Biochemical and Biophysical Research Communications 392 (2010) 41–46

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Plasma membrane calcium pump and sodium–calcium exchanger in maintenance and control of calcium concentrations in platelets

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ARTICLE INFO

Article history:
Received 18 December 2009
Available online 4 January 2010

Keywords:
Plasma membrane calcium ATPase
Store-operated calcium entry
Sodium–calcium exchanger
Cytosolic calcium concentration
Dynamics of calcium concentration

ABSTRACT

The purpose of this research was to elucidate the activity of the mechanisms responsible for control of cytosolic calcium concentration in platelets by modeling the time-course of the concentration changing in response to discharge of the intracellular stores or store-operated calcium entry (SOCE). The parameters estimated as a result of model fitting to experimental data are related to physiological or pathological state of the cells. It has been shown that: (a) the time-course is determined by the passive calcium fluxes and activities of the corresponding mechanisms; (b) the decline in the concentration (after its rise) develops due to activity of plasma membrane calcium ATPase (PMCA) both in the case of discharge of the stores of platelets contained in calcium-free medium and in the case of SOCE; (c) impulsive extrusion of calcium in response to its sudden influx, presumably, is the main function of PMCA; (d) the function of sodium–calcium exchanger (NCX) is to extrude calcium excess by permanent counteracting its influx.

The role of cytosolic free calcium in various cellular processes is well known (see, e.g., [1–3]). Calcium concentration in cytosol of platelets is ~50 nM while in the extracellular medium it is ~1 mM. In various cellular compartments the concentration is ~2 orders higher than in cytosol. It is clear that 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., [4]).

There are no direct quantitative data of store-operated calcium entry (SOCE) and on the activity of sodium–calcium exchanger (NCX). There is, however, a large volume of detailed experimental data concerning the biochemical pathways and mechanisms of calcium influx into and its elimination from platelets (see, e.g., [5–11]). Control experiments of SOCE investigations [6–11] and others provide valuable information concerning the fluxes via the plasma membrane and the activities of plasma membrane calcium ATPase (PMCA) and NCX. It is evident that proper analysis of this information is not possible without application of mathematical models, and, on the other hand, reasonable simplification of real biological systems is necessary to make modeling practicable.

The passive flux via the plasma membrane is counteracted by the activities of PMCA and NCX located in the plasma membrane. Experimental data concerning the activity of NCX in platelets are rather fragmentary. From the experiments carried out in calcium-free medium (cytosolic calcium concentration being rather low), PMCA has been concluded to be the main mechanism of calcium removal from cytosol [6], the contribution of NCX (if any) being insignificant. The role of NCX which is allosteric [12] at high cytosolic calcium concentrations is expected to be quite different.

PMCA in the cells of various types has been shown to be responsible for so called 'overshoot' of intracellular calcium concentration in response to calcium entry (see, e.g., [14]). In platelets contained in calcium-free medium, it responds to sudden increase in cytosolic calcium concentration by a pulse of calcium extrusion [15,16]. PMCA's capability to function both in pulse and permanent modes, however, has to be analyzed in more detail.

In this work, therefore, the known models of PMCA activity are modified to take into account the possibility of PMCA being active in the absence of sudden calcium influx into cytosol. Besides, the previous studies are extended to include the contribution of the NCX activity into dynamics of cytosolic calcium concentration in platelets. Both calcium influx from the stores and that from the extracellular medium (SOCE) are considered. The models are intended to relate the dynamics of cytosolic calcium concentration in platelets with the properties of the mechanisms responsible for the maintenance of the concentration under conditions close to normal.

Methods

Standard software ("Maple" to solve differential equations and "UN-SCAN-IT gel" to digitize curves) was used.

Results (modeling)

The pathways of calcium fluxes in platelets and mechanisms responsible for control of calcium concentrations are schematically

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doi:10.1016/j.bbrc.2009.12.153

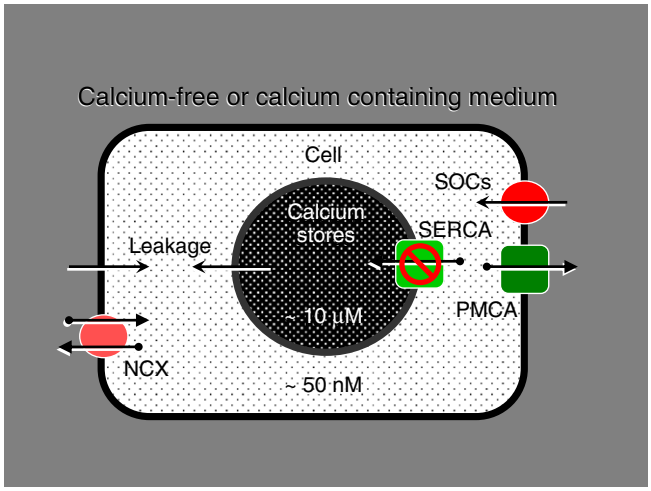

Fig. 1. Cartoon depicting the cell, calcium stores, pathways and mechanisms of calcium fluxes. Only the pathways and mechanisms of the main concern are taken into account here. Dots on the arrows' tails are intended to symbolize the "handling" of calcium ions one by one by the ATPase or NCX (macro)molecules, the dotless arrows symbolizing the passive leakage.

depicted in Fig. 1. In the absence of the activity of SERCA (it being inhibited),

$$\begin{cases} \frac{dC}{dt} = -\alpha(C-c), \\ \frac{dc}{dt} = \beta(C-c) + \mathrm{SOCE} - X - P, \end{cases} \tag{1}$$

where C and c are calcium concentrations in calcium stores and cytosol, respectively; α and β, relative rates of change in calcium concentration in the stores and cytosol; X and P, terms characterizing the contributions of NCX and PMCA.

In the case of discharge of calcium stores of platelets suspended in calcium-free medium, there is no influx from the medium (SOCE = 0), the contribution of NCX may be neglected as well [6] ($X = 0$), so system (1) is reduced to system (11) in [16] which is solved both for $P = 0$ and $P > 0$. This approach is applied for SOCE as well. In corresponding experiments calcium influx from the medium usually is made possible upon the discharge of the stores ($C - c = 0$), SERCAs being inhibited. Then two additional mechanisms of control of calcium fluxes (SOCs and NCX) have to be taken into account. System (1), therefore, is reduced to

$$\frac{dc}{dt} = \mathrm{SOCE} - X - P. \tag{2}$$

The permeability of SOCs for calcium ions like that of any channels declines over time [17]; the decline may be considered to be monoexponential. The channels are assumed to be open before the moment of changing the medium from calcium-free to calcium containing, the calcium influx, therefore, may be considered to be

$$\mathrm{SOCE} = S\exp(-\varepsilon t), \tag{3}$$

where ε is rate constant of the decline in the channels' permeability, S, initial SOCE.

Since NCX is allosteric (see, e.g., [12]), the dependence of its activity on calcium concentration, presumably, is sigmoid (see, e.g., [18]). For NCX having four binding sites its activity

$$X = x\frac{c(c+\kappa\gamma)^3\gamma}{(c+\kappa\gamma)^4 + \kappa^3\gamma^4(1-\kappa)} \tag{4}$$

definitions of the parameters being presented in Table 1.

PMCA is either in passive (P), active (A) or "sleeping" (S) states and undergoes P → A → S transitions [16], the rates of P → A and A → S transitions being different, in general. When the discharge of the stores is facilitated by ionomycin, the rates do not differ considerably in a range of ionomycin concentrations [15]. So there is good reason to assume (for simplicity) the both transitions to proceed with the same rate, i.e., to consider the scheme of PMCA transitions to be

$$\mathrm{P} \xrightarrow{\lambda} \mathrm{A} \xrightarrow{\lambda} \mathrm{S}, \tag{5}$$

where λ is relative rate constant of the transitions. That leads to (see Appendix A)

$$a = \lambda^2 t\exp(-\lambda t), \tag{6}$$

a denoting PMCA activity. The model is depicted in Fig. 2A.

According to scheme (5), however, PMCA upon stimulation ends up in the "sleeping" state (S) becoming inactive again (see also Eq. (6) and Fig. 2A). To take into account the possibility of PMCA to be active again, the following scheme of transitions

$$\begin{array}{ccccc} \mathrm{P} & \xrightarrow{\lambda} & \mathrm{A} & \xrightarrow{\lambda} & \mathrm{S} \\ \uparrow & \leftarrow & \xleftarrow{\omega} & \leftarrow & \lrcorner \end{array} \tag{7}$$

has to be considered where ω is rate constant of S → P transition. That leads to system of equations

$$\begin{cases} \frac{dp}{dt} = -\lambda p + \omega s, \\ \frac{da}{dt} = \lambda p - \lambda a, \\ \frac{ds}{dt} = \lambda a - \omega s \end{cases}, \tag{8}$$

where a, p and s are normalized probabilities of PMCA to reside in states A, P and S. Analytical solution of this system (see [16]) is

Table 1
Model parameters estimated[a] as a result of fitting the models to the experimental data.

Parameter	Notation	Estimate
Initial calcium concentration in cytosol before store discharge (nM)	c_0	49
Initial calcium concentration in cytosol before SOCE (nM)	$c_{0,\ \mathrm{SOCE}}$	120
Relative rate of decrease in calcium concentration in the stores (s^{-1})	α	0.01
Relative rate of increase in calcium concentration in cytosol (s^{-1})	β	0.0005
Relative rate of PMCA transitions (stores) (s^{-1})	$\lambda_{\mathrm{stores}}$	0.015
Relative rate of PMCA transitions (SOCE) (s^{-1})	λ_{SOCE}	0.03
Efficacy of PMCA (stores) (10^6 ions per cell)	$E_{\mathrm{PMCA,\ stores}}$	2.5
Efficacy of PMCA (SOCE) (10^6 ions per cell)	$E_{\mathrm{PMCA,\ SOCE}}$	19.2
Factor of proportionality (nM s^{-1})	x	0.002
Number of calcium-binding sites on the NCX	n	4
Equilibrium dissociation constant characterizing calcium affinity for NCX (μM)	γ	50
Cooperativity factor characterizing change of the affinity upon binding calcium ion	κ	0.02
Initial rate of increase in calcium concentration in cytosol as a result of SOCE (nM s^{-1})	S	46
Relative rate of decline of channels' permeability (s^{-1})	ε	0.01

[a] Values of parameters n, γ, κ, S and ε are arbitrary.

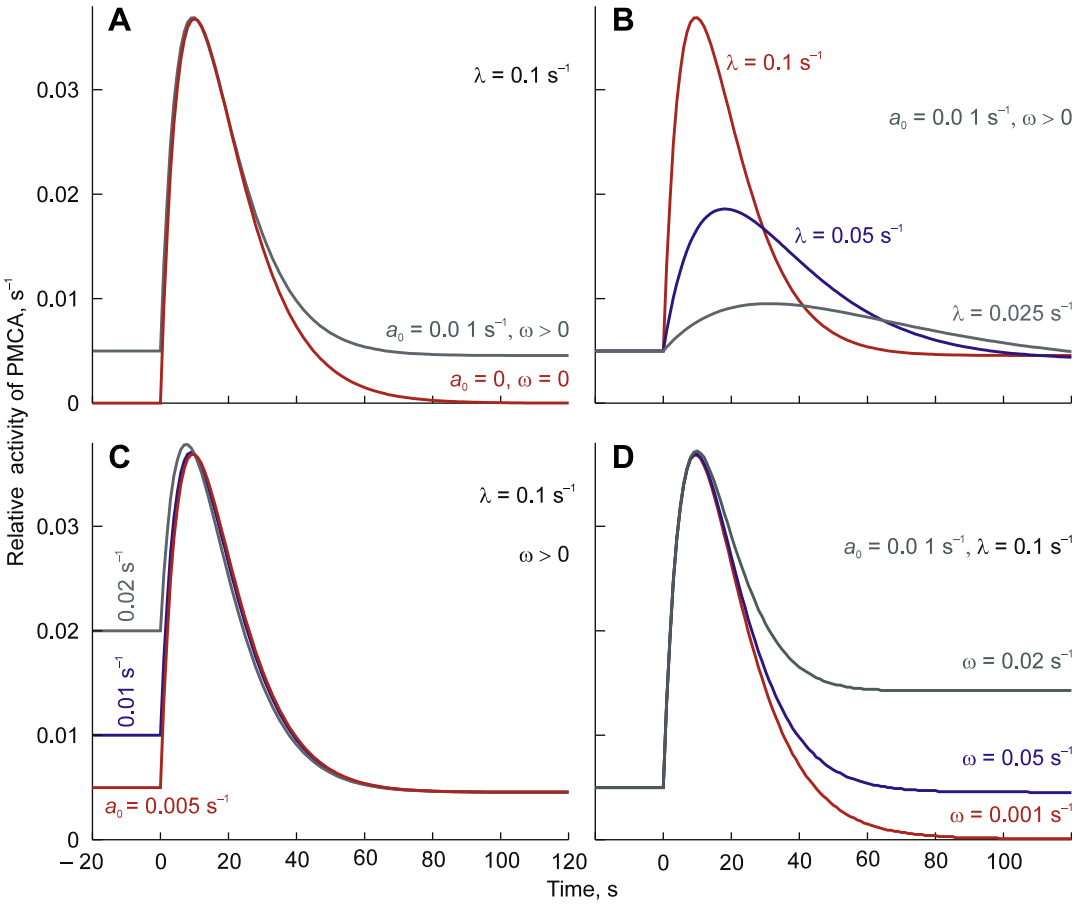

Fig. 2. Dynamics of PMCA activity. (A) Models of the activity (solution of system (8)) without or with S → P transition, without ($a_0 = 0$) or with ($a_0 > 0$) background activity. (B) Models for different values of λ (the rates of P → A transitions), ω (the rate of S → P transition) and a_0 (background activity) being the same, as in (A). (C) Models for different values of a_0, λ and ω being the same, as in (A). (D) Models for different values of ω, λ and a_0 being the same, as in (A).

impractical to be presented here; the models with respect to a (the unknown of interest), taking into account the initial conditions (let $a(0) = a_0$, $p(0) = \lambda - a_0$, $s(0) = 0$, where a_0 is the background activity of PMCA) are depicted in Fig. 2. For $a_0 = 0$ the solution is as follows:

$$a_{\text{cycle}} = \frac{\lambda\omega}{\lambda+2\omega} + \frac{\left(-\lambda\omega\sqrt{\omega(\omega-4\lambda)} - \lambda\omega^2 + \lambda^2\omega + 2\lambda^3\right)\exp\left(-\frac{2\lambda+\omega-\sqrt{\omega(\omega-4\lambda)}}{2}t\right)}{2(\lambda+2\omega)\sqrt{\omega(\omega-4\lambda)}} + \frac{\left(-\lambda\omega\sqrt{\omega(\omega-4\lambda)} + \lambda\omega^2 - \lambda^2\omega - 2\lambda^3\right)\exp\left(-\frac{2\lambda+\omega+\sqrt{\omega(\omega-4\lambda)}}{2}t\right)}{2(\lambda+2\omega)\sqrt{\omega(\omega-4\lambda)}}. \quad (9)$$

It can be shown that if the rate of S → P transition is very low

$$\lim_{\omega\to 0} a_{\text{cycle}} = \lambda^2 t\exp(-\lambda t)$$

i.e., Eq. (9) is reduced to Eq. (6).

Assuming the transitions of PMCA (scheme (5)) to proceed with the same rate, λ, Eq. (17) in [16] can be simplified (see Appendix A):

$$c_{\text{stores, extrusion}} = c_{0,\text{stores}} + (c_\infty - c_{0,\text{stores}})(1-\exp(-(\alpha+\beta)t)) - \frac{\alpha}{\alpha+\beta}\left(1 + \frac{\beta\lambda^2\exp(-(\alpha+\beta)t)}{\alpha(\alpha+\beta-\lambda)^2} + \frac{(\alpha+\beta)((\beta\lambda-1)\lambda t-1)((\alpha-\lambda)^2+\alpha\beta)\exp(-\lambda t)}{\alpha(\alpha+\beta-\lambda)^2}\right)E_{\text{PMCA, stores}}, \quad (10)$$

where $c_{0,\text{ stores}}$ is the initial calcium concentration in cytosol, c_∞, the asymptotical concentration (upon store discharge) in absence of its extrusion, $E_{\text{PMCA, stores}}$, the efficacy of PMCA in the case of store discharge. This model is presented in Fig. 3.

When calcium influx via SOCs and its extrusion by PMCA proceed simultaneously Eq. (2) has to be modified as follows:

$$\frac{dc}{dt} = S\exp(-\varepsilon t) - x\frac{c(c+\kappa\gamma)^3\gamma}{(c+\kappa\gamma)^4 + \kappa^3\gamma^4(1-\kappa)} - E_{\text{PMCA, SOCE}}\lambda^2 t\exp(-\lambda t), \quad (11)$$

where $E_{\text{PMCA, SOCE}}$ denotes PMCA efficacy. The numerical solution of the above equation is presented in Fig. 3.

Discussion

In the resting state constant concentrations of calcium ions in the cell and its compartments have to be maintained. All the ion channels are closed in this case, there being only (uncontrolled) leakage of the ions. These passive ion fluxes have to be countered actively by the fluxes generated by the corresponding mechanisms. The passive flux via the plasma membrane is supposed to be counteracted by the activities of PMCA and NCX located in the plasma membrane. The exchanger can operate in either direction but in this case it is removing calcium ions from cytosol. The leakage via the plasma membrane and the activities of NCX and PMCA, presumably, determine the resting (or background) calcium concentration in cytosol. The leakage from the stores is counteracted by the activity of SERCAs located in the endomembranes, these fluxes

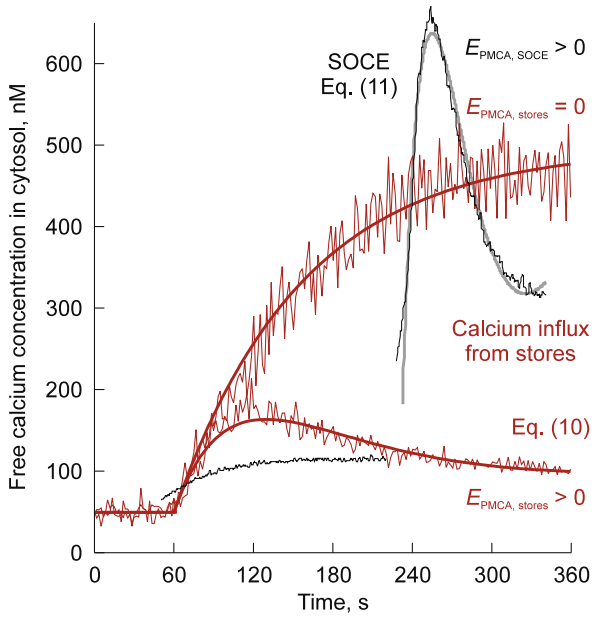

Fig. 3. Dynamics of cytosolic calcium concentration resulting from discharge of the stores facilitated by ionomycin and as a result of SOCE. The smooth lines correspond to the models (Eq. (10), solution of Eq. (11)), the zigzagged ones, to experimental data taken from Refs. [13,15].

determining calcium concentration in the stores (see [19]). The leakage from mitochondria is balanced by the activity of NCX and that of the uniporter located in the mitochondrial membranes. To maintain the equilibrium of calcium fluxes the above transporters have to operate permanently (see Fig. 1).

Dynamics of cytosolic calcium concentration

When calcium fluxes are evoked experimentally, the above ion transporters counter the changes in the fluxes. The SERCAs do not lower cytosolic calcium concentration appreciably when its rise results from the influx from the stores of another type with SERCAs inhibited (see Figs. 2–4 in [19]). The activity of SERCA located in the endomembrane is controlled by the transmembrane difference of calcium concentrations [16,19]. The capability of SERCAs to lower calcium concentration in cytosol, presumably, is rather limited (if any) unless the stores are empty or depleted considerably. The same seems to be applicable to mitochondria as well (see [6]). No experimental data are available concerning the activity of the NCX located in the plasma membrane of platelets in response to change in cytosolic calcium concentration. No activity of NCX is observed in platelets contained in calcium-free medium (see [6]). The activity of PMCA located in the plasma membrane differs essentially from that of SERCAs, namely, it is pulse-like [16].

The models presented here are intended to relate quantitatively the data concerning the change of cytosolic calcium concentration in response to experimentally induced calcium fluxes with the properties of the mechanisms responsible for the maintenance of the concentration. Application of fluorescent indicators makes calcium concentration measurements highly sensitive [20]. Still it should be kept in mind that while the mechanisms in question "handle" ion fluxes (which are essentially temporal, say, nM s^{-1}) the results of their activity manifest themselves in the quantity of ions transferred (and accumulated) over a period of time causing a change in ion concentration (being non-temporal, nM). Mathematically, a concentration is an integral function with respect to a flux, all the "sharpnesses" of the original functions being "smoothed" in the integral functions. The contribution of a separate flux (and the activity of the mechanism, respectively) to the change may be distorted, therefore, by the "noise" accompanying experimental data. Possible differences in PMCA activity dynamics are hardly discernible, therefore, in the cumulative data of cytosolic calcium concentration (see Fig. 3).

Any differences in the rates of PMCA transitions (P → A and A → S; see scheme (5)) if not large enough, cannot be observed in the cumulative data of cytosolic calcium concentration. And that does not make much sense in considering the rates to be different. Likewise, Eq. (6) rather than solution of system (8) was used in Eq. (11), the rate of S → P transition, ω, being not possible to estimate from the same data.

In the case of SOCE, beside one more mechanism of calcium elimination (NCX), calcium redistribution is essentially different from that resulting from store discharge because of essential difference of calcium sources. Indeed, the quantity of calcium ions in the stores is essentially finite whereas that in the medium does not appreciably change during the experiments. The transmembrane difference of calcium concentrations, therefore, declines during the discharge, but that in the case of SOCE may be considered to remain constant. The extracellular concentration of calcium in SOCE experiments usually was 300 μM, that one in cytosol rising below 2 μM (see, e.g., [6–11,13,21]); the decrease in the transmembrane concentration, therefore, can be ignored. It is assumed, therefore, that SOCE may be modeled by Eq. (3) during the experiments.

As mentioned above, in SOCE experiments calcium influx into cytosol from the medium is made possible upon the addition of calcium into the medium rather than from the moment the stores are being depleted when the platelets are maintained in calcium-free medium (see, e.g., [6–11,13,21]). That implies tacitly that corresponding channels are supposed to remain open until the moment up to the change in composition of the medium, at least; on the other hand, the channels are considered to have short open times [17,22]. More specific data are not available. Inevitable indeterminacy is also bound to the activity of NCX.

The model generating the curve with a maximum (rise and decline) of cytosolic calcium concentration (Fig. 3) is in agreement with findings obtained on the cells other than platelets. So-called 'overshoot' in cytosolic calcium concentration is explained by delayed increase in the rate of calcium clearance by PMCA (see, e.g., [14]).

Possible molecular mechanisms underlying PMCA activity

Penheiter et al. [23] consider two states (open and closed) of PMCA. After binding calmodulin, the open form undergoes the isomerization. The transitions considered above seem to correspond to the isomerization of PMCA. Calmodulin may also bind to the closed form of PMCA, destabilizing it [23]. Johnson [24] on the basis of investigations of calmodulin–target interactions presents three states of PMCA; it should be noted, however, that there are four possibilities given that PMCA states depend both on its autoinhibitory domain and its relation to calmodulin. The active state of PMCA considered in the schemes above corresponds, presumably, to both its autoinhibitory domain not blocking access of calcium ions to its binding site and the (macro)molecule being associated with calmodulin. Calmodulin binding is supposed to trigger conformational change of PMCA that removes autoinhibition [24]. That is related, presumably, to the P → A transition of PMCA. The A → S transition corresponds to the translocation of calcium ion from the side of the (macro)molecule facing cytosol to that facing the outside of the cell; the ion thereby vacates the binding site of PMCA on its cytosolic side. That results in either blocking access of calcium ions to the binding site by the autoinhibitory domain or calmodulin dissociation from PMCA. The P → A transition (and new cycle) is initiated, presumably, by another calcium ion binding to the vacant site of PMCA. The duration of the vacancy

contains information concerning calcium concentration, which may be estimated by PMCA. That information, presumably, constitutes the PMCA memory. Caride and co-authors [25] suggest that the memory effect is due to calmodulin remaining bound to PMCA. It follows, therefore, that the A → S transition is likely to result in blocking access of calcium ions to the binding site of PMCA by the autoinhibitory domain rather than unbinding calmodulin.

The rates of PMCA transitions (P → A and A → S) should be expected, therefore, to be related to the duration of the vacancy. The rates have been shown to depend on the (initial) rate of calcium influx into cytosol from the stores [15]. That, presumably, remains true for SOCE as well. Delayed increase in the rate of calcium extrusion from cytosol by the PMCA observed in human T cells [14] is related, presumably, to the P → A transition.

It follows from the solution of system (8) with respect to a (see also Eq. (9)) that

$$\lim_{t\to\infty} a_{\text{cycle}} = \frac{\lambda\omega}{\lambda + 2\omega} \tag{12}$$

irrespective of the initial conditions. In contrast, the initial conditions (corresponding to the relative number of PMCA (macro)molecules residing in passive (P), active (A) and "sleeping" (S) states) are of importance in determining the transient phase of PMCA activity (see Fig. 2). The ultimate states of PMCA (macro)molecule resulting from the transitions (scheme (7)) are different from the initial ones. That means that the relative number of the (macro)molecules residing in the "sleeping" state is >0, and both the numbers of the (macro)molecules residing in passive and active states are equal. No further transition of the (macro)molecule, therefore, can occur, once it is in the S state. In order to renew the cycle of transitions (7), the initial conditions have to be reset. The resetting, presumably, can be realized by the initiating stimulus.

Considering the rate constants of PMCA transitions (P → A and A → S) to be different as in the original scheme [16] would not produce essentially different results making, however, the scheme (and corresponding equations) impracticable. It is postulated, irrespective of possible modifications of the scheme, that the rate of rise in cytosolic calcium concentration (supposed to initiate the transitions) induces fast transit of the PMCA (macro)molecule from the "sleeping" (S) to the passive (P) state (the unstimulated transit (S → P) being very slow) leaving the active (A) state unchanged. This is the simplest possible way to realize the peak in the activity of PMCA in response to increase in cytosolic calcium concentration.

There is good reason to believe the peak in the activity of PMCA to be the fundamental feature of this ATPase, namely, its ability to extrude from cytosol a sudden influx of calcium. It can be seen that this ability (which is determined by the integral over time or by the area under the curve above the background activity) is decreased if its background activity (the rate of S → P transition, ω) is increased. High background activity of PMCA, therefore, would be disadvantageous in view of its capability to extrude a large quantity of calcium in a short pulse. There are no direct data, however, to test this conclusion.

As evidence of impulsive (with low background) activity of PMCA can be interpreted the results of experiments of SOCE. There is no observable decline in the cytosolic calcium concentration or the decline being very slow before the change in the medium composition but the decline is fast (after its sudden rise) after the change. The absence of observable removal of calcium from cytosol of platelets contained in calcium-free medium by NCX (see [6]) could be explained by the change (arising because of the change in the medium) of electrochemical potential driving the exchanger. Indeed, the exchanger under similar conditions operates in reverse mode [11].

The models (Eq. (10) and numerical solution of Eq. (11)) were fitted to experimental data (Fig. 3); parameters estimated as a result of model fitting are presented in Table 1. Agreement of model (10) with the data [15] means that no difference in the rates of P → A and A → S transitions can be found from these data. The solution of Eq. (11) is also in agreement with the data [13], but the equation contains several free parameters ($S, \varepsilon, \gamma, \kappa$), their values being arbitrary because of shortage of experimental data necessary for the estimation; S, e.g., could be estimated from the time-course of SOCE without calcium extrusion by PMCA (making $P = 0$ in Eq. (2)). Effects of change of these parameters can be compensated by changes of others.

The models developed and analyzed here contain rather few parameters. Model (10) contains α, β, λ, $E_{\text{PMCA, stores}}$, $c_{0,\text{ stores}}$ and c_∞; model (11), S, ε, $c_{0,\text{ SOCE}}$ $E_{\text{PMCA, SOCE}}$, λ, γ, κ and x. While the parameters α, β or S are determined by the conditions of the experiments, c_∞, by the conditions of the cell (calcium content in the stores), the others (ε, λ, x, γ, κ, $E_{\text{PMCA, stores}}$ and $E_{\text{PMCA, SOCE}}$), presumably, characterize the activity of the mechanisms responsible for the maintenance and control of calcium concentrations in platelets (depending also on the conditions of the cell and the experiments). "Responsible" rather than just "involved" should be emphasized here: agreement of the models with experimental data does not suggest (not excluding, however) involvement of any mechanisms other than those considered in the models; on the other hand, the parameters are related to the fluxes of calcium ions in platelets and are sufficient to make the models to agree with the data. Parameters of models characterizing the activities of SOCs, PMCA and NCX (S, ε, $E_{\text{PMCA, stores}}$, $E_{\text{PMCA, SOCE}}$, λ, x, γ and κ) supposed to remain the same both under experimental and physiological conditions) are expected to correlate with physiological or pathological state of the cells.

Conclusions

All the parameters of the models are related to the properties of the mechanisms responsible for the maintenance and control of calcium concentrations in platelets. The values of the parameters are expected to correlate with physiological or pathological state of the cells. The main results of this work can be summarized as follows:

1. The dynamics of cytosolic calcium concentration are determined by the passive calcium fluxes and activities of the mechanisms responsible for the maintenance and control of calcium concentrations in the cells.
2. The decline in cytosolic calcium concentration (after its rise) develops due to activity of PMCA both in the case of calcium influx into cytosol resulting from the discharge of the stores of platelets contained in calcium-free medium and in the case of SOCE.
3. Impulsive extrusion of calcium from cytosol in response to its sudden influx, presumably, is the main function of PMCA. Its activity is likely to be low in the resting state.
4. The function of NCX is to extrude calcium excess from cytosol by permanent counteracting its influx; the activity of NCX also is limiting cytosolic calcium concentration in the case of SOCE.

Acknowledgments

The author thanks Professor Rimantas Daugelavičius for stimulating discussions and Doctors Dainius Rusakevičius for his help in graphical presentation and Romas Lenkaitis for reading the manuscript and helpful comments.

Appendix A

Under assumption that $\kappa = \lambda$ in Eq. (10) in [16] it follows that

$$\lim_{\kappa\to\lambda} \frac{\kappa\lambda}{\kappa - \lambda}(\exp(-\lambda t) - \exp(-\kappa t)) = \lambda^2 t \exp(-\lambda t) \tag{A1}$$

what is expressed by Eq. (6) in the main text. Likewise, it follows from Eq. (17) in [16] that

$$\begin{aligned}\lim_{\kappa\to\lambda}\Bigg(1+\frac{\beta\kappa\lambda\exp(-(\alpha+\beta)t)}{\alpha(\alpha+\beta-\kappa)(\alpha+\beta-\lambda)}\\ +\frac{\alpha+\beta}{\alpha(\kappa-\lambda)}\left(\frac{(\alpha-\kappa)\lambda\exp(-\kappa t)}{\alpha+\beta-\kappa}-\frac{(\alpha-\lambda)\kappa\exp(-\lambda t)}{\alpha+\beta-\lambda}\right)\Bigg)\\ =1+\frac{\beta\lambda^2\exp(-(\alpha+\beta)t)}{\alpha(\alpha+\beta-\lambda)^2}\\ +\frac{(\alpha+\beta)((\beta\lambda-1)\lambda t-1)((\alpha-\lambda)^2+\alpha\beta)\exp(-\lambda t)}{\alpha(\alpha+\beta-\lambda)^2}\end{aligned}\tag{A2}$$

what leads to Eq. (10) in the main text.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.12.153.

References

[1] T.J. Rink, S.O. Sage, Calcium signaling in human platelets, Annu. Rev. Physiol. 52 (1990) 431–449.
[2] A.R. Means, Calcium, calmodulin and cell cycle regulation, FEBS Lett. 347 (1994) 1–4.
[3] R. Rizzuto, T. Pozzan, E. Carafoli, Ca^{2+} on the move: ways and means to translate a multifarious signal, Trends Pharmacol. Sci. 23 (2002) 348–350.
[4] T. Pozzan, R. Rizzuto, P. Volpe, J. Meldolesi, Molecular and cellular physiology of intracellular calcium stores, Physiol. Rev. 74 (1994) 595–636.
[5] S. Jenner, S.O. Sage, Two pathways for store-mediated calcium entry in human platelets, Platelets 11 (2000) 215–221.
[6] J.A. Rosado, S.O. Sage, Regulation of plasma membrane Ca^{2+}-ATPase by small gtpases and phosphoinositides in human platelets, J. Biol. Chem. 275 (2000) 19529–19535.
[7] J.A. Rosado, S.O. Sage, Coupling between inositol 1,4,5-trisphosphate receptors and human transient receptor potential channel 1 when intracellular Ca^{2+} stores are depleted, Biochem. J. 350 (2000) 631–635.
[8] J.A. Rosado, S.O. Sage, Phosphoinositides are required for store-mediated calcium entry in human platelets, J. Biol. Chem. 275 (2000) 9110–9113.
[9] J.A. Rosado, S.O. Sage, Farnesylcysteine analogues inhibit store-regulated Ca^{2+} entry in human platelets: evidence for involvement of small GTP-binding proteins and actin cytoskeleton, Biochem. J. 347 (2000) 183–192.
[10] M.T. Harper, S.O. Sage, PAR-1-dependent $pp60^{src}$ activation is dependent on protein kinase C and increased: evidence that $pp60^{src}$ does not regulate PAR-1-dependent Ca^{2+} entry in human platelets, J. Thromb. Haemost. 4 (2006) 2695–2703.
[11] A.G. Harper, S.O. Sage, A key role for reverse Na^+/Ca^{2+} exchange influenced by the actin cytoskeleton in store-operated Ca^{2+} entry in human platelets: evidence against the de novo conformational coupling hypothesis, Cell Calcium 42 (2007) 606–617.
[12] J.P. Reeves, M. Condrescu, Allosteric activation of sodium–calcium exchange activity by calcium: persistence at low calcium concentrations, J. Gen. Physiol. 122 (2003) 621–639.
[13] A.G. Harper, M.J. Mason, S.O. Sage, A key role for dense granule secretion in potentiation of the Ca^{2+} signal arising from store-operated calcium entry in human platelets, Cell Calcium 45 (2009) 413–420.
[14] D.M. Bautista, M. Hoth, R.S. Lewis, Enhancement of calcium signalling dynamics and stability by delayed modulation of the plasma-membrane calcium-ATPase in human T cells, J. Physiol. 541 (2002) 877–894.
[15] A. Juška, P.C. Redondo, J.A. Rosado, G.M. Salido, Dynamics of calcium fluxes in human platelets assessed in calcium-free medium, Biochem. Biophys. Res. Commun. 334 (2005) 779–786.
[16] A. Juška, Dynamics of calcium fluxes in nonexcitable cells: mathematical modeling, J. Membr. Biol. 211 (2006) 89–99.
[17] M.D. Bootman, T.J. Collins, C.M. Peppiatt, L.S. Prothero, L. MacKenzie, P. De Smet, M. Travers, S.C. Tovey, J.T. Seo, M.J. Berridge, F. Ciccolini, P. Lipp, Calcium signaling – an overview, Semin. Cell Dev. Biol. 12 (2001) 3–10.
[18] A. Juška, Minimal models of multi-site ligand-binding kinetics, J. Theor. Biol. 255 (2008) 396–403.
[19] A. Juška, I. Jardin, J.A. Rosado, Physical properties of two types of calcium stores and SERCAs in human platelets, Mol. Cell. Biochem. 311 (2008) 9–18.
[20] A.B. Parekh, J.W. Putney Jr., Store-operated calcium channels, Physiol. Rev. 85 (2005) 757–810.
[21] J.A. Rosado, S.O. Sage, The actin cytoskeleton in store-mediated calcium entry, J. Physiol. 526 (2000) 221–229.
[22] M.D. Bootman, P. Lipp, M.J. Berridge, The organisation and functions of local Ca^{2+} signals, J. Cell Sci. 114 (2001) 2213–2222.
[23] A.R. Penheiter, Z. Bajzer, A.G. Filoteo, R. Thorogate, K. Torok, A.J. Caride, A model for the activation of plasma membrane calcium pump isoform 4b by calmodulin, Biochemistry 42 (2003) 12115–12124.
[24] C.K. Johnson, Calmodulin, conformational states, and calcium signaling. A single-molecule perspective, Biochemistry 45 (2006) 14233–14246.
[25] A.J. Caride, A.R. Penheiter, A.G. Filoteo, Z. Bajzer, Á. Enyedi, J.T. Penniston, The plasma membrane calcium pump displays memory of past calcium spikes. Differences between isoforms 2b and 4b, J. Biol. Chem. 276 (2001) 39797–39804.