Ca^{2+} Signaling in Atrial Myocytes: Evidence from 2-D Rapid Confocal Imaging

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Atrial myocytes lacking t-tubules, have two functionally separate groups of ryanodine receptors (RyRs): those at the periphery colocalized with dihydropyridine receptors (DHPRs) and those at the cell interior not associated with DHPRs. It has been thought that during action potential Ca^{2+} influx through the voltage dependent Ca^{2+} channels (DHPRs) triggers peripheral Ca^{2+} release, which in turn propagates into the center of atrial myocytes to trigger contraction of myofilaments. Here, using rapid (240 Hz) two-dimensional confocal Ca^{2+} imaging in voltage clamped rat atrial myocytes, we have examined: 1) the extent to which the two sets of RyRs are controlled by Ca^{2+} current (I_{Ca}) or Ca^{2+} diffusion, and 2) control mechanisms of Ca^{2+} propagation wave which occurs physiologically, to understand detailed atrial Ca^{2+} signaling.

Activation of I_{Ca} first triggered rapid releases of peripheral Ca^{2+} at discrete subsarcolemmal sites (within 14 ms), producing local Ca^{2+} transients with a single component. The central Ca^{2+} release produced both fast rising component (within 14 ms) and slower and delayed components (>14 ms). I_{Ca}-gated peripheral Ca^{2+} release propagated into the center of cell with a velocity of ≈230 μm/sec, causing a ≈34 ms delay of slow central Ca^{2+} release. The propagation of I_{Ca}-triggered Ca^{2+} release into the cell center occurred sequentially at sarcomeric distances (≈2 μm). Peripheral Ca^{2+} release and the fast component of central Ca^{2+} release were resistant to high concentrations of Ca^{2+} buffers (2 mM EGTA plus 1 mM Fluo-3), while the slower component of central Ca^{2+} release was completely abolished by high concentrations of Ca^{2+} buffers. The voltage dependence of the peripheral and central releases was bell-shaped and closely related to the magnitude and duration of I_{Ca}. The velocity of Ca^{2+} propagation wave was rather constant and independent of the magnitude of I_{Ca}, suggesting involvement of other mechanisms in the regulation of the propagation. The results suggest that peripheral release sites are directly governed by Ca^{2+} influx through the DHPRs and that central release sites are controlled by Ca^{2+} diffusion and I_{Ca} as well.

To understand control mechanisms for the central Ca^{2+} propagation wave we further examined the role of inositol 1,4,5-trisphosphate receptor (IP_3R) and mitochondria, localized in close proximity to the RyRs, on the local Ca^{2+} signaling in rat atrial myocytes.

Incubation with xestospongin C (XeC), a blocker of IP_3R, partially suppressed the magnitudes of the central and peripheral Ca^{2+} releases. The suppressive effect of XeC on focal Ca^{2+} releases was apparent at the delayed (> 16 ms) central, but not the peripheral and fast central Ca^{2+} release sites. Mitochondrial staining showed longitudinal alignments with 2-•m separation in both ventricular and atrial myocytes. Depolarization by rapid application of high K^+ solution triggered brief mitochondrial uptake and release of peripheral Ca^{2+}, which was followed by slow delayed uptake of central and peripheral Ca^{2+} by the mitochondria. Our data suggest that the activation of IP_3R during depolarization and mitochondrial Ca^{2+} handing may serve as a cofactor for the activation of nonjunctional RyRs right next to the DHPR-coupled peripheral RyRs with large gaps between them.

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