ONLINE SUPPLEMENTARY MATERIAL

Calculations to estimate the total amount of Ca^{2+} released during the ER Ca^{2+} content and the ER Ca^{2+} permeability.

Cells were bathed in a series of solution changes to evoke two kinds of $[Ca^{2+}]_i$ transients as described in text and Fig. 4A. The first represented Ca^{2+} released from the ER when SERCA was blocked with BHQ and the second following a KCl depolarization permitted calculation of extrusion by the PMCA (J_{PMCA}) as a function of $[Ca^{2+}]_i$ (Fig. 4B). Because the calcium flux changes induced by the initial BHQ application ($J_{BHQ}(t)$) were a result of both release from the ER ($J_{release}$) and Ca^{2+} extrusion via the PMCA, the $J_{release}(t)$ had to be calculated as the difference between J_{BHQ} and J_{PMCA} at each time point (Fig. 4C). The drop in the ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) at different time points was calculated based on the equation:

$$\Delta\left[Ca^{2+}\right]_{ER}(t) = -\frac{v_i}{v_{ER}\kappa_{ER}}\int_t^{end} J_{release}\kappa_i dt = \frac{v_i}{v_{ER}\kappa_{ER}}\Delta\left[Ca^{2+}\right]^i_{ER}(t)$$

(21), in which v_i and v_{ER} are the volumes of the cytoplasm and the ER, respectively, and κ_i and κ_{ER} are the calcium binding ratios of the cytoplasm and the ER, respectively. Assuming that $[Ca^{2+}]_{ER}$ equilibrates with $[Ca^{2+}]_i$ after BHQ application, we calculated the initial content in the ER according to the equation:

$$\left[Ca^{2+}\right]^i_{ER}(0) = \left[Ca^{2+}\right]_{i,end}\kappa_i + \int_0^{end} J_{release}\kappa_i dt$$

The change in $[Ca^{2+}]_{ER}$ over time is defined as

$$\left[Ca^{2+}\right]^i_{ER}(t) = \left[Ca^{2+}\right]^i_{ER}(0) - \int_0^{end} J_{release}\kappa_i dt\,,$$

as shown in Fig. 4D. The relative permeability of the ER was estimated according to the equation,

$$P_{ER}(t)\left[\frac{v_i}{v_{ER}\kappa_{ER}}\right] \approx -\frac{J_{release}(t)}{\left[Ca^{2+}\right]^i_{ER}(t)}$$ (Fig. 4E) (21).