Stress-induced thiol modification and phosphorylation in striated muscle disorders

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The ryanodine receptor (RyR) is ligand-gated calcium release channel found embedded in the membrane of the intracellular calcium store (the sarcoplasmic reticulum) of skeletal and cardiac muscle. The RyR serves to release calcium from the intracellular store in response to an action potential, in a process known as excitation–contraction coupling. The RyR is a >2MDa homotetramer at the centre of a multiprotein calcium release complex which extends from the extracellular space to the lumen of the sarcoplasmic reticulum. RyR activity (and the amount of calcium released to elicit contraction) is set by integrated effects of associated ions, co-factors and proteins, the level of calcium within the SR and through covalent modification by redox reactions and phosphorylation. The RyR contains numerous serine and cysteine residue that are vulnerable to chronic covalent modification under pathological conditions. Such conditions include malignant hyperthermia and muscular dystrophy in skeletal muscle and ischemic, heart failure and chemotherapy-induced cardiotoxicity in the heart.

Work over the last decade has highlighted the importance of maintaining robust calcium release during contraction and in minimizing calcium release, or leak, through the RyR during relaxation. Calcium leak occurs in both the heart and skeletal muscle and appears to underpin many of the serious and fatal disorders associated with the RyR. In the heart, phosphorylation due to chronic β-adrenergic stimulation and thiol modification via chronic reactive oxygen/nitrogen species production renders the cardiac RyR (RyR2) leaky (Shan *et al.*,2012), and our recent data has shown that drug modification of free thiols on RyR2 may underlie some of the cardiotoxicity associated with chemotherapy treatment (Hanna *et al.*, 2011). In skeletal muscle, RyR leak found in core myopathies (reviewed in Meissner, 2010), muscular dystrophies (Billinger, *2009*) and associated with muscle weakness in ageing (Andersson *et al.*, 2011) is attributed to thiol modification of the channel.

Our results show that 1) RyR2 oxidation and phosphorylation are induced in several arrhythmogenic models, 2) acute treatment with anthracyclines (chemotherapeutic agents) not only results in RyR2 phosphorylation, oxidation and dysfunction in cardiac muscle, but also significantly oxidizes the skeletal RyR1 and 3) the phosphorylation and oxidation status of the RyR2 can in part, influence channel responsiveness to store calcium load. These results and those of others illustrate that RyR leak makes a major contribution to serious skeletal and cardiac myopathies and highlights the significant role that phosphorylation and oxidation play in conferring this channel dysfunction.

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