Zinc controls RyR2 activity during excitation-contraction coupling

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Cardiac excitation-contraction (EC) coupling is a process which governs contractility of the heart through the controlled release of Ca$^{2+}$ from the sarcoplasmic reticulum (SR). The type-2 ryanodine receptor (RyR2) is the route through which Ca$^{2+}$ is released from the SR providing the necessary driving force for cellular contraction. In heart failure, RyR2-channels become abnormally active, or ‘leaky’, and are unable to remain closed during diastole resulting in unwanted irregular contractile and electrical activity. Defective Zn$^{2+}$ handling has been shown to contribute to the cellular pathology of certain cardiomyopathies which give rise to impaired contractility including heart failure. This is likely a consequence of altered EC coupling as a result of modified RyR2 function. How zinc impacts upon the contractile force and the release of calcium from intracellular stores in heart is not fully understood.

In the recent study by Woodier and co-workers it was shown that cytosolic Zn$^{2+}$ can act as a high affinity activator of RyR2. In the aforementioned study, single RyR2 channels were incorporated into phospholipid bilayers under voltage-clamp conditions and the direct action of Zn$^{2+}$ at the cytosolic face of the channel studied. This approach enabled the study of RyR2 function under tight control of the chemical environment. Concentrations of free Zn$^{2+}$ ≤ 1 nM potentiated RyR2 activity but the presence of activating levels of cytosolic Ca$^{2+}$ was a requirement for channel activation. At concentrations of free Zn$^{2+}$ > 1 nM, the main activating ligand became Zn$^{2+}$ and the requirement of Ca$^{2+}$ for channel activation was removed. Under these conditions channel gating was altered and RyR2 gated in exceptionally long-lived open states. The ability of Zn$^{2+}$ at a concentration of 1 nM to directly activate RyR2 reveals that RyR2 has a much higher affinity for Zn$^{2+}$ than Ca$^{2+}$ (by ~3-orders of magnitude). These data suggest that RyR2-mediated Ca$^{2+}$-homeostasis is intimately related to intracellular Zn$^{2+}$ levels. Woodier et al. also showed that Zn$^{2+}$ modulated both the frequency and amplitude of Ca$^{2+}$-waves in cardiomyocytes in a concentration-dependent manner. Reduction of the concentration of intracellular Ca$^{2+}$ to sub-activating concentrations did not abolish Ca$^{2+}$-waves in the presence of 1 nM Zn$^{2+}$. This suggests that RyR2 gating is altered under these conditions whereby RyR2 gates in a Ca$^{2+}$-independent manner with Zn$^{2+}$ the sole activating ligand. These data indicate that channel dysregulation, through aberrant Zn$^{2+}$ homeostasis, may play a fundamental role in the generation of heart failure and other arrhythmic diseases.

Cardiomyocytes contain a small but measurable pool of free Zn$^{2+}$ in the cytosol reported to be ~100 pM. Since small changes in the Zn$^{2+}$ level will have a marked effect on RyR2 activity, this becomes highly relevant when we consider that concentrations of Zn$^{2+}$ have recently been reported to be transiently elevated to ~50 nM during Zn$^{2+}$-signaling events. Live-cell detection of intracellular Zn$^{2+}$ and Ca$^{2+}$ using selective fluorophores reveal that intracellular Zn$^{2+}$ concentrations are altered during cardiac EC coupling and that spatio-temporal fluctuations in free Zn$^{2+}$ levels are comparable to those of Ca$^{2+}$. Extracellular Zn$^{2+}$ can also enter cardiomyocytes through the L-type Ca$^{2+}$ channel in a similar manner to Ca$^{2+}$. These Zn$^{2+}$ fluctuations may serve to modulate RyR2 activity highlighting a potential role for Zn$^{2+}$ in fine-tuning graded Ca$^{2+}$-release events to control the force and duration of
cardiac contractions. Under certain pathophysiological conditions including heart failure, diabetes and ischemia, concentrations of intracellular Zn\textsuperscript{2+} are chronically elevated and are reported to be in the high nanomolar range (c.a. 30 nM).\textsuperscript{2,8} Under such conditions RyR2 will decouple from the regulatory effects of cytosolic Ca\textsuperscript{2+} and be under control of Zn\textsuperscript{2+}. This may contribute toward abnormally high RyR2 channel activity which is associated with heart failure and fatal arrhythmias.

The role of Zn\textsuperscript{2+} as a high affinity activator of RyR2 able to modulate channel function in the absence of Ca\textsuperscript{2+} represents a paradigm shift in our understanding of how RyR2 is activated during physiological contractions. Under certain pathophysiological conditions including heart failure, diabetes and ischemia, concentrations of intracellular Zn\textsuperscript{2+} are chronically elevated and are reported to be in the high nanomolar range (c.a. 30 nM).\textsuperscript{2,8} Under such conditions RyR2 will decouple from the regulatory effects of cytosolic Ca\textsuperscript{2+} and be under control of Zn\textsuperscript{2+}. This may contribute toward abnormally high RyR2 channel activity which is associated with heart failure and fatal arrhythmias.

Figure 1. A model to show how zinc may regulate RyR2-mediated sarcoplasmic reticulum calcium release. Physiological conditions: During the resting phase of the cardiac cycle RyR2 is closed as the cytosolic [Ca\textsuperscript{2+}] is sub-activating (100 nM). The normal trigger for RyR2 activation during systole is a transient rise in [Ca\textsuperscript{2+}] (≥ 1 μM). Zn\textsuperscript{2+}-dyshomeostasis: If the intracellular [Zn\textsuperscript{2+}] rises >1 nM, the dependency on Ca\textsuperscript{2+} for RyR2 openings is removed and Zn\textsuperscript{2+} becomes the activating ligand. This leads to abnormally active channels.

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