Estimation of RyR permeability in living muscle cells (Workshop in Ulm, April 2005)

Aim

In studies of EC coupling it is important to assess the permeability changes of the ryanodine receptors in functioning muscle cells.

Introduction

There is no method available to directly measure the time course of the flux of Ca^{2+} release from the SR. In contrast the Ca^{2+} entry flux from the extracellular spacecan be electrically recorded by the Ca^{2+} inward current. Information on the Ca^{2+} release flux is, however hidden in the myoplasmic Ca^{2+} transient measured with a fluorescent calcium indicator dye. Calcium in the myoplasm increases upon depolarization due to the voltage-activated opening of the ryanodine receptors. In skeletal muscle, the ryanondine receptors in the junctional face membrane of the SR are remote-controlled using an allosteric link to the voltage-sensing DHP receptors in the transverse tubules (figure, panel A). Increasingly stronger depolarization leads to a progressive increase in the Ca^{2+} signal amplitude.

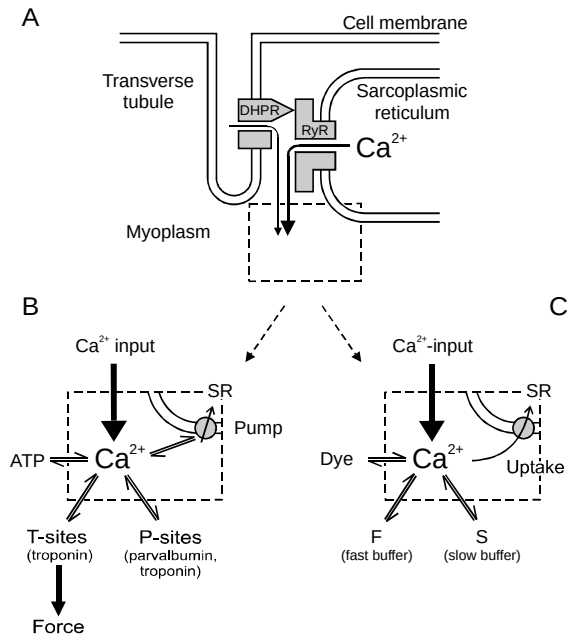

FIGURE Ca^{2+} input and removal in skeletal muscle.
(A) Schematic diagram of the control of ryanodine receptor (RyR)-mediated Ca^{2+} release from the SR by the transverse tubular dihydropyridine receptor (DHPR). (B) Main compartments to which released Ca^{2+} is distributed (Baylor and Hollingworth, 1998; Jiang et al., 1999): Fast binding sites on troponin C (T-sites), slow binding sites on troponin C and parvalbumin (P-sites), fast calcium binding by ATP, and binding and transport by the SR Ca^{2+}-ATPase. (C) Simplified reaction scheme to account for binding and transport of released Ca^{2+}: 1. Fast binding assumed to be instantaneous and linear. 2. Slow binding assumed to be dominated by EGTA in the experimental conditions. 3. Uptake assumed to be proportiona1 to free calcium.

The Ca^{2+} buffering effect of the indicator influences the time course of the cellular Ca^{2+} transient. In addition there are multiple cellular calcium binding proteins which

participate in shaping the calcium transient: Troponin C which regulates the cross bridge cycle of the contractile proteins, the SERCA pump that refills the SR, and parvalbumin which helps to remove calcium form troponin in fast twitch muscle cells. Also ATP binds some Ca^{2+}. The figure (panel B) explains the typical intracellular calcium buffering situation in a muscle cell under physiological conditions.

Calculations

If one knows the properties of all the calcium binding reactions in a muscle cell one can determine the total Ca^{2+} release from the indicator signal. One can first calculate the free calcium concentration using calibration data of the indicator. Using free calcium, one can calculate how much calcium is bound to each of the other buffers, and finally, by summing everything up, how much calcium flowed into the myoplasm during the depolarization and with which time course. With a computer and the appropriate software this would be only a few mouse clicks away from our measured data. Unfortunately, we have not all the information that we need, i.e. all the concentrations of the buffers and their calcium binding rate constants. By using a trick, we therefore simplify the situation: We fill the cells with a lot of EGTA (up to 15 mM) to dominate the cell's calcium buffering by EGTA. This allows us to ignore most of the other buffers in the calculation and we can use a simpler reaction model to describe intracellular Ca^{2+} binding that takes into account mainly EGTA (panel C of the figure; S represents EGTA). We can now find optimal values for the rate constants in the model using a non-linear fitting procedure of model-generated traces to measured traces ("removal model fit") and can use the result to determine the voltage-activated Ca^{2+} release flux and finally the Ca^{2+} permeability of the SR, i.e. the signal that shows the actual "gating" of the release pathway.

Practical procedure for the workshop

In the workshop we will go step by step through this procedure to turn your recorded fluorescent transients into permeability.

Note: If you want to read more about this: Schuhmeier & Melzer 2004. Voltage-dependent Ca^{2+} fluxes in skeletal myotubes determined using a removal model analysis. J. Gen. Physiol. 123: 33-51