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Dynamics of Calcium Fluxes in Nonexcitable Cells: Mathematical Modeling

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Abstract. Mathematical models simulating the dynamics of calcium redistribution (elicited by experimental interference with the pathways of calcium fluxes) in cellular compartments have been developed, based on a minimal scheme of the pathways of calcium fluxes in nonexcitable cells suspended in calcium-free medium. The models are consistent with available experimental data. All parameters are quantitatively related to the intrinsic properties of calcium adenosine triphosphatases (ATPases) and cellular membranes; there is no interdependence between the parameters. The models can be used as the basis for quantitative analysis and interpretation of experimental data. The activities of plasma membrane and sarcoendoplasmic reticulum calcium ATPases (PMCA and SERCAs) are governed by different mechanisms. PMCA is likely to undergo transitions from inactive to active to "dormant" (not identical to the initial) and back to inactive states, the mean duration of the cycle lasting for minutes or longer. The sequence of the transitions is initiated, presumably, by an increase in cytosolic calcium concentration. The transition of PMCA from inactive to active (at least at low rates of increase in cytosolic calcium concentration) is likely to be slower than that from active to dormant. SERCA, presumably, transits from inactive to active state in response to increases in calcium leakage from calcium stores. Whereas PMCA extrudes excess calcium (a definite quantity of it) in a short pulse, SERCA retakes calcium back into the stores permanently at a high rate. The models presented here may be the best means for the moment to quantitatively relate the dynamics of calcium fluxes in nonexcitable cells with known or putative properties of the mechanisms underlying activation of calcium ATPases.

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Key words: Cytosolic free calcium — Calcium stores — Endomembrane permeability — Calcium leakage — Calcium extrusion — Calcium reuptake — Calcium ATPases — Ionomycin

Introduction

The role of cytosolic free calcium in various cellular processes is well known (*see*, e.g., Rink & Sage, 1990; Rizzuto, Pozzan & Carafoli, 2002). Calcium concentration in cytosol is considerably lower than that in extracellular medium or various cellular compartments or organelles. Cytosolic calcium concentration is maintained or changed depending on calcium fluxes into (passive) or out of (active, proceeding with energy expenditure) cytosol (*see*, e.g., Pozzan et al., 1994). The dynamics of the changes, however, are researched to a much lesser extent. They are difficult to relate to the properties of the molecular devices (when known) and mechanisms responsible for the above changes. In view of the overwhelming complexity of the pathways of calcium flux in cells (*see*, e.g., Pozzan et al. 1994; Rizzuto et al. 2002) and the variety of mechanisms involved, the necessity of mathematical models is evident; on the other hand, reasonable simplification of real biological systems is inevitable to make modeling feasible.

Some experimental material concerning the pathways of calcium flux in human platelets has been presented (Juška et al., 2005). As a matter of fact, the work was based on mathematical models, which were not presented as being of secondary importance for the paper. The models, however, would be helpful in getting deeper insight into the processes related to calcium flux in platelets and other nonexcitable cells.

In the present study, mathematical models simulating the dynamics of calcium redistribution (elicited by experimental interference with the pathways of calcium flux) in cellular compartments are

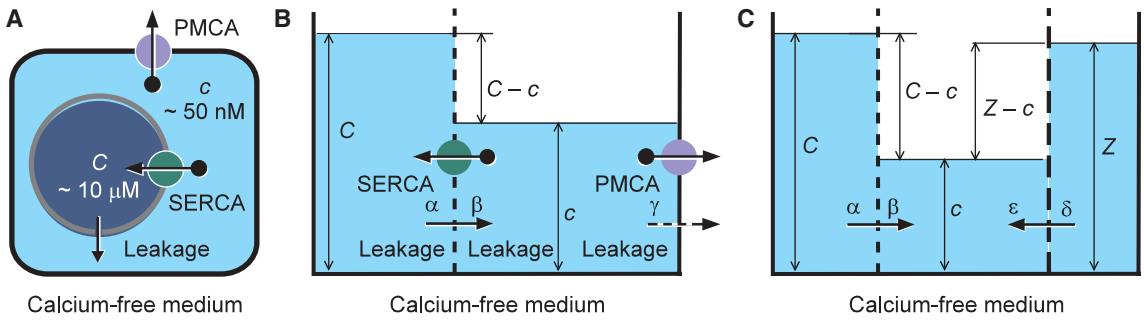

Fig. 1. (*A*) Cartoon depicting the pathways of calcium fluxes in cells maintained in calcium-free medium. (*B*) Hydrodynamic diagram dynamically equivalent to cartoon *A* with respect to calcium fluxes. The heights of liquid columns in communicating vessels of different cross-section correspond to calcium concentrations in the stores (*C*, left compartment) and cytosol (*c*, the right one). α and β, rate constants of change of calcium concentrations in the stores (α) and cytosol (β); in general, $\beta \neq \alpha$ (for more detail, see Discussion). Only the pathways considered in the present study are depicted. (*C*) Schematic representation of the pathways of calcium flux from the stores of two types, both elimination of calcium excess from cytosol being blocked and possible outward leakage not taken into account. The right compartment represents the stores of a different type; δ and ε, rate constants, *Z*, calcium concentration. *Dots on the arrows' tails* symbolize that calcium ions are supposed to be handled one by one by the ATPase (macro)molecules.

developed. The models are based on a minimal scheme of the pathways of calcium flux in nonexitable cells suspended in calcium-free medium. Besides providing the basis for analysis and interpretation of experimental data, the models allow us to relate the dynamics of calcium redistribution to the properties of cellular membranes and activities of both plasma membrane (PMCA) and sarcoendoplasmic reticulum (SERCA) calcium adenosine triphosphatases (ATPases). These calcium ATPases are likely to undergo transitions: PMCA from inactive to active to "dormant" (not identical to the initial) in response to increases in cytosolic calcium concentration and SERCA to active in response to increases in leakage from calcium stores.

Modeling

The known models of transport of molecules or ions across biological membranes designed to solve specific problems cannot be applied here because of the simplifications made, which are justifiable in each specific case but not acceptable here. In the model of solute transport through a membrane (e.g., Hobbie, 1997), the volume of the larger compartment is assumed to be infinite. The volumes of all the compartments, however, had to be taken into consideration in the case of calcium redistribution between cellular compartments in the present study.

A minimal scheme of the pathways of calcium flux in nonexitable cells maintained in calcium-free medium is depicted in Figure 1*A*. To better visualize the fluxes depending on the difference in calcium concentrations in the stores and cytosol, the scheme is substituted by a hydrodynamic analogue (Fig. 1*B*). For the purposes of the present analysis, both diagrams can be considered equivalent. Indeed, calcium leakage from the stores to cytoplasm (through ion-permeable membrane) takes place due to the difference in calcium concentrations in those compartments; liquid leakage from one vessel to the other (through a porous partition) takes place due to the difference in the heights of liquid columns in the vessels. The quantity of calcium in a cellular compartment equals the product of its concentration in the compartment and the volume of the compartment; the quantity of liquid in a vessel equals the product of the height of the liquid column and the area of cross section of the vessel. The rate (absolute or relative) of change in calcium concentration in a cellular compartment corresponds to the (absolute or relative) rate of change in the height of the liquid column in a vessel.

Only the pathways and mechanisms of main concern to this study are taken into account here. Possible roles of other mechanisms and pathways that might contribute to calcium redistribution in the cell will be discussed in the last section. As long as the pathways of calcium flux in cells remain undisturbed, the fluxes are in dynamic equilibrium. Permeability of cellular membranes can be increased experimentally by ionomycin (Beeler, Jona & Martonosi, 1979) or other ionophores; elimination of calcium excess from cytosol can be selectively blocked by inhibiting calcium ATPases.

In the absence of calcium in extracellular medium and there being no outward leakage, upon inhibition of both PMCA and SERCA there remains only the pathway of calcium leakage from the stores into cytosol, which leads to calcium redistribution in the above cellular compartments. As a result, calcium concentration in the stores (or liquid column height in the vessel with the higher column), *C*, declines; the rate of decline (i.e., the first derivative of *C*) may be assumed to be proportional to the difference of its

concentrations in the stores and cytosol (or in the heights of liquid columns in communicating vessels, as depicted in Fig. 1*B*), the coefficient of proportionality being α. The rate of rise in cytosolic calcium concentration, c, is similar, the respective coefficient being β. The two statements above constitute the main assumptions and provide a model of the process of calcium redistribution between calcium stores and cytosol occurring due to permeability of endomembrane in the absence of outward leakage and activity of calcium ATPases. All the other models below are extensions of this one and are based on the assumptions as above and others specified additionally in each case. The above verbal model expressed in mathematical terms makes a system of differential equations with respect to calcium concentrations in the compartments (the notations being the same as in Fig. 1*B*):

$$\begin{cases} \frac{\mathrm{d}C}{\mathrm{d}t} &= -\alpha(C-c), \\ \frac{\mathrm{d}c}{\mathrm{d}t} &= \beta(C-c). \end{cases} \tag{1}$$

The solution of this system with respect to c (the concentration of interest), taking into account initial conditions (let $c(0) = c_0$, $C(0) = C_0$), is

$$c = c_0 + \frac{\beta}{\alpha+\beta}(C_0 - c_0)\left(1 - \mathrm{e}^{-(\alpha+\beta)t}\right) \tag{2}$$

It should be kept in mind that the initial calcium concentration in the stores, C_0, is not accessible for direct measurement. It can be found, however, on the basis of mass conservation.

As a result of redistribution, the asymptotic concentrations of calcium in both the stores and cytosol are equal. The latter is observable experimentally. In the absence of outward leakage, the total quantity of calcium in the cell (in the stores and cytosol) remains constant in the process of its redistribution. It can be written, therefore,

$$c_0 \times V_{\mathrm{cytosol}} + C_0 \times V_{\mathrm{stores}} = c_\infty \times \left(V_{\mathrm{cytosol}} + V_{\mathrm{stores}}\right)$$

where c_∞ is asymptotic calcium concentration in cytosol and V_{cytosol} and V_{stores}, volumes of the compartments. It follows from the above equation that

$$C_0 = \frac{V_{\mathrm{cytosol}} + V_{\mathrm{stores}}}{V_{\mathrm{stores}}} c_\infty - \frac{V_{\mathrm{cytosol}}}{V_{\mathrm{stores}}} c_0.$$

In most cases, the volumes are not known. However, the rate constants (α and β) of change in calcium concentrations in the respective compartments are related to the permeability of endomembrane and the volumes of the compartments as follows:

$$\alpha = \frac{permeability}{V_{\mathrm{stores}}},\ \beta = \frac{permeability}{V_{\mathrm{cytosol}}}$$

(so, in general, $\beta \neq \alpha$). Thus,

$$\frac{V_{\mathrm{stores}}}{V_{\mathrm{cytosol}}} = \frac{\beta}{\alpha}.$$

This ratio is not known. It will be assumed here (as in Juška et al., 2005) to be 0.05, which is in the range presented by Rink and Sage (1990). A little algebra leads to

$$C_0 = \frac{\alpha+\beta}{\beta} c_\infty - \frac{\alpha}{\beta} c_0 \tag{3}$$

Now Eq. 2 can be simplified (let c_{influx} mean cytosolic calcium concentration resulting solely from its influx from the stores):

$$c_{\mathrm{influx}} = c_0 + (c_\infty - c_0)\left(1 - \mathrm{e}^{-(\alpha+\beta)t}\right). \tag{4}$$

Taking into account possible outward leakage of calcium (let the rate constant of leakage be γ), the system of equation 1 has to be modified as follows (*see* Fig. 1):

$$\begin{cases} \frac{\mathrm{d}c}{\mathrm{d}t} &= \beta(C-c) - \gamma c, \\ \frac{\mathrm{d}C}{\mathrm{d}t} &= -\alpha(C-c). \end{cases} \tag{5}$$

A similar analysis can be applied in the case of several types of stores. If the stores are of two types, the system of equations can be written based on Figure 1*C*:

$$\begin{cases} \frac{\mathrm{d}c}{\mathrm{d}t} &= \beta(C-c) + \varepsilon(Z-c), \\ \frac{\mathrm{d}C}{\mathrm{d}t} &= -\alpha(C-c), \\ \frac{\mathrm{d}Z}{\mathrm{d}t} &= -\delta(Z-c). \end{cases} \tag{6}$$

It should be noted that in this case the initial calcium concentrations in the stores (of two types), C_0 and Z_0, not being accessible for direct measurement, cannot be found as in the previous case.

In the absence of outward leakage and one of the two calcium ATPases (PMCA or SERCA) being inhibited, there remain two fluxes: (passive) influx of calcium from the stores and its extrusion by PMCA or reuptake into the stores by SERCA (*see* Fig. 1). Therefore, the contribution of the activity of calcium ATPases to the rate of change in cytosolic calcium concentration has to be taken into account and the equations of system 1 have to be modified.

Whereas the rate of calcium influx from the stores to cytosol is supposed to depend on the transmembrane difference of calcium concentrations (*see above*), that of elimination of calcium excess from

cytosol should be thought to depend on the activity of calcium ATPases. Extrusion of the excess (as shown in Juška et al., 2005) is transitory, its rate rising and declining to zero afterward. The rise and decline in the rate of extrusion has to be attributed, presumably, to the activity of PMCA observed in the ensemble measurements. PMCA (its total population in the cell) may be supposed, therefore, to make transitions in its activity from inactive to active and to "dormant" (different from the initial) state:

$$\mathrm{I} \xrightarrow{\kappa} \mathrm{A} \xrightarrow{\lambda} \mathrm{D} \tag{7}$$

where I, A and D are the states (inactive, active and dormant) of PMCA and κ and λ are rate constants or relative rates of transitions. The above transitions are supposed to occur in response to increased cytosolic calcium concentration (for more detail, *see below*, Activity of Calcium ATPases).

Let i, a and d denote relative concentrations of PMCA (macro)molecules residing in respective state at a given moment, t. It is reasonable to assume that $i + a + d = 1$. Based on scheme 7, a system of equations can be written:

$$\begin{cases} \frac{\mathrm{d}i}{\mathrm{d}t} &= -\kappa i, \\ \frac{\mathrm{d}a}{\mathrm{d}t} &= \kappa i - \lambda a, \\ \frac{\mathrm{d}d}{\mathrm{d}t} &= \lambda a. \end{cases} \tag{8}$$

The solution with respect to a (the unknown of main interest here), taking into account the initial conditions (let $i_0 = 1$, $a_0 = 0$, $d_0 = 0$) is

$$a = \frac{\kappa}{\kappa - \lambda}\left(\mathrm{e}^{-\lambda t} - \mathrm{e}^{-\kappa t}\right). \tag{9}$$

PMCA activity (let it be A), it seems reasonable to assume, is proportional to the above solution, a, the coefficient of proportionality being the rate constant, λ:

$$A = \frac{\kappa\lambda}{\kappa - \lambda}\left(\mathrm{e}^{-\lambda t} - \mathrm{e}^{-\kappa t}\right). \tag{10}$$

To take into account the total activity of PMCA producing an observable effect in the ensemble measurements, a scale multiplier (let it be E_{PMCA}) has to be introduced. Let PMCA be active and SERCA inhibited (*see* Fig. 1). Then system 1 has to be modified as follows:

$$\begin{cases} \frac{\mathrm{d}c}{\mathrm{d}t} &= \beta(C - c) - \frac{\kappa\lambda}{\kappa - \lambda}\left(\mathrm{e}^{-\lambda t} - \mathrm{e}^{-\kappa t}\right)E_{\mathrm{PMCA}}, \\ \frac{\mathrm{d}C}{\mathrm{d}t} &= -\alpha(C - c). \end{cases} \tag{11}$$

Let SERCA be active and PMCA inhibited. In contrast to calcium extrusion, neither its reuptake back into the stores nor the rate of reuptake declines to zero. Upon (experimental) increase of calcium leakage (above background level), reuptake also increases (see Fig 1 in Juška et al., 2005). So does SERCA activity. By analogy with PMCA, it seems reasonable to suppose that SERCA undergoes similar transitions from inactive to active state, not entering, however, into dormant state. The relative concentration of SERCA carrying calcium ions back into the stores (let it be b) can be found the same way as above (background activity being ignored):

$$b = (1 - \mathrm{e}^{-\mu t}) \tag{12}$$

where μ is the rate constant of transition. Then, as above, system 1 can be modified as follows:

$$\begin{cases} \frac{\mathrm{d}c}{\mathrm{d}t} &= \beta(C - c) - \beta(1 - e^{-\mu t})E_{\mathrm{SERCA}}, \\ \frac{\mathrm{d}C}{\mathrm{d}t} &= -\alpha(C - c) + \alpha(1 - e^{-\mu t})E_{\mathrm{SERCA}} \end{cases} \tag{13}$$

where E_{SERCA} is a scale multiplier taking into account the total activity of SERCA (macro)molecules in the process (for more detail, *see below*).

Let both PMCA and SERCA be active. Then system 1 can be further modified (*see* equations 11 and 13):

$$\begin{cases} \frac{\mathrm{d}c}{\mathrm{d}t} = \beta(C - c) - \beta(1 - \mathrm{e}^{-\mu t})E_{\mathrm{SERCA}} \\ \qquad - \frac{\kappa\lambda}{\kappa - \lambda}\left(e^{-\lambda t} - e^{-\kappa t}\right)X_{\mathrm{PMCA}}, \\ \frac{\mathrm{d}C}{\mathrm{d}t} = -\alpha(C - c) + \alpha(1 - \mathrm{e}^{-\mu t})E_{\mathrm{SERCA}} \end{cases} \tag{14}$$

where X_{PMCA} is a scale multiplier taking into account the activity of PMCA when SERCA is active (the activity of SERCA is supposed to be independent of the activity of PMCA; the result of the activity of the latter, however, as observed by Juška et al. [2005], may depend on the activity of SERCA; for more detail, *see below*).

Discussion

Intracellular Calcium Redistribution

The redistribution of calcium is modeled by equation 4. The model is presented in Figure 2 (curve *a*), its parameters taken from Table 2 in Juška et al. (2005) for 50 nM ionomycin concentration. As seen from equation 4, the shape of the model curve does not depend on the above assumption concerning the ratio of the volumes. (The shape of the cell [*see*, e.g., Paul et al. 1999] and, consequently, its volume (as well as the volumes of its compartments) undergo changes during the redistribution; the dynamics of the changes, however, not being known, are not taken into account in the model.) It should be emphasized that it is the sum (α + β) which has to be considered the observable rate constant (or relative rate) of calcium redistribution, while α and β, taken separately, can be observed only under extreme conditions (namely, α can be viewed as rate constant

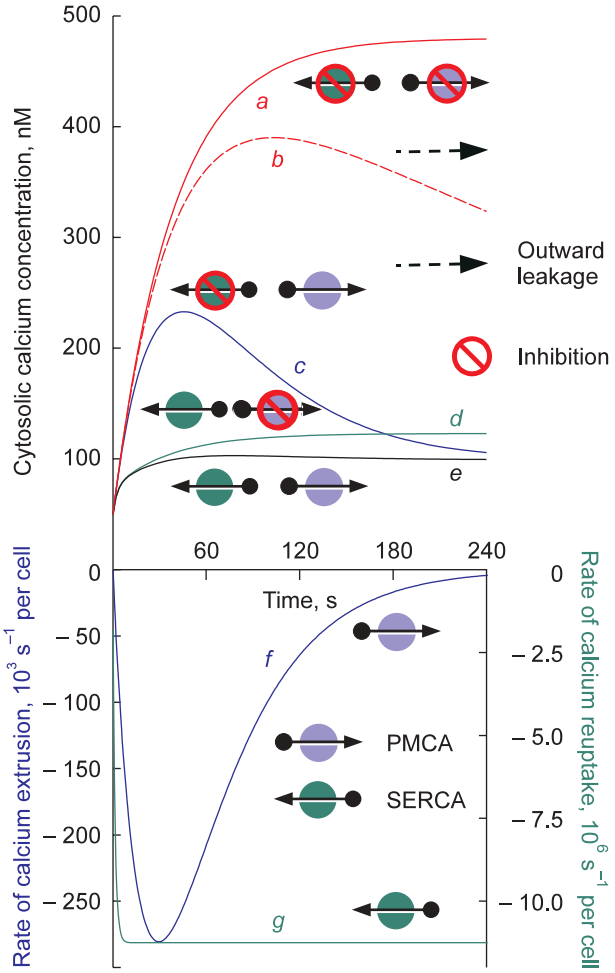

Fig. 2. Dynamics of cytosolic calcium concentration in cells upon modification of the pathways of calcium influx, its reuptake and extrusion to extracellular medium. Curves *a* (c_0 = 50 nM, c_∞ = 480 nM, α = 0.025 s^{-1} at 50 nM ionomycin [Juška et al., 2005], β = 0.00125 s^{-1}) represent dynamics of calcium redistribution in the absence of its elimination (extrusion or reuptake); *b* (γ = 0.002 s^{-1}), dynamics of calcium redistribution as a result of both its influx and outward leakage; *c* (κ = 0.025 s^{-1} and λ = 0.023 s^{-1} [or κ = 0.023 s^{-1} and λ = 0.025 s^{-1}], E_{PMCA} = 400 nM), dynamics of calcium redistribution as a result of both its influx and extrusion; *d* (μ = 0.4 s^{-1}), dynamics of calcium redistribution as a result of both its influx and reuptake; *e*, dynamics of calcium redistribution as a result of its influx, reuptake and extrusion; *f*, rate of calcium extrusion; *g*, rate of calcium reuptake. The meaning of the icons near the curves is the same as in Figure 1.

[or relative rate] of the discharge of the stores into an open space of infinite volume [β = 0 in this case] and β, as the rate constant of rise in calcium concentration in cytosol as a result of its influx from a source of infinite capacity [while α = 0]). Whereas endomembrane permeability, as mentioned above, can be increased experimentally by ionophores, the basal leakage of calcium being controlled by various mechanisms (Camello et al., 2002) cannot be reduced experimentally. The model is in agreement with experimental data (Juška et al., 2005). The sum α + β can be estimated rather accurately from model fitting to the data.

A possible contribution of calcium channels of endomembrane to the dynamics of calcium redistribution in the absence of its reuptake back into the stores and extrusion of its excess, presumably, can be safely ignored. Indeed, by conducting ion flow from higher to lower concentrations, the channels would only increase (passive) leakage (mediated or not by ionophores), i.e. the rate of redistribution, having no effect on the dynamics.

Calcium redistribution is accompanied by changes in transmembrane electric potential differences. With respect to these differences, an electric circuit diagram (dynamically similar to the scheme presented in Fig. 1*B*) and corresponding system of differential equations (similar to system 1) can be composed whose solution would be similar to that of equation 2. Taking into account the electric potentials, therefore, is not expected to bring anything of interest into the system considered. Besides, no contribution (if any) of the potentials to calcium redistribution can be revealed by the methods used.

Calcium redistribution (in the absence of its reuptake back into the stores and extrusion of its excess) being a passive process, there seems to be no sense in considering the ATP/ADP ratio in the process.

The solution of system 5, which takes into account outward leakage of calcium under initial conditions as above, is

$$
\begin{aligned}
&c_{\text{influx \& leakage}} \\
&= \frac{c_0}{2}\left(1 + e^{-\zeta t}\right)e^{-(\alpha+\beta+\gamma-\zeta)t/2} \\
&- \frac{c_0(\alpha+\beta+\gamma)}{2\zeta}\left(e^{-(\alpha+\beta+\gamma-\zeta)t/2} - e^{-(\alpha+\beta+\gamma+\zeta)t/2}\right) \\
&+ \frac{c_\infty(\alpha+\beta)}{\zeta}\left(e^{-(\alpha+\beta+\gamma-\zeta)t/2} - e^{-(\alpha+\beta+\gamma+\zeta)t/2}\right)
\end{aligned}
\tag{15}
$$

where

$$\zeta = \sqrt{\alpha^2 + 2\alpha(\beta-\gamma) + (\beta+\gamma)^2}.$$

It can be seen that for γ = 0 (no outward leakage of calcium) ζ = α + β, and the above equation is reduced to equation 4. If γ ≠ 0, it can be estimated rather accurately from model fitting to experimental data. (A possible contribution of calcium channels in plasma membrane to calcium redistribution in the comparments could be revealed by the above model.) This model is presented in Figure 3 (curve *b*). It is interesting to note that no outward leakage of calcium was observed even at ionomycin concentrations as high as 100 nM (Juška et al., 2005).That means that plasma membrane is

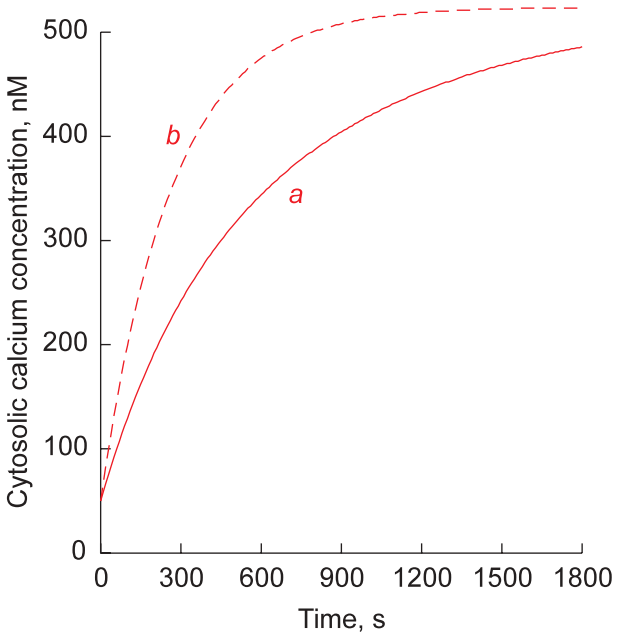

Fig. 3. Dynamics of calcium redistribution between stores of two types and cytosol. Curve *a* represents the time course of cytosolic calcium concentration as a result of its influx from the stores with parameters $\alpha = 0.0036\ s^{-1}$ (as in Juška et al., 2005, no ionomycin), $\beta = 0.00009\ s^{-1}$, $C_0 = 15\ \mu M$, $\delta = \alpha/3 = 0.0012\ s^{-1}$, $\varepsilon = \beta/3 = 0.00003\ s^{-1}$, $Z_0 = C_0/3 = 5\ \mu M$; *b*, same, with parameters $\delta = \alpha$, $\varepsilon = \beta$, C_0 and Z_0 as in *a*.

considerably less susceptible to treatment by ionomycin than endomembrane.

Calcium Redistribution Between Stores of Two Types and Cytosol

The solution of system 6 with respect to c taking into account two types of calcium stores, under initial conditions as above, is impractical to present here in full. It can be simplified, however, as follows:

$$c_{\text{two types}} = c_0 + P e^{-(\alpha+\beta+\delta+\varepsilon-\xi)t/2} + Q e^{-(\alpha+\beta+\delta+\varepsilon+\xi)t/2}$$

where

$$\xi = \sqrt{(\alpha+\beta-\delta)^2 - 2(\alpha-\beta-\delta)\varepsilon + \varepsilon^2},$$

where P and Q are algebraic combinations of c_0, C_0, Z_0, α, β, δ, ε and ξ.

The model curve is plotted in Figure 3.

As seen in Figure 3, the model curve (*a*) is monotonic, its shape visually not very different from a monoexponential curve (cf. curve *b*). At the same time, this model contains three extra parameters (two rate constants and an initial calcium concentration, Z_0, in the stores of a different type) in comparison with model 4. Whereas the sum $\alpha + \beta$ in equation 4 (as well as γ in 15) can be estimated rather accurately from model fitting to experimental data, this cannot be done in the present case (because of too many parameters). Keeping in mind a high level of noise in the fluorescence measurements (the method used for the assessment of cytosolic free calcium concentration) and the rather low accuracy of experimental data, the model seems of little value. It can justify, however, ignoring the fact that stores are of several different types (Pozzan et al., 1994; Pizzo, Fasolato & Pozzan, 1997; Pinton, Pozzan & Rizzuto, 1998) and considering them as a homogeneous pool, as done by Juška et al., (2005). While investigating the net redistribution of calcium, there is no reason (and no way) to consider the stores being different, if respective rate constants are close enough. Indeed, it can be seen from equation 15 that $\xi = 2\beta$ for $\delta = \alpha$ and $\varepsilon = \beta$, and there remains only one exponential with the rate constant $\alpha + 2\beta$. If, however, the stores (calcium content in them or endomembrane permeability) of a particular type are of special interest, all the other stores should be selectively discharged before experimental investigation of this particular type (Juška et al., in preparation).

Calcium Influx from Stores and Fluxes Generated by Calcium ATPases

The solution of system 11, which takes into account both calcium influx and extrusion of its excess from cytosol, under initial conditions as above, is

$$c_{\text{influx \& extrusion}} = c_0 + (c_\infty - c_0)\left(1 - e^{-(\alpha+\beta)t}\right) - \frac{\alpha}{\alpha+\beta}\left(1 + \frac{\beta\kappa\lambda}{\alpha(\alpha+\beta-\kappa)(\alpha+\beta-\lambda)} e^{-(\alpha+\beta)t} + \frac{\alpha+\beta}{\alpha(\kappa-\lambda)}\left(\frac{(\alpha-\kappa)\lambda}{\alpha+\beta-\kappa} e^{-\kappa t} - \frac{(\alpha-\lambda)\kappa}{\alpha+\beta-\lambda} e^{-\lambda t}\right)\right) E_{\text{PMCA}}. \quad (17)$$

This model is presented in Figure 2 (curve *c*), its parameters taken from Table 2 in Juška et al., (2005) for 50 nM ionomycin concentration). The model is in agreement with experimental data (Juška et al., 2005). Parameters κ and λ can be estimated rather accurately from model fitting to the data. It can be seen that when PMCA is inhibited ($E_{\text{PMCA}} = 0$), the above equation is reduced to equation 4.

As can be seen from equation 17, the asymptotic calcium concentration in cytosol (for $t \to \infty$) as a result of its influx from the stores and extrusion of its excess is

$$c_{\text{influx \& extrusion},\infty} = c_\infty - \frac{\alpha}{\alpha+\beta} E_{\text{PMCA}}. \quad (18)$$

The solution of system 13, which takes into account both calcium influx into and its reuptake from cytosol

back into the stores, under initial conditions as above, is

$$c_{\text{influx \& reuptake}} = c_0 + (c_\infty - c_0)\left(1 - e^{-(\alpha+\beta)t}\right) - \frac{\beta}{\alpha+\beta}\left(1 + \frac{\mu}{\alpha+\beta-\mu}e^{-(\alpha+\beta)t} - \frac{\alpha+\beta}{\alpha+\beta-\mu}e^{-\mu t}\right)E_{\text{SERCA}}. \quad (19)$$

This model is presented in Figure 2 (curve *d*), its parameters (α and β) being the same as in model 4. The model is in agreement with experimental data. Parameters E_{SERCA} and μ can be estimated rather accurately from model fitting to the data. It can be seen that when SERCA is inhibited ($E_{\text{SERCA}} = 0$), the above equation is reduced to equation 4.

As can be seen from equation 19, the asymptotic calcium concentration in cytosol (for $t \to \infty$) as a result of its influx from the stores and its reuptake back into stores is

$$c_{\text{influx \& reuptake},\infty} = c_\infty - \frac{\beta}{\alpha+\beta}E_{\text{SERCA}}. \quad (20)$$

The solution of system 14, which takes into account calcium influx, its reuptake back into the stores and extrusion of its excess from cytosol, under initial conditions as above, is

$$c_{\text{influx \& extrusion}} = c_0 + (c_\infty - c_0)\left(1 - e^{-(\alpha+\beta)t}\right) - \frac{\beta}{\alpha+\beta}\left(1 + \frac{\mu}{\alpha+\beta-\mu}e^{-(\alpha+\beta)t} - \frac{\alpha+\beta}{\alpha+\beta-\mu}e^{-\mu t}\right)E_{\text{SERCA}} - \frac{\alpha}{\alpha+\beta}\left(\begin{array}{l}1 + \frac{\beta\kappa\lambda}{\alpha(\alpha+\beta-\kappa)(\alpha+\beta-\lambda)}e^{-(\alpha+\beta)t} \\ \quad + \frac{\alpha+\beta}{\alpha(\kappa-\lambda)}\left(\frac{(\alpha-\kappa)\lambda}{\alpha+\beta-\kappa}e^{-\kappa t} - \frac{(\alpha-\lambda)\kappa}{\alpha+\beta-\lambda}e^{-\lambda t}\right)\end{array}\right)X_{\text{PMCA}}. \quad (21)$$

The asymptotic calcium concentration in cytosol (for $t \to \infty$) as a result of its influx from the stores, its reuptake back into stores and extrusion of its excess from cytosol is

$$c_{\text{influx \& reuptake \& extrusion},\infty} = c_\infty - \frac{\beta}{\alpha+\beta}E_{\text{SERCA}} - \frac{\alpha}{\alpha+\beta}X_{\text{PMCA}} \quad (22)$$

As observed in (Juška et al., 2005) and in Figure 1 in that paper, the asymptotic calcium concentration in cytosol when PMCA is active is about the same independently of SERCA being active or not. It can be assumed, therefore, that

$$-\frac{\beta}{\alpha+\beta}E_{\text{SERCA}} - \frac{\alpha}{\alpha+\beta}X_{\text{PMCA}} = -\frac{\alpha}{\alpha+\beta}E_{\text{PMCA}} \quad (23)$$

from where

$$X_{\text{PMCA}} = \begin{cases} 0, & \text{if PMCA is inhibited,} \\ E_{\text{PMCA}} - \frac{\beta}{\alpha}E_{\text{SERCA}}, & \text{if PMCA is active.} \end{cases} \quad (24)$$

Taking into account the above, equation 21 is reduced to either equation 19, 17 or 4 if either SERCA or PMCA (or both) is inhibited. This equation, therefore, can be considered a generalized model simulating both passive and active calcium fluxes in nonexcitable cells. The model is presented in Figure 2 (curve *e*), its parameters being the same as for curves *b*, *c* and *d*. The model is in agreement with experimental data presented in Juška et al., (2005).

As mentioned above, there are several types of calcium stores (Papp et al., 1991; Cavallini, Coassin & Alexandre, 1995); two types of corresponding SERCAs are identified (Cavallini et al., 1995). Experimentally, activities of SERCAs of these types can be observed separately by selectively inhibiting other pathways of calcium excess elimination but for the particular type of SERCA (Juška et al., in preparation). System 13 and equation 19 can be used to characterize the activity of SERCA of a particular type, other calcium ATPases being selectively inhibited.

Activity of Calcium ATPases

Transitions 7 and equation 10 can be viewed as responses of PMCA to calcium influx into cytosol elicited by an experimental increase of endomembrane permeability. It is essential that system 11, whose solution is model 17, makes use of equation 9, which is based on the assumption concerning the transitions 7 of PMCA. The dynamics of calcium extrusion from cytosol (*see* Fig. 2, curve *f*) are assumed, therefore, to be determined entirely by the activity of PMCA. One might attempt, however, to explain the decline in the rate of calcium excess extrusion, e.g., by a decline in cytosolic calcium concentration. Indeed, ATPase activity might depend on cytosolic calcium concentration, which, being low (~50 nM), is about or below the dissociation constant of an ATPase (macro)molecule and calcium ion ($K_{½}$ < 0.5–1 μM [Żylińska & Soszyński, 2000; Caride et al., 2001a]). The decline in cytosolic calcium concentration (in the case of its extrusion), however, is not large enough to account for the (considerable) decline in the rate of elimination of its excess (besides, the decline in the rate is greater when the

corresponding decline in the concentration is smaller [Juška et al., 2005]).

Scheme 7 has been postulated on the basis of analysis of the dynamics of calcium redistribution in a cell (Juška et al., 2005) rather than from considering any molecular mechanism which might be responsible for the dynamics. It has to conform to the findings concerning molecular aspects of the activity of calcium ATPases however. Presumably, it does conform to the available data. PMCA activity has been shown to be relatively low in the absence of calmodulin; binding of calmodulin to a specific domain stimulates the activity (Penniston & Enyedi, 1998). The open form (with calmodulin bound) has higher ATPase activity than the closed one (in the absence of calmodulin) (Penheiter et al., 2003). Later, this two-state view was modified. It was found that the state of PMCA without calmodulin bound is inactive, with the autoinhibitory domain blocking the active site. Another one, with calmodulin bound and the autoinhibitory domain dissociated, is active. The third corresponds to calmodulin bound but the autoinhibitory domain not dissociated (Osborn et al., 2004). Several major reaction intermediates of the ATPases have been revealed by X-ray crystallography and structural investigations (Stokes & Green, 2003; Young & Stokes, 2004). These studies, however, cannot provide information concerning the dynamic characteristics of ATPase. It should be emphasized that the existence of the inactive, active and dormant (or, in terms of molecular biology, without calmodulin bound and with the autoinhibitory domain blocking the active site, with calmodulin bound and the autoinhibitory domain dissociated and with calmodulin bound but the autoinhibitory domain not dissociated) states of PMCA, being necessary, is not a sufficient condition for the activity of PMCA to be transient: it is necessary that PMCA undergoes transitions 7. PMCA is known to be activated by a sudden increase in calcium concentration (Caride et al., 2001a). The increase provides, presumably, an external stimulus eliciting the sequence of transitions. The increase in cytosolic calcium concentration need not, presumably, be "sudden" (as stated by Caride et al., 2001a) to elicit the transitions. The transitions most probably occur as a result of (slow) fluctuations of the concentration in the absence of any interference with the pathways of calcium flux.

Transitions 7 not being reversible lead eventually all the PMCA (macro)molecules to the dormant state. That is in good agreement with experimental data obtained on platelet suspension in calcium-free medium (Juška et al., 2005). Indeed, as a result of calcium extrusion, cytosolic calcium concentration remains above the initial concentration (further extrusion, therefore, being possible), suggesting the absence of PMCA activity. Under physiological conditions, however, calcium leaks from the extracellular medium to cytosol, resulting in its excess which has to be extruded. PMCA, therefore, has to be active permanently; i.e., it cannot remain in the dormant state. Consequently, one more transition, $D \rightarrow I$ (closing the cycle), is necessary. The mechanism by which this transition is controlled remains unclear.

The system of equations, based on the scheme of a closed cycle, $I \rightarrow A \rightarrow D \rightarrow I$, has been considered and solved. Its solution with respect to a is not presented here. It results in $a > 0$ for $t \rightarrow \infty$, which means that PMCA activity never declines to zero and can, therefore, extrude excess of calcium fom cytosol. With the rate of transition $D \rightarrow I$ approaching zero, the solution of the system of equations is reduced to equation 9.

The rate constants (relative rates) of transitions (parameters κ and λ) have been shown to depend on the initial rate of increase in cytosolic calcium concentration (Juška et al., 2005). This is related, presumably, to the memory effect of PMCA (Caride et al., 2001b). Indeed, to be able to respond to the rate of change in calcium concentration, PMCA has to compare the current concentration with that a moment earlier; i.e., the earlier concentration has to be "remembered."

It is interesting to note that the right-hand part of equation 10 is symmetrical with respect to parameters κ and λ:

$$A(\kappa, \lambda) \equiv A(\lambda, \kappa) \tag{25}$$

That means that curve f (as well as c) in Figure 3 will remain unchanged when κ and λ interchange. It is not possible, therefore, to determine experimentally (by fitting corresponding models to the data) which estimate belongs to which parameter. As seen from Table 2 in Juška et al., (2005), in which estimates of model parameters are presented, $\kappa > \lambda$ in a range of ionomycin concentrations (for lower α). It seems more likely, however, that $\kappa < \lambda$ for low α; i.e., it is parameter κ to which the lower estimate should be assigned. Indeed, at low rate of change in cytosolic calcium concentration, transition $I \rightarrow A$ (inactive to active) is expected to be slow, i.e. $\kappa < \lambda$ (*see* scheme 7; that is consistent with the studies on the rate of activation of PMCA suggesting that the rate of binding of calmodulin to isoform 4b of the pump is relatively slow [Penniston & Enyedi, 1998]). Such an assignement of the estimates to the parameters characterizing the transitions of PMCA and interpretation of the meaning of parameters κ and λ as relative rates of transitions 7 seems more adequate, therefore, than that in Juška et al., (2005). It should be remembered, however, that when the Juška et al., (2005) manuscript was in preparation, scheme 7 did not exist and relationship 25 was not known.

Since

$$\int_0^\infty \frac{\kappa\lambda}{\kappa-\lambda}\left(e^{-\lambda t}-e^{-\kappa t}\right)dt = 1 \tag{26}$$

for any κ and λ, from the point of view of the theory of probabilities the right-hand part of equation 10 can be viewed as a probability density function of a random variable, *T*. Transitions 7 can be viewed, therefore, as a random process, and PMCA (macro)molecules involved can be considered to reside in state A (as in any other) for a random period of time. The mean duration, Θ, of residence of the (macro)molecule in this state is, therefore,

$$\Theta = \int_0^\infty \frac{\kappa\lambda}{\kappa-\lambda}\left(e^{-\lambda t}-e^{-\kappa t}\right)t\mathrm{d}t = \frac{1}{\kappa}+\frac{1}{\lambda}. \tag{27}$$

For $\alpha = 0.025\ s^{-1}$ (50 nM ionomycin concentration), $\Theta_{PMCA} = 84$ s (see κ and λ in Table 2 of Juška et al., 2005). For lower α (lower ionomycin concentrations or none), Θ_{PMCA} is higher. That is in accordance with the rate of activation by calmodulin of isoform 4b of PMCA (half-time ~1 min) and that of inactivation (~20 min) obtained under essentially different conditions (Caride et al., 2001b).

The right-hand part of equation 10 multiplied by E_{PMCA} and by d*t* represents the work (i.e., the quantity of calcium transferred) performed by all the PMCA in time interval $t + dt$. Integrated over time from 0 to ∞, it yields the net work performed by the PMCA involved in the transitions during the period of its activity (see equation 26). E_{PMCA}, therefore, can be viewed as the net quantity of calcium (per cell) carried across the plasma membrane by all the population of PMCA (macro)molecules involved. It seems reasonable to suppose this parameter to be related to the number of PMCA (macro)molecules taking part in the process. One PMCA (macro)molecule may be thought to carry across the membrane one calcium ion per activity cycle. Then E_{PMCA} would also mean the number of PMCA (macro)molecules on the plasma membrane. This interpretation of the meaning of E_{PMCA}, however, does not seem realistic. Indeed, E_{PMCA} was estimated to be 2.4×10^6 calcium ions/cell in platelets (Juška et al., 2005). That is higher than the number of copies of so-called Band 3 protein (chloride/bicarbonate exchanger)/cell (1.2×10^6) in erythrocytes (Wang, 1994). PMCA is a low-abundance protein in nearly all cells, whereas Band 3 is a major erythrocyte membrane protein.

It is interesting to note that E_{PMCA} does not depend on α (relative rate of calcium effflux from the stores) while E_{SERCA} slightly declines with the rise of α (Juška et al., 2005). It would be of interest to obtain experimental data concerning E_{PMCA} under different conditions (e.g., in a range of cytosolic calcium concentrations).

Calcium ATPases (both PMCA and SERCA) deal with the quantity of calcium by carrying it across the membrane rather than with (the rate of change of) its concentrations (in the compartments partitioned by the membrane), which appear in the equations of system 1. The contribution of the ATPases, therefore, had to be adjusted to the equations. The dimension of the additional terms of the equations taking into account the contribution of the ATPases (equations in systems 11 and 13) is, therefore, concentration per unit of time. That of E_{PMCA} and E_{SERCA} is concentration (quantity of matter per unit of volume, or just per cell). Calcium ions are handled by the ATPase (macro)molecules, presumably one by one (this idea is symbolized in Figs. 1 and 2 by dots attached to the arrows' tails).

Whereas the result of PMCA activity is elimination of calcium excess from cytosol, that of SERCA is calcium reuptake back into the stores, keeping both the inward and outward fluxes in dynamic equlibrium. The activities of these ATPases are supposed, therefore, to be governed by different mechanisms (see equations 10 and 12). As stated above, the models based on these assumptions are in agreement with experimental data.

The meaning of E_{SERCA} is less clear than that of E_{PMCA}. As can be seen from the equations of system 13, for $t \to \infty$ and any α (or β), $E_{SERCA} = C_\infty - c_\infty$. This means that the activity of SERCA depends on the difference of calcium concentrations across the membrane being independent of the initial concentration in cytosol. (The above independence has been confirmed experimentally by Juška et al., [in preparation].) The meaning of the product αE_{SERCA} (*see* system 13) is clearer: it is absolute rate of ion transport by SERCA across the endomembrane. It remains unclear how αE_{SERCA}, i.e., the activity of SERCA, is controlled by α, i.e., the rate of change in calcium concentration in the stores. The memory effect seems to be involved here as in the case of PMCA. Interpretation of $E_{SERCA}\ \beta/(\alpha+\beta)$ follows immediately from equation 20: it is the change in asymptotic calcium concentration in cytosol resulting from the activity of SERCA.

The background activity (in the absence of ionomycin) of SERCA2b in platelets has been estimated as $\alpha E_{SERCA} \approx 270 \times 10^3\ s^{-1}$ per cell (Juška et al., in preparation). Taking the turnover rate of calcium transport by SERCA2b to be $\sim 5\ s^{-1}$ (Graupner, Erler & Meyer-Hermann, 2005) results in 54×10^3 (macro)molecules of SERCA2b involved in calcium transport per endomembrane in a cell. At 50 nM of ionomycin, $\alpha_{50} E_{SERCA} \approx 2.03 \times 10^6\ s^{-1}$ per cell (406×10^3 [macro]molecules of SERCA2b, respectively). This may be thought to be about the number of

SERCA2b (macro)molecules on the endomembrane of the cell.

The rates of calcium extrusion and reuptake (*see* systems 11 and 13) are plotted in Figure 2 (curves *f* and *g*). Both qualitative and quantitative differences in the rates are striking: while PMCA extrudes a definite quantity of calcium in a short pulse, SERCA reuptakes it with a constant (reached shortly after the change in membrane permeability) high rate (cf. scales in Fig. 2).

It follows from the above analysis that all the parameters of the models discussed correspond to the intrinsic properties of the mechanisms involved; the dynamics of calcium flux, being determined by model parameters, depend therefore on the permeability of cellular membranes and the dynamic characteristics of calcium ATPases. On the other hand, the dynamics of the fluxes, e.g., in abnormal platelets, are different from those in normal ones (*see*, e.g., Saavedra et al., 2004). The models would be helpful in identifying the mechanisms responsible for the differences. It should be noted that the models are rather simple, being expressed as finite combinations of elementary functions.

Possible Mechanisms and Pathways of the Fluxes not Considered in the Study

Mitochondria are known to accumulate calcium and to act as its temporary stores (Nicholls & Chalmers, 2004). They may be expected to take up excess calcium from the cytosol during calcium redistribution. Although the mechanisms of calcium uptake by mitochondria (Gunter & Pfeiffer, 1990; Parekh, 2003) are essentially different from those of PMCA, system 11 and equation17 can be applied in this case as a first approximation. The contribution of mitochondria to the net concentration of cytosolic calcium, therefore, would manifest itself in the shape of the dynamics of calcium redistribution (see curve *c* in Fig. 2; the decline would not necessarily be so pronounced). Experimentally, however, no involvement of mitochondria in the process of calcium redistribution was observed in platelets in a range of ionomycin concentrations (Juška et al., 2005). This may be related to the application of ionomycin to elicit calcium redistribution. Ionomycin facilitates calcium efflux from rat liver mitochondria (Kauffman, Taylor & Pfeiffer, 1980) rather than uptake of its excess. In this case, mitochondria would play the role of (releasable) calcium stores (Parekh, 2003) rather than being a sink for its excess. In the absence of ionomycin, mitochondria would, perhaps, take up the excess calcium.

In platelets, no contribution of Na, Ca exchange to calcium extrusion has been found (Rosado & Sage, 2000), PMCA playing the major role in the extrusion at low calcium concentrations (Rink & Sage, 1990). In other nonexcitable cells, possible involvement of the exchange in calcium extrusion would manifest itself in the shape of the dynamics of calcium redistribution in the absence of its reuptake and extrusion of its excess (see curve *c* in Fig. 1). Since the exchange proceeds with the energy expenditure, its (formal) modeling might be similar to that of the ATPases.

The schemes depicted in Figure 1, as well as the models developed, are supposed to reflect calcium fluxes in a separate cell. The results of modeling are applicable for ensemble measurements obtained in a suspension of cells (*see*, e.g., Rosado & Sage 2000, Juška et al., 2005). The ATPases located on separate membranes are not likely to interact or cross-talk. No feedback is supposed to take place. The models analyzed here cannot give rise to spikes or oscillations of cytosolic calcium concentration: presumably, some other elements (besides ATPases) have to be included in the schemes (and corresponding systems of equations) to produce this effect. On the other hand, if spikes or oscillations of calcium concentration normally arise but the molecular mechanisms responsible for them are inhibited, the above analysis can be usefully applied. In general, the models are applicable whenever and as long as the simplifications made are justifiable.

Conclusions

All the parameters of the models developed are related to the intrinsic properties of calcium ATPases and cellular membranes; there is no interdependence between the parameters. Due to their simplicity, the models are applicable for routine fitting to experimental data. This would provide the basis for quantitative analysis and interpretation of the data and facilitate identification of the mechanisms responsible for the differences in the dynamics of calcium fluxes in normal and abnormal cells.

The activities of PMCA and SERCA are governed by different mechanisms. PMCA is likely to undergo transitions from inactive to active to "dormant" (not identical to the initial) and back to inactive states, the mean duration of the cycle lasting for minutes or longer. The sequence of the transitions is initiated, presumably, by an increase in cytosolic calcium concentration. The transition of PMCA from inactive to active state (at least at low rates of increase in cytosolic calcium concentration) is likely to be slower than that from active to dormant state. SERCA, presumably, transits from inactive to active state in response to an increase in calcium leakage from calcium stores. Whereas PMCA extrudes excess calcium (a definite quantity of it) in a short pulse, SERCA retakes calcium back into the stores permanently at a high rate.

The models discussed may be the best means for the moment to quantitatively relate the dynamics of calcium fluxes in nonexcitable cells with known or putative properties of the mechanisms underlying activation of calcium ATPases.

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