A basic model of calcium homeostasis in non-excitable cells

Christina H. Selstø, Peter Ruoff*

Department of Chemistry, Bioscience, and Environmental Engineering, University of Stavanger, Stavanger, Norway

Supporting Information S2 Text

Description of PMCA rate equations, cytosolic Ca^{2+} set point and determination of V_{max}, K_M and turnover number values

The rate of the PMCA pump is given by the Michaelis Menten equation in Eq. S1

$$v = \frac{d(Ca^{2+})}{dt} = \frac{V_{\max}\cdot(Ca^{2+})}{K_{\mathrm{M}} + Ca^{2+}} \tag{S1}$$

The cytosolic Ca^{2+} set-point of 0.1 μM is determined by a negative feedback composed of the PMCA-CaM pump complex (PMCA) and its activation by Ca^{2+}. To keep the steady state level of Ca^{2+} at a certain set-point, integral control by negative feedback is used where the difference (error, e) between ${Ca^{2+}}_{set}$ and Ca^{2+} is calculated and integrated over time. The other part of the negative feedback loop, $PMCA{\cdot}M^*$, needs to have zero-order degradation in order for this to happen. With first order degradation, integral control is missing, except in a few other cases e.g. by invoking auto-catalysis with regards to E.

Rate equations for Ca^{2+} and the activate PMCA-M pump complex ($PMCA{\cdot}M^*$) can be written as (see scheme in Fig 4):

$$\begin{aligned}\frac{dCa^{2+}}{dt} &= k_1{\cdot}Ca^{2+}_{ext} - k_2{\cdot}Ca^{2+} - \frac{k_3{\cdot}(PMCA{\cdot}M^*){\cdot}Ca^{2+}}{k_4 + Ca^{2+}} \\ &\quad - 4{\cdot}k_8{\cdot}M{\cdot}Ca^{2+} + 4{\cdot}k_9{\cdot}(M{\cdot}Ca^{2+}_4)\end{aligned} \tag{S2}$$

$$\frac{d(PMCA{\cdot}M^*)}{dt} = k_5{\cdot}(PMCA{\cdot}M){\cdot}(Ca^{2+}) - \frac{k_6{\cdot}(PMCA{\cdot}M^*)}{k_7 + (PMCA{\cdot}M^*)} \tag{S3}$$

By setting $d(PMCA{\cdot}M^*)/dt = 0$, and assuming that K_M (k_7) is much smaller than $PMCA{\cdot}M^*$, $K_M \ll PMCA{\cdot}M^*$, the cytosolic Ca^{2+} set-point by the PMCA is:

$$Ca^{2+}_{set} = Ca^{2+}_{ss} = \frac{k_6}{k_5{\cdot}(PMCA{\cdot}M)} \tag{S4}$$

PMCA kinetics

The $V_{\max}$ for the PMCA pump was determined using several experimental data. The calculation is based on Scharff and Foder who reports a maximum rate of 60 μmol/min(g protein) for PMCA in erythrocytes, which converts to 1 μmol/s(g protein) [1]. By taking into account different relevant values for the cell the rate of PMCA in a single cell can be calculated. The average cell volume of erythrocytes is 100 μm^3, and has a density of 1.1 [2]. The cell protein (cp) number has been found to be cp = 0.3 g protein / ml. First the mass of protein (x), in grams, in one red blood cell is found in the following manner

$$\frac{x}{100{\cdot}10^{-18}m^3} = \frac{0.3g}{100{\cdot}10^{-6}m^3} \Rightarrow x\,(g\,protein) = \frac{0.3g{\cdot}10^{-16}m^3}{10^{-6}m^3} = 0.3{\cdot}10^{-10}g$$

The velocity is given as 1 μmol/s(g protein) which can now be written as $v_{cell}/0.3 \cdot 10^{-10}$ g and the velocity is calculated to be

$$\Rightarrow v_{\text{cell}} = 0.3{\cdot}10^{-10}\mu mol/s$$

or in μM (μmol/liter)

$$\frac{0.3g{\cdot}10^{-10}\mu mol}{s{\cdot}V_{\text{cell}}} = \frac{0.3g{\cdot}10^{-10}\mu mol}{s{\cdot}10^{-13}l} = 3{\cdot}10^{2}\mu M/s$$

Vanagas et al. diluted the membrane protein level and found PMCA to be more active at lower levels of membrane protein present. By using their numbers of Ca^{2+} through the PMCA, two values for V_{max} could be calculated, respectively with 0.03 nmol Ca^{2+} per mg protein per hour, and 0.116 nmol Ca^{2+} per mg protein per hour. Before the velocities of the two Ca^{2+} concentrations could be found, the values were converted to fitting mathematical terms

$$\frac{0.03nmol}{h{\cdot}(mg\,protein)} = \frac{8.3{\cdot}10^{-6}\mu mol}{s{\cdot}(g\,protein)}$$

$$\frac{0.116nmol}{h{\cdot}(mg\,protein)} = \frac{3.2{\cdot}10^{-5}\mu mol}{s{\cdot}(g\,protein)}$$

The mass of protein (g) in one single red blood cell was found to be

$$\frac{x}{100{\cdot}10^{-18}m^3} = \frac{0.3\,g}{1{\cdot}10^{-6}m^3} \Rightarrow x\,(g\,protein) = \frac{0.3g{\cdot}10^{-15}m^3}{10^{-6}m^3} = 3.0{\cdot}10^{-9}\,g$$

By employing the same way for finding the velocity as the previous V_{max} calculations, the velocites for the cell was found by taking into account the protein mass in the cell. For $8.3 \cdot 10^{-6}$ μmol / s $\cdot$ g protein

$$\frac{8.3{\cdot}10^{-6}\mu mol}{s{\cdot}(gprotein)} = \frac{v_{\text{cell}}}{3.0{\cdot}10^{-9}g}$$

$$v_{\text{cell}} = \frac{8.3 \cdot 10^{-6} \cdot 3.0 \cdot 10^{-9} \mu mol}{s} = 2.5 \cdot 10^{-14} \mu mol/s$$

And given in μM by dividing with the cell volume of 10^{-13} L:

$$v_{\text{cell}} = \frac{2.5 \cdot 10^{-14} \mu mol}{s \cdot 10^{-13} L} = \frac{25 \mu mol}{s \cdot L} = 25 \mu M/s$$

The following calculations found the velocity for $3.2 \cdot 10^{-5}$ μmol / s $\cdot$ g protein

$$\frac{3.2 \cdot 10^{-5} \mu mol}{s \cdot (g\, protein)} = \frac{v_{\text{cell}}}{3.0 \cdot 10^{-9} g}$$

$$V_{\text{cell}} = \frac{3.2 \cdot 10^{-5} \cdot 3.0 \cdot 10^{-9} \mu mol}{s} = 9.6 \cdot 10^{-14} \mu mol/s$$

Given in μM by dividing with the cell volume of 10^{-13} L:

$$v_{\text{cell}} = \frac{9.6 \cdot 10^{-14} \mu mol}{s \cdot 10^{-13} L} = \frac{96 \mu mol}{s \cdot L} = 96 \mu M/s$$

The PMCA concentration of 10^{-2} μM that is chosen for the model was also found there.

The $V_{\max}$ value was also subsequently calculated by utilization of experimental results from research on cochlear hair cells [3]. The Ca^{2+} extrusion rate was estimated to be of about 200 Ca^{2+} ions per second per pump in the hair cells. Assuming that the cell has approximately 2×10^6 PMCA pump molecules the total pump rate (per cell) can be calculated as

$$200 \cdot 2 \cdot 10^{-10} mol/s = 6.6 \cdot 10^{-16} mol/s = 6.6 \cdot 10^{-10} \mu mol/s$$

This value divided by cell volume, $3.927 \cdot 10^{-12}$ l, gives the Ca^{2+} extrusion rate of

$$v = \frac{6.6 \cdot 10^{-10} \mu mol}{s \cdot 3.927 \cdot 10^{-12} l} = 168 \mu M/s \approx 2 \cdot 10^2 \mu M/s$$

This value is comparable to the rate calculated from experimental data extracted from Scharff and Foder [1]. The PMCA concentration was estimated to be 1 μM/s from their experimental data, notably 2 orders of magnitude larger than the data from Vanagas et al. [4]. Based on the similar $V_{\max}$ values calculated from both Scharff and Foder, and Chen et al. experiments, the previously assumed PMCA concentration of 10^{-2} μM in the model appears to be too low.

References

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