Unique actions of junctin and triadin on skeletal muscle ryanodine receptor calcium release channels

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Excitation contraction (EC) coupling is the process which initiates skeletal muscle contraction. Depolarisation of the sarcolemma triggers Ca^{2+} release through ryanodine receptor (RyR) calcium release channels in the sarcoplasmic reticulum (SR) of skeletal muscle. The Ca^{2+} binding protein calsequestrin (CSQ1) interacts with the skeletal muscle type 1 ryanodine receptor (RyR1) in the lumen of the SR and strongly inhibits RyR1 channel activity. The membrane associated proteins triadin and junctin are thought to mediate a functional interaction between CSQ1 and the RyR1 which conserves Ca^{2+} stores in the lumen of the sarcoplasmic reticulum (SR). The individual actions of triadin and junctin on RyR1 and their contribution to CSQ1 interactions with RyR1 are reported here. Our aims were to determine: 1) the influence of triadin and junctin (either individually or together) on purified RyR1 activity and 2) the ability of the anchoring proteins (either individually or together) to mediate the inhibitory effect of CSQ1 on RyR1 and to prevent an increase in RyR1 activity when the concentration of Ca^{2+} in the SR falls.

New Zealand male white rabbits were euthanized by a captive bolt and back and leg muscle used to prepare skeletal muscle proteins. We obtained highly purified CSQ1, triadin, junctin and RyR1 from skeletal muscle using a combination of skeletal SR protein solubilization, gradient centrifugation and SDS and native preparative gel electrophoresis. RyR channel activity was measured in lipid bilayers. Purified RyR1 channels were reconstituted into artificial planar lipid bilayers which separate two chambers which are equivalent to the cytoplasmic and SR luminal compartments of the fibres and we examined the actions of purified triadin, junctin and CSQ on channel activity.

Addition of either triadin or junctin to the luminal side of RyR1 channels embedded in lipid bilayers increased channel open probability by increasing channel open time and stabilised channel openings to the maximum conductance. Junctin/triadin competition studies showed an additive effect of adding these proteins to the RyR, indicating that triadin and junctin interacted with independent sites on RyR1, confirming previous observations from our laboratory. Purified CSQ1 inhibited the reconstituted triadin/junctin/RyR1 complex without reducing the number of maximum conductance openings. In addition the CSQ1/triadin/junctin/RyR1 complex responded to low luminal $[Ca^{2+}]$ in the same way as native RyR1 channels. Therefore, triadin and junctin alone are sufficient to transmit the inhibitory effect of CSQ1 to RyR1 channels and other associated proteins in the native SR are not essential for this action. CSQ1 inhibited RyR1 channels associated with junctin alone, but not RyR1 channels associated only with triadin. In addition the complex between purified CSQ1, junctin and RyR1 supported the inhibition seen when native RyR1 channels are briefly exposed to low luminal Ca^{2+} which may occur during repetitive activity. The CSQ1/triadin/RyR1 complex did not support channel inhibition indicating that triadin is not essential for communication between CSQ1 and the RyR1.

These novel results show that, contrary to expectation, junctin alone is responsible for mediating signals between CSQ1 and RyR1. The rapid Ca^{2+} efflux *in vivo* may be reinforced by the actions of triadin and junctin in stabilizing RyR1 channel gating to enhance channel function. The activation achieved by triadin and junctin binding to RyR1 is negated by the antagonistic effect of CSQ1 on junctin activation. Therefore junctin underlies the role of CSQ1 as a key regulator of the amount of Ca^{2+} released from the SR and is thus an essential component of a tightly interacting machine that *in vivo* ensures functional EC coupling and SR Ca^{2+} release.