Voltage-dependent and -independent Ca^{2+} entry into skeletal muscle during excitation-contraction coupling

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Excitation-contraction coupling (EC coupling) is the process that links the activation of the surface membrane of a muscle to the force response produced. This process is reliant on a rapid and large change in cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_{cyto}$) due to release activated from the internal Ca^{2+} store, the sarcoplasmic reticulum (SR). The change in $[Ca^{2+}]_{cyto}$ regulates the movement of the contractile proteins in the muscle to produce force. Also, during EC coupling there can be an input of Ca^{2+} across the surface membrane, or from the invagination of this membrane, the tubular (t-) system. In skeletal muscle, this influx of Ca^{2+} is not strictly required for a contracture to occur, but its importance may be in the longer-term regulation of function or may indeed have more subtle, immediate consequences that are not clear from present measurements. Several pathways can be responsible for an influx of Ca^{2+} into skeletal muscle fibres during EC coupling. These can be voltage-dependent, including L-type Ca^{2+} current, action potential-activated Ca^{2+} current (APACC) and excitation-coupled Ca^{2+} entry (ECCE); or voltage-independent, including store-operated Ca^{2+} entry (SOCE), stretch-activated Ca^{2+} entry or otherwise activated to enter through transient receptor protein (TRP) channels. It should be noted that the conditions that prevail under bouts of EC coupling are the conditions that activate voltage-dependent and –independent Ca^{2+} entry during EC coupling in skeletal muscle (Allen *et al*. 2005; Launikonis *et al.*, 2010).

The nature of these voltage-dependent and -independent pathways in skeletal muscle have been examined by a number of groups using either fully differentiated fibres or myotubes. A problem that does arise here is that properties derived on myotubes are often assumed to be very similar to that in the fully differentiated muscle. There are significant differences in myotube and adult fibre physiology and membrane ultrastructure, making extrapolation of results derived on one cell to other not a simple matter. The examination of Ca^{2+} currents in myotubes may have been necessary in some instances because conventional electrophysiological methods are difficult or do not have the sensitivity to measure such tiny Ca^{2+} currents in adult fibres. Furthermore, the use of pharmacological agents commonly used to block Ca^{2+} entry pathways are, unfortunately, non-specific in most cases. A combination of these factors may have lead to a misrepresentation of certain pathways existing in myotubes or adult fibres.

In adult, mammalian skeletal muscle fibres it has been possible to image Ca^{2+} in the lumen of the t-system using a low-affinity Ca^{2+}-sensitive fluorescent dye. The dye is trapped in the t-system of skinned fibres, where the surface membrane has been removed by microdissection. With this preparation, Ca^{2+} movement across the t-system membrane is visualized as a net change in the Ca^{2+}-dependent fluorescence signal emitted from the t-system lumen. This can be monitored in conjunction with a spectrally separate Ca^{2+}-sensitive dye in the cytoplasm to monitor changes in $[Ca^{2+}]_{cyto}$, which will be predominately due to fluxes across the SR membrane. This preparation has allowed the identification and characterization of SOCE during voltage-independent Ca^{2+} release and also APACC, which has been identified and observed to occur during single and trains of action potentials in adult mammalian skeletal muscle fibres.

Allen DG, Whitehead NP, Yeung EW. (2005) *Journal of Physiology*, **567**: 723-735.
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