Ryanodine receptor Ca\(^{2+}\) sensitivity and excitation-contraction coupling in muscular dystrophy and heart failure: similar and yet different

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DUCHENE MUSCULAR DYSTROPHY (DMD) is a severe inherited disease of striated muscle caused by a mutation of the dystrophin gene (7). Dystrophin is part of a large complex that forms a transmembrane bridge between nonsarcomeric actin on the intracellular end and the extracellular matrix on the other. Disruption of this complex is thought to result in plasma membrane instability and defective cellular signaling. Although the primary pathological defect is severe muscle wasting, improvements in the treatment of this aspect of the disease have made the cardiac abnormalities associated with it of increasingly greater importance. These abnormalities eventually lead to dilated cardiomyopathy and heart failure (8).

Cardiac dysfunction in heart failure is, in part, due to a number of alterations in Ca\(^{2+}\) handling in the myocyte. These usually lead to depressed cardiac Ca\(^{2+}\) transients and to decreased contractile function. Typically, these defects in Ca\(^{2+}\) handling include depressed sarcoplasmic reticulum (SR) Ca\(^{2+}\) pump function and/or increased Na\(^{+}/Ca^{2+}\) exchange activity (16, 17). Both of these effects lead to decreased SR [Ca\(^{2+}\)]. SR Ca\(^{2+}\) release is steeply dependent on this factor (1).

In addition to these effects, cardiac myocytes from failing hearts typically exhibit an increase in the diastolic leak of Ca\(^{2+}\) from the SR (20). This increase is primarily due to increased ryanodine receptor isof orm 2 (RyR2) Ca\(^{2+}\) sensitivity. There are a number of different mechanisms by which this might take place. One of the most well known of these is via phosphorylation of the RyR2. Although the exact mechanisms are controversial (4), studies have generally shown that the Ca\(^{2+}\) sensitivity of the RyR2 may be increased by phosphorylation, either by PKA and/or Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) (13, 26, 29).

In the current issue of American Journal of Physiology-Heart and Circulatory Physiology, the study by Ulrich et al. (25) addressed changes in cellular signaling associated with DMD by measuring alterations in excitation-contraction coupling and Ca\(^{2+}\) handling in dystrophin-deficient mdx mice. In one set of experiments, the authors measured Ca\(^{2+}\) oscillations in response to slow elevations in intracellular [Ca\(^{2+}\)]. The authors found that these oscillations occur significantly earlier, indicating that RyR2 Ca\(^{2+}\) sensitivity is enhanced in mdx myocytes. This increase is consistent with changes in other models of heart failure and is of pathophysiological significance. Depolarizing Na\(^+\) inward Na\(^{+}/Ca^{2+}\) exchange activity may result from the directed leak of Ca\(^{2+}\) toward the sarcolemmal membrane, particularly as Ca\(^{2+}\) accumulates within the narrow junctional cleft and just under the membrane (19). This, in combination with the upregulation of Na\(^{+}/Ca^{2+}\) exchange and the downregulation of the inward rectifier and transient outward K\(^{+}\) currents (18), may lead to the generation of delayed afterdepolarizations and triggered arrhythmias (28), such as those seen in DMD.

One additional factor that may make this pathogenic mechanism more serious in DMD is the increased SR [Ca\(^{2+}\)] that is suspected to occur. Increased voltage-dependent, mechanical stress-induced Ca\(^{2+}\) influx is likely to occur via membrane ruptures, stretch-activated channels, or other pathways (30, 27). Increased SR [Ca\(^{2+}\)] steeply increases diastolic SR Ca\(^{2+}\) release, making the above mechanism for potentiating triggered activity more deadly. Ironically, the increased RyR2 Ca\(^{2+}\) sensitivity, itself, may play a role in limiting this increase. Cardiac myocytes when exposed to low concentrations of caffeine and stimulated at steady state reduce SR [Ca\(^{2+}\)] via a negative feedback mechanism involving increased Na\(^{+}/Ca^{2+}\) exchange and increased Ca\(^{2+}\)-dependent inactivation of the L-type Ca\(^{2+}\) current. SR Ca\(^{2+}\) release is therefore reduced such that Ca\(^{2+}\) transient magnitude returns to near control levels (24). Such a mechanism may serve to limit SR Ca\(^{2+}\) overload in dystrophic myocytes.

Interestingly, the authors found that RyR2 Ca\(^{2+}\) sensitivity in dystrophic myocytes was decreased by reducing agents, indicating that an elevated production of reactive oxygen species (ROS) may be the primary cause for the effect in dystrophic mice. Indeed, high ROS production is becoming increasingly better appreciated for its role in the pathogenesis of heart failure. Increased ROS have been measured in mdx mice (11), and it has been previously shown that oxidative stress during heart failure can lead to RyR2 oxidation, elevated RyR2 Ca\(^{2+}\) sensitivity, and increased leak (21). Although not discussed, another possible, less direct mechanism may be through the activation of CaMKII. This molecule was recently shown to have persistent activity when methionine residues near its regulatory domain are oxidized (6). Phosphorylation of the RyR2 by CaMKII is known to increase its Ca\(^{2+}\) sensitivity (26, 29) and increase SR Ca\(^{2+}\) leak (5).

Although the Ulrich et al. study naturally concentrated upon the effects of RyR alteration within the cardiac myocyte, a valid direction for future research may be the evaluation of the effects of oxidation upon the skeletal muscle isoform of the RyR (RyR1). Increased RyR1 activity and SR Ca\(^{2+}\) leak have been implicated in malignant hyperthermia (15), central core disease (22, 23), and muscle fatigue (3). Chronic SR Ca\(^{2+}\) leak may lead to elevated myoplasmic Ca\(^{2+}\) levels, contributing to muscle damage and cell death. What role increased RyR1 Ca\(^{2+}\) sensitivity might play in altering Ca\(^{2+}\) homeostasis in muscular dystrophy is an open question (2).

Finally, and importantly, the authors also measured L-type Ca\(^{2+}\) currents using the whole cell patch-clamp technique while Ca\(^{2+}\) transients were measured simultaneously. The efficiency of excitation-contraction coupling was measured as “gain” or the magnitude of the Ca\(^{2+}\) transient divided by the...
peak current. Although initial findings at matched SR $[Ca^{2+}]$ showed little difference in excitation-contraction coupling between dystrophic myocytes and control myocytes, lowering external $[Ca^{2+}]$ uncovered a hypersensitivity in the excitation-contraction coupling mechanism.

This result is consistent with the interpretation of enhanced RyR2 $Ca^{2+}$ sensitivity in dystrophic myocytes. Yet it is surprising in that it stands in stark contrast to what is found in other models of heart failure to date, where a deficiency in excitation-contraction coupling is detected (e.g., Refs. 27–30) despite the fact that heart failure myocytes also show an increase in RyR2 $Ca^{2+}$ sensitivity (13, 20). This decreased excitation-contraction coupling is undoubtedly a factor, in addition to those mentioned above, in causing the decreased $Ca^{2+}$ transient magnitude and contractility in these other forms of failure. What causes this change is unknown.

Ullrich et al. note that mdx mice at the age used in the study (6–12 mo) typically begin to develop dilation, but they are not in overt failure. Therefore, it is perhaps this difference in the stage and degree of failure development that is responsible for the divergence from the results of other studies in other models. In any case, the fact that DMD myocytes show characteristics similar to other forms of heart failure, such as the increased RyR2 $Ca^{2+}$ sensitivity, but do not show the excitation-contraction coupling defect and, in fact, show the opposite, should be noted, and further study is warranted.

REFERENCES


