Recirculation Fraction as a functional readout of SERCA2a activity in mouse ventricular cardiomyocytes

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Abstract

Background: SERCA2a is an ATPase responsible for Ca^{2+} reuptake into the sarcoplasmic reticulum (SR) during diastole. Its expression and activity are reduced in various forms of cardiac dysfunction, making it a promising therapeutic target. Current assays to evaluate SERCA2a activity are time-consuming and expensive, highlighting the need for rapid, predictive tools. The recirculation fraction (RF), defined as the fraction of Ca^{2+} released from SR during a contraction that is successfully re-sequestered by SERCA before the next contraction, has been proposed as a functional indicator of SERCA activity.

Aim: To evaluate RF as a SERCA-specific index by integrating *in vitro* epifluorescence experiments with *in silico* modeling, systematically modulating SERCA activity and analyzing the resulting RF dynamics.

Materials and Methods: Ca^{2+} dynamics were measured using epifluorescence in isolated mouse ventricular cardiomyocytes (CMs) electrically stimulated at 1 Hz and maintained at 37 °C. Following a 40 s stimulation pause the amplitude of the Ca^{2+} transients (CaTs) markedly increased (post-rest potentiation) to decay thereafter along a multi-exponential course. The analysis focused on the magnitude and time course of the post-pause CaTs recovery toward steady state. Experiments were performed under control (CTRL) conditions, SERCA inhibition (CPA 10 µM), and SERCA stimulation. The latter was achieved with ISTAROXIME (ISTA 20 µM, a mixed SERCA stimulator and NaK pump inhibitor, or PST3093 1 µM a pure SERCA stimulator). The contribution of the sodium–calcium exchanger (NCX) was also assessed by inhibiting it with SEA0400 (1 µM). Simulations with a computational (*in silico)* mouse model based on Li et al. 2009 were performed to simulate Ca^{2+} dynamics under the same stimulation protocol and treatments.

Results: In CTRL, CaTs post-pause exhibited an exponential-like recovery toward baseline, with a smooth and gradual return to steady state. In the presence of CPA, the CaT decay was enhanced and markedly slower. Effects opposite to that of CPA, and qualitatively similiar to eachother were observed with ISTA and PST3093. The effect of PST3093 was slightly larger than that of ISTA. SEA0400 modified CaT recovery to a lesser extent, but in a direction similar to that of ISTA and PST3093. All these observed experimental trends were well reproduced by the *in silico* simulations.

Conclusions: All the results converge with the expectedly reciprocal roles of SERCA and NCX in determining the RF. The identified response patterns are clearly discernible and might therefore be used as high-throughput readouts of the functional expression of the two transport systems under pharmacological treatments. In silco reproduction of the patterns assists in detailed understanding of the underlying mechanisms. Further analysis will be devoted to identify numerical parameters providing the best description of the observed patterns and to subject them to predictivity (sensitivity+specificxity) analysis.