional form of the spectral density arising from the so-called model-free treatment of Lipari and Szabo is especially appealing on several grounds. This treatment provides for relatively accurate descriptions of the internal motions underlying relaxation in terms of two root parameters: the so-called generalized order parameter and the effective correlation time. The former provides a measure of the spatial amplitude of the motion, and the latter effectively provides an upper limit on the correlation time(s) of the motion(s) contributing to relaxation. There is no requirement to specify the exact physical nature of the internal motion beyond the requirment that they

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