

Imaging tubular system, sarcoplasmic reticulum and myoplasmic calcium with novel fluorescence methods

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The precise control of contraction in skeletal muscle critically depends on the rapid and precise delivery of Ca^{2+} from the specialized internal store, the sarcoplasmic reticulum (SR), which is under voltage control from the tightly apposed tubular (t-) system. Regulation of Ca^{2+} in the SR and cytoplasm during periods of muscle work is complex and involves influxes of Ca^{2+} from the t-system. A major problem is that measurements of voltage and store-dependent Ca^{2+} fluxes from the t-system of skeletal muscle are not possible with standard electrophysiological techniques. Methods to simultaneously image Ca^{2+} in two subcellular compartments of single muscle fibres using laser scanning confocal microscopy have recently been developed. This has largely involved trapping one Ca^{2+} sensitive dye in the t-system of a mechanically skinned fibre and introducing a second spectrally separate Ca^{2+} indicator to the cytoplasm. By stimulating release of Ca^{2+} directly from SR or *via* t-system depolarization, voltage dependent and independent fluxes across the t-system can be spatiotemporally resolved against the release flux of Ca^{2+} from SR. These measurements have identified an action potential-induced Ca^{2+} flux across the t-system and an ultra-rapid store-operated Ca^{2+} entry (SOCE) mechanism in skeletal muscle. This has led to the development of a working model for SOCE that is relevant within the large Ca^{2+} release fluxes initiated by excitation-contraction coupling. Potential roles of the action potential-induced Ca^{2+} flux in skeletal muscle remain speculative.