Analysis of intracellular calcium concentration dynamics in human platelets following calcium influx from the stores or extra-cellular medium

A. Juška

Vilnius Gediminas Technical University, Saulėtekio al. 11, LT-2040 Vilnius, Lithuania

The dynamics of intracellular calcium concentration – a potential source of information about the mechanisms involved in calcium influx and efflux – has virtually never been analysed. In the present study, an attempt has been made to perform a formal analysis of the dynamics and to interpret the results of the analysis in terms of known or putative mechanisms responsible for changes in intracellular calcium concentration. The results of the analysis suggest that calcium influx and efflux are governed by stochastic processes of opening calcium influx channels in the plasma membrane or the activation of Ca^{++}-ATPase (macro)molecules and termination of the open state of the channels or the activity of the macromolecules. Calcium extrusion from the cells proceeds via two independent, spatially and temporally separated pathways.

Key words: calcium influx, calcium extrusion, mathematical models

INTRODUCTION

In platelets as in many other cells, calcium influx from the stores or extra-cellular medium proceeds via calcium channels in the cellular membranes. The opening of calcium channels can be stimulated by agonists; calcium efflux results from its extrusion by Ca^{++}-ATPase and (or) re-uptake into the stores [1]. Under experimental conditions, the net result of calcium influx and efflux is usually monitored as the change in fluorescence of a calcium-sensitive dye in response to various stimuli [1–3]. Analysis of the change dynamics – a potential source of information about the mechanisms involved in calcium influx and efflux – has virtually never been done. The aim of the present study, therefore, was to perform a formal analysis of the dynamics and to interpret the results of the analysis in terms of the known or putative mechanisms responsible for changes in intracellular calcium concentration.

METHODS

Experimental data used in this work are taken from publications of S. O. Sage and co-workers [2–3]. The methods of obtaining the data are described in the original papers. On the basis of generally accepted or postulated mechanisms of calcium influx and efflux, differential equations for calcium concentration are composed. These equations are solved in some cases, simplified solutions being provided in the others, resulting in quantitative or qualitative models of calcium concentration dynamics.

RESULTS AND DISCUSSION

The results of the analysis are presented in Figs. 1 and 2 and Table. It is evident from the inspection of the curves that they are non-linear, non-monotonic, and often bi-phasic (see also Figs. 1, 4, 6 and 7 in [2]). These dynamics are a cumulative result of several independent processes. In the first series of experiments, ionomycin was added to enable calcium efflux from the stores, together with thapsigargin to prevent its re-uptake back into the stores. Figure 1 illustrates the dynamics of intracellular calcium concentration as a result of its re-distribution between the cytosol and the stores (curve **a**) or redistribution and its loss due to calcium extrusion into the calcium-free medium (curve **b**). Calcium redistribution (curve **a**) is modelled by the following equation:

$$c_i = c_{i0}\,\mathrm{e}^{-\alpha t} + c_\infty\left(1-\mathrm{e}^{-\alpha t}\right), \qquad (1)$$

where α is the rate constant of calcium redistribution, c_{i0} is the initial and c_∞ is the final intracellular

calcium concentration. It can be seen that the rate calcium loss due to its extrusion is essentially non-monotonic. This is best interpreted as a net result of the activity of Ca^{++}-ATPase (macro)molecules whose entry into and exit from the active state are governed by simple random processes such as the Poisson processes, namely, the molecules are switched at random into the active (the mean lag being θ) state in which they spend a random period of time (the mean duration being τ). The rate of calcium extrusion, therefore can be expressed as

$$e = A\frac{\lambda+\mu}{\mu}\lambda e^{-\lambda t}\left(1-e^{-\mu t}\right), \qquad (2)$$

where $\lambda = 1/\tau$, $\mu = 1/\theta$ and A is extrusion efficacy. The net loss of calcium concentration due to its extrusion is shown in Fig. 1 d; it is subtracted from the calcium redistribution dynamics (curve **a**) to give curve **b**. This is not very accurate for the rising part of the curve, but qualitatively is quite reasonable for its declining part.

In the second series, thrombin was used to stimulate calcium influx from the stores (Fig. 2A) or from both the extra-cellular medium and the stores (Fig. 2B). In contrast to the first series, here the rate of increase in intracellular calcium concentration is greater, the increase lasting a very short period of time (note that the extracellular calcium concentration here is 1 mM). The rate of calcium extrusion into calcium-containing medium (Fig. 2B) is essentially bimodal; it is not bimodal in the case of calcium-free medium (Fig. 2A), in both cases, however, the dynamics of the rate can be seen to be made of two independent temporally separated components. That means, presumably, that two types of Ca^{++}-ATPase are involved in both cases. The first one (responsible for the pulse of calcium extrusion immediately following the stimulation by thrombin) is associated, presumably, with the plasma membrane, the second one being associated with the membrane of the stores.

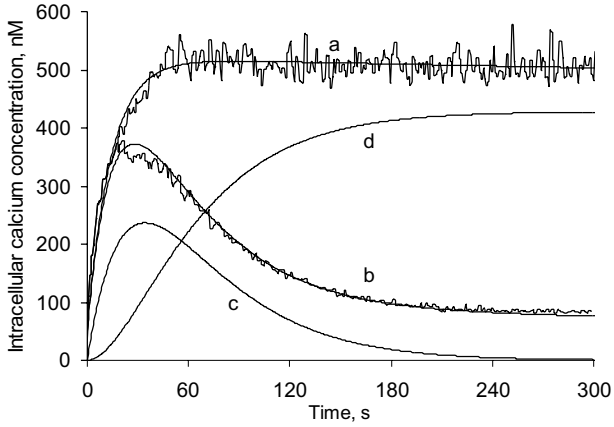

Fig. 1. Calcium redistribution in human platelets following its influx from the stores enabled by ionomycin (50 nM) (its re-uptake being prevented by thapsigargin (1 μM) and extrusion into the medium by $LaCl_3$ (curve a) and calcium redistribution with its extrusion (curve b). Smooth lines model the rate of calcium extrusion (c) and cumulative loss (d). Experimental data are taken from Fig. 1B [3] by copying the figure with Acrobat Reader from the PDF file and digitising the curves

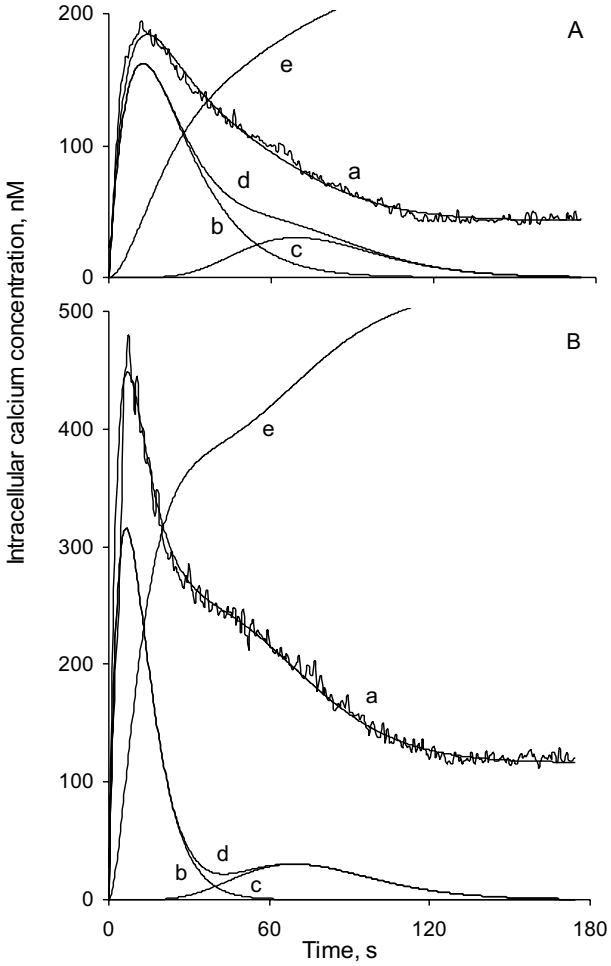

Fig. 2. Calcium redistribution in human platelets following its influx from the stores stimulated by thrombin (0.1 unit/ml), re-uptake into the stores and extrusion into calcium-free medium (A); the same, but calcium-containing (1 mM) medium (B). Smooth lines model the rate of calcium extrusion by two types of Ca^{++}-ATPase (b and c), the resulting rate of extrusion (d) and cumulative loss (e). Experimental data are taken from Fig. 1 [2] in the same way as above

Whereas the dynamics of calcium re-uptake remains the same both in the case of calcium-free and calcium containing medium, the dynamics of calcium extrusion differs considerably in all three cases (see Figs. 1, 2 and Table), the extrusion being faster (the mean duration of Ca^{++}-ATPase activity being shorter) in the case of calcium-containing me-

Table. **Time constants of calcium extrusion in human platelets**

Parameter	Notation	Estimate		
		Conditions*		
		1	2A	2B
Calcium extrusion pulse duration (s)	τ	40.0	13.3	6.7
Lag period of the extrusion pulse (s)	θ	100	100	100

* As specified in legends of Fig. 1 and Fig. 2.

dium and under stimulation of calcium entry by thrombin.

Received 10 December 2002
Accepted 18 April

References

1. Rink TJ, Sage SO. Annu Rev Physiol 1990; 52: 431–49.
2. Rosado JA, Sage SO. Biochem J 2000; 347(1): 183–92.
3. Rosado JA, Sage SO. Biol Chem 2000; 275: 19529–35.

A. Juška

KALCIO KONCENTRACIJOS KAITOS ŽMOGAUS KRAUJO PLOKŠTELĖSE ANALIZĖ

S a n t r a u k a

Kalcio koncentracijos kaita – potencialus informacijos apie kalcio patekimo į ląstelę ir pašalinimo iš jos būdus šaltinis – iš esmės yra nenagrinėta. Darbo tikslas – išanalizuoti šią kaitą ir išreikšti ją žinomų ar spėjamų mechanizmų, lemiančių kalcio judėjimą, sąvokomis. Analizės rezultatai rodo, kad kalcio patekimas į ląstelę ir pašalinimas iš jos valdomas stochastinių procesų: atsitiktinai atsidaro kalcio patekimo į ląstelę kanalai ar aktyvuojamos jį pašalinančios (makro)molekulės ir panašiai kanalai uždaromi ar sustabdomas kalcio šalinimas. Kai išorinėje terpėje yra kalcio, jo pašalinimas iš vidaus vyksta dviem skirtingais nepriklausomais keliais ne tuo pačiu metu.