Role of phosphorylation in the Ca^{2+} regulation of Ca^{2+} release channels in skeletal muscle

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During excitation contraction coupling, the action potential depolarises the voltage-dependent L-type Ca^{2+} channels in the sarcolemma and T-tubules, leading to a release of Ca^{2+} from the sarcoplasmic reticulum (SR, the main intracellular Ca^{2+} store) and a rise in cytoplasmic Ca^{2+} which stimulates muscle contraction. In skeletal muscle, the Ca^{2+} release channels in the SR (RyR1 isoform) are stimulated *via* a direct protein-protein interaction with the L-type Ca^{2+} channels whereas in cardiac muscle it is the inflow of Ca^{2+} into the cytoplasm through the L-type Ca^{2+} channels that activates the cardiac ryanodine receptors (RyR2). Cardiac and skeletal RyR isoforms are modulated differently by intracellular Ca^{2+}. Cardiac RyRs have a complete reliance on Ca^{2+} for opening in which Ca^{2+} in the cytoplasm and SR lumen produce a synergistic activation of the channel (Laver & Honen, 2008). On the other hand, skeletal RyRs can open in the complete absence of Ca^{2+} or any other activating ligand and intracellular Mg^{2+} is required to inhibit RyR1 and prevent SR Ca^{2+} release during muscle relaxation (Laver *et al*., 2004). In this study, we explore the effect of endogenous phosphorylation of RyR1 on its ability to open in the absence of Ca^{2+}.

RyR1 was isolated from rabbit skeletal muscle as described previously (Laver *et al.*, 1995). RyRs were incorporated into artificial lipid bilayers and channel gating was measured by single channel recording. RyR open and closed times were measured in the presence various concentrations of cytoplasmic and luminal [Ca^{2+}] and in the presence of cytoplasmic ATP or AMP-PCP (2 mmol/l). The effect of endogenous phosphorylation of RyR1 was assessed by incubating SR vesicles containing RyR1 with Protein Phosphatase1 (PP1, 20 units/mg SR protein) for 5 min at 30°C. PP1 removes phosphate groups from serine residues.

RyR1 in their endogenous phosphorylation state were quite active in the virtual absence of cytoplasmic and luminal Ca^{2+} (1 nmol/l cytoplasmic and < 10 μmol/l luminal). Under these conditions they had an opening rate of 100 ± 50 s^{-1} (n=4). The opening rate showed no significant dependence on luminal [Ca^{2+}] between 10 μmol/l and 2 mmol/l. The opening rate increased with increasing cytoplasmic [Ca^{2+}] up to 1000 s^{-1} with half activation at 1 μmol/l. RyR1 treated with PP1 had zero opening rate in the absence cytoplasmic and luminal Ca^{2+}. Opening rates increased to 2 ± 0.5 s^{-1} in the presence of 0.1 mmol/l luminal Ca^{2+} and 170 ± 80 s^{-1} in the presence of 0.1 mmol/l cytoplasmic Ca^{2+}.

The results indicate that endogenous phosphorylation is important for maintaining the level of activity of RyR1 in skeletal muscle. Interestingly, dephosphorylation of RyR1 makes them reliant on Ca^{2+} for their activation and in that regard makes them very similar cardiac RyRs. This suggests that a key functional difference between cardiac and skeletal muscle may depend on phosphorylation of a serine residue in RyR1.

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