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LETTER

Response to Qian and Colvin: Zinc-mediated Regulation of the Cardiac Ryanodine Receptor Occurs via Multiple Binding Sites

This is a response to a letter by Qian and Colvin (1).

We would like to thank Qian and Colvin for their interest in our recent publication (2) 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 (2,2′-(ethylenedioxy)dianiline-*N*,*N*,*N*′,*N*′-tetraacetic acid) is not a Ca^{2+}-specific chelator and can also bind Zn^{2+} when present. The purpose of the experiment represented in Fig. 4 was to show that Zn^{2+} can directly activate RyR2 when levels of Ca^{2+} are subactivating 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 (Figs. 1 and 3). The estimates of free Zn^{2+} levels in the presence of BAPTA offered by Qian and Colvin 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 (1). 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.

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1. Qian, C., and Colvin, R. A. (2016) Zinc modulation of cardiac ryanodine receptor gating: alternate interpretation of the interplay between zinc and calcium. *J. Biol. Chem.* **291,** 4266
2. Woodier, J., Rainbow, R. D., Stewart, A. J., and Pitt, S. J. (2015) Intracellular zinc modulates cardiac ryanodine receptor-mediated calcium release. *J. Biol. Chem.* **290,** 17599–17610

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