An action potential activated Ca^{2+} current in skeletal muscle

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Ca^{2+} entry into cells is a fundamental process that enables cells to regulate cytoplasmic [Ca^{2+}], [Ca^{2+}] in intracellular stores and many Ca^{2+}-dependent intracellular processes from gene expression to muscle contraction. Cardiac cells have an absolute requirement for Ca^{2+} entry via the L-type Ca^{2+} channel upon membrane excitation to induce Ca^{2+} release from the sarcoplasmic reticulum (SR) and consequently activate the contractile apparatus. The L-type Ca^{2+} channel also exists in skeletal muscle but the duration of action potentials (APs) in skeletal fibres is too brief (2-5 ms), compared to that in cardiomyocytes (100-200 ms), to activate the channel to any degree. Instead, the α-subunit of the L-type Ca^{2+} channel in skeletal muscle acts as a voltage sensor, which directly activates Ca^{2+} release from the SR. This is not to say that skeletal muscle L-type Ca^{2+} channels cannot pass Ca^{2+}, they simply require a relatively long period of depolarization that does not occur under normal physiological conditions. Yet, there is evidence for Ca^{2+} entry associated with periods of low frequency excitation of skeletal muscle (Gissel & Clausen, 1999), but the pathway of Ca^{2+} entry during normal excitation in skeletal muscle fibres has not been identified due to inherent limitations in the techniques used to record very small Ca^{2+} fluxes during normal excitation. Our aim was to use a recently developed fluorescence technique (Launikonis & Rìos, 2007) to identify whether there is a t-system Ca^{2+} current associated with normal excitation in skeletal muscle.

The Animal Ethics Committee at Rush Medical Centre approved the use of animals in this project. Male rats (3 months old) were killed by asphyxiation and the extensor digitorum longus (EDL) muscles were removed. Intact fibres were exposed to a Na^+-based physiological solution containing mag-indo-1 salt. Fibres were mechanically skinned, trapping the dye in the t-system, and transferred to a chamber containing a K^+-repriming solution with rhod-2. Net changes in the finite t-system [Ca^{2+}] ($[Ca^{2+}]_{t-sys}$) of the skinned fibre, $d[Ca^{2+}]_{t-sys}/dt$, could be equated to t-system Ca^{2+} current (Launikonis & Rìos, 2007). The chamber was equipped with platinum electrodes that ran parallel to the mounted fibre. In other experiments, skinned fibres without dye in the t-system were bathed in a K^+-repriming solution with indo-5F and rhod-2. The indo analogues with rhod-2 were simultaneously imaged during field stimulation on a Leica SP-2 confocal microscope in linescan mode, with the scanning line positioned parallel to the long axis of the fibre. The group scanning speed of the three lasers used to excite mag-indo-1 (or indo-5F) and rhod-2 was 1.9 ms/line.

Imaging the cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyto}$) transient during field stimulation of skinned fibre preparations in the presence of rhod-2 and indo-5F in the bathing solution produced a uniform and rapid (~ 5 ms) increase in $[Ca^{2+}]_{cyto}$ as indicated by both dyes. This imaging technique allowed calibration of the Ca^{2+} transient in skinned fibres for the first time (with indo-5F) and the parallel imaging of rhod-2 provided a reference for EC coupling viability the next group of experiments. Under our imaging conditions, $\gamma K_{D,Ca}$ of indo-5F was 2.38 μM and indicated a peak $[Ca^{2+}]_{cyto}$ of 1.1 μM following excitation. Following correction of the raw $[Ca^{2+}]_{cyto}$ calibration for the slow off rate of indo-5F (75 s^{-1}), a peak $[Ca^{2+}]_{cyto}$ of 4 μM was estimated to be reached in about 2 ms. Thus skinned fibres release Ca^{2+} at a normal rate and magnitude in response to physiological excitation. Simultaneous imaging of cytoplasmic rhod-2 and t-system trapped mag-indo-1 showed that there was indeed an influx of Ca^{2+} into the cell following an AP when $[Ca^{2+}]_{t-sys}$ was 0.2 mM or greater. The current decayed exponentially and lasted approximately 70 ms. Subsequent APs produced no further t-system Ca^{2+} current in the following 200 ms, even though Ca^{2+} was released from sarcoplasmic reticulum, thus defining an inactivation period for this current. When $[Ca^{2+}]_{t-sys}$ was about 0.1 mM, a transient rise in $[Ca^{2+}]_{t-sys}$ was observed almost concurrently with the increase in $[Ca^{2+}]_{cyto}$ following the action potential. The change in direction of Ca^{2+} flux was consistent with changes in driving force for Ca^{2+}. This is the first direct demonstration of a marked Ca^{2+} flux that inactivates, associated with an AP in skeletal muscle.

Gissel H & Clausen T. (1999) *American Journal of Physiology*, **276:** R331-9.
Launikonis BS & Rìos E. (2007) *Journal of Physiology*, **583:** 81-97.