

Response to Colvin and Qian: Zinc-mediated regulation of the cardiac ryanodine receptor occurs via multiple binding sites

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We would like to thank Colvin & Qian for their interest in our recent publication [1] where we demonstrate for the first time that Zn^{2+} acts as a high affinity activator of the cardiac ryanodine receptor (RyR2). We are aware that BAPTA is not a Ca^{2+} -specific chelator and can also bind Zn^{2+} when present. The purpose of the experiment represented in Figure 4, was to show that Zn^{2+} can directly activate RyR2 when levels of Ca^{2+} are sub-activating rather than to provide the absolute Zn^{2+} concentration required for Zn^{2+} -dependent channel openings. This was addressed in the experiments carried out in the absence of BAPTA, where our data reveal that Zn^{2+} is the primary activating ligand of RyR2 at concentrations >1 nM (Figures 1 & 3). The estimates of free Zn^{2+} levels in the presence of BAPTA offered by Colvin & Qian in no way alter the interpretation of our data and it is unclear why this led them to speculate that the action of Zn^{2+} is through a single site on the channel [2]. A single site model is not consistent with the finding that 100 pM Zn^{2+} sensitizes Ca^{2+} -mediated RyR2 activity yet higher concentrations of Zn^{2+} (1-100 nM) enable switching from Ca^{2+} -dependent to Ca^{2+} -independent gating. Thus separate Zn^{2+} sites must exist to enable Ca^{2+} -sensitization and Zn^{2+} -activation, respectively. A single site model is also not consistent with the observation that very high concentrations of Zn^{2+} (1 mM) abolish all channel openings. Collectively, our data highlight a new and important role for intracellular Zn^{2+} in shaping Ca^{2+} -dynamics in cardiomyocytes and that this is mediated through Zn^{2+} binding at multiple sites on RyR2.

References

1. Woodier J, Rainbow RD, Stewart AJ & Pitt SJ. (2015) Intracellular zinc modulates cardiac ryanodine receptor-mediated calcium release. *J Biol Chem* **290**, 17599-610.
2. Colvin RA & Qian C. (2016) Zinc modulation of cardiac RyR2 gating: Alternate interpretation of the interplay between zinc and calcium. *J Biol Chem* (eLetter to the Editor)