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Computer simulation of a cytosolic calcium oscillator

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A new interpretation of existing data permits us to define a model capable of accounting for agonist-induced Ca^{2+} oscillations in the cytosol of electrically non-excitable cells. The model only requires one Ca^{2+} store, which contains Ca^{2+} channels controlled by inositol 1,4,5-trisphosphate and Ca^{2+}. Computer simulations may generate different experimentally observed patterns of Ca^{2+} oscillations.

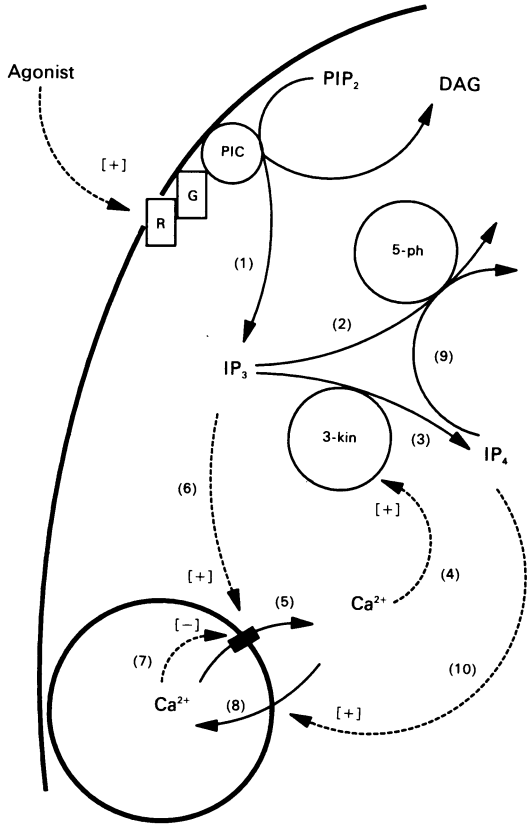
INTRODUCTION

Discharge of internal Ca^{2+} stores in the cytosol of electrically non-excitable cells can be triggered by an elevated level of intracellular inositol 1,4,5-trisphosphate (IP_3) (Berridge & Irvine, 1989). As observed in single-cell systems, a continuous agonist stimulation of IP_3 formation may produce periodic Ca^{2+} spikes with a frequency in the range of 1–10/min (Berridge & Galione, 1988). The oscillatory pattern of the Ca^{2+} response depends on the types of cell and agonist. For instance, in hepatocytes the frequency, but not the amplitude, is modulated by the vasopressin concentration (Woods *et al.*, 1986), whereas in pancreatic acinar cells the amplitude increases with cholecystokinin concentration (Tsunoda *et al.*, 1990).

Several models have been proposed to delineate feedback loops allowing Ca^{2+} oscillations (Berridge & Galione, 1988). However, the existence of a cytosolic Ca^{2+} oscillator has been theoretically demonstrated in only two models (Meyer & Stryer, 1988; Goldbeter *et al.*, 1990). Here another model, based on the interpretation of published experimental data, is analysed. Computer simulations demonstrate that this model can generate observed characteristics of the IP_3-induced Ca^{2+} response.

THEORY AND RESULTS

The proposed model defined in Scheme 1 is based on well-documented facts (Berridge & Irvine, 1989). The interaction of an agonist with its receptor (R in Scheme 1) stimulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by the G (guanine-nucleotide-regulatory)-protein (G in Scheme 1)-coupled phosphoinositidase C (PIC) and thus accelerates the formation of diacylglycerol (DAG) and IP_3 (reaction 1, Scheme 1). IP_3 is phosphorylated to inositol 1,3,4,5-tetrakisphosphate (IP_4) (reaction 3) by a 3-kinase (3-kin in Scheme 1), the $V_{max.}$ of which is increased by Ca^{2+} (reaction 4) (Takazawa *et al.*, 1989). Both IP_3 and IP_4 are dephosphorylated by a 5-phosphatase (5-ph in Scheme 1) (reactions 2 and 9). Only two pools of Ca^{2+} are considered, namely the cytosol and an IP_3-sensitive store. Indeed, in contrast with the other systems theoretically studied (Meyer & Stryer, 1988; Goldbeter *et al.*, 1990), our model will not require other sources of Ca^{2+} to account for Ca^{2+} oscillations. The identity of the IP_3-sensitive store is still uncertain, but, by definition, the membrane of such a vesicle contains IP_3 receptors which are linked to Ca^{2+} channels. The efflux of Ca^{2+} from the vesicle (reaction 5) is accelerated by IP_3 (reaction 6). On the other hand, the store is refilled by an ATP-driven Ca^{2+} pump (reaction 8).



Scheme 1. Definition of the model

Further abbreviations: R, receptor; G, G-protein; 3-kin, 3-kinase; 5-ph, 5-phosphatase. [+] and [−] stand for positive and negative controls respectively.

On the basis of this first description (i.e. ignoring for the time being reactions 7 and 10), the kinetic equations describing the time courses of free cytosolic concentrations of IP_3, IP_4 and Ca are as follows:

$$\frac{d[IP_3]}{dt} = v_1 - v_2 - v_3 f_4 \quad (1)$$

Abbreviations used: IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIC, phosphoinositidase C; G-protein, guanine-nucleotide-regulatory protein; IP_4, inositol 1,3,4,5-tetrakisphosphate; DAG, diacylglycerol; $[Ca^{2+}]_{tot.}$, total amount of Ca^{2+}; ISP_3, inositol trisphosphothioate; ED_{50}, effective dose producing half-maximal effect.

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$$\frac{d[IP_4]}{dt} = v_3 f_4 - v_9 \quad (2)$$

$$\frac{d[Ca^{2+}]}{dt} = v_5 f_6 - v_8 \quad (3)$$

v_i is the activity of an enzyme or channel involved in reaction i and acting on a certain component X (⟶ in Scheme 1). f_j is the fractional effect on reaction i of a component X due to the interaction described by reaction j (⇢ in Scheme 1). It is assumed that the reactions obey the Hill equation and thus are characterized by an activity

$$v = V/[1+(K/[X])^h]$$

or by an effect

$$f = 1/[1+(K/[X])^h]$$

where V, K and h are defined as maximal activity, effective dose producing half-maximal effect (ED_{50}) and Hill coefficient respectively. The model assumes that the total amount of Ca^{2+} ($[Ca^{2+}]_{tot.}$) is constant and is distributed between the cytosolic pool and the IP_3-sensitive stores. Theoretical analysis shows that this model cannot generate Ca^{2+} oscillations, despite the existence of the Ca^{2+} feedback loop (reaction 4). Only stable stationary states are obtained for any value of the kinetic parameters.

Theoretical studies on the nature and multiplicity of stationary states came to the conclusion that stable oscillations may be produced by the adequate combination of a negative feedback and an autocatalytic process (Tyson, 1975). Now we will show that an autocatalytic process may be defined on the basis of the interpretation of published observations. Joseph *et al.* (1989) reported that the addition of Ca^{2+} to a cerebellum microsomal preparation diminished both Ca^{2+} efflux and IP_3 binding to the receptors. They suggested that cytosolic Ca^{2+} may contribute to Ca^{2+} oscillations by inhibiting the IP_3-induced Ca^{2+} efflux, allowing the refilling of the stores. However, this interpretation is not supported by the mathematical analysis of the model, which again presents stable steady states (simulation not shown). Now it was shown in the same study that the uptake of Ca^{2+} by the vesicles is directly related to the external Ca^{2+} concentration. Thus another interpretation of the data is that Ca^{2+} efflux is inhibited by intra- rather than extra-vesicular Ca^{2+}, even in the submicromolar range, since this efflux must be corrected for the variable calcium load of the vesicle. This interpretation is supported by the study of Willems *et al.* (1990), which showed that the IP_3-induced Ca^{2+} efflux from vesicles preloaded with submicromolar concentrations of Ca^{2+} does not depend on the extravesicular Ca^{2+} concentration in the submicromolar range. This proposed negative control of calcium efflux (reaction 7 in Scheme 1) may be viewed as an autocatalytic process, since a decrease of Ca^{2+} concentration in the vesicle, and consequently an increase of free Ca^{2+} concentration, favours the Ca^{2+} efflux.

On the basis of this description, eqn. (3) becomes:

$$\frac{d[Ca^{2+}]}{dt} = v_5 f_6 / f_7 - v_8 \quad (3')$$

Even in the absence of any co-operativity in the considered reactions (i.e. all the Hill coefficients are equal to 1), the model may generate Ca^{2+} oscillations (Fig. 1), at least if the kinetics of Ca^{2+} movements across the vesicle membrane are faster than the kinetics of IP_3 metabolism. Sustained oscillations only appear at IP_3 production rates (v_1) between two critical values. When Ca^{2+} movements are relatively slow, the oscillations exhibit a sinusoidal shape, with a period not obviously dependent on v_1 (Figs. 1*a*–1*c*). By contrast, the peak and baseline of the oscillation are both modulated by v_1. Fig. 1(*c*) is reminiscent of the Ca^{2+} response observed with acinar pancreatic cells in the presence of 0.1 μM-acetylcholine (Osipchuk *et al.*, 1990). If Ca^{2+} movements are relatively faster, the amplitude becomes independent of v_1, but an enlargement of the peak showing an unrealistic shape is observed (not shown). Thus the proposed model contains the required cross-coupling between IP_3 and Ca^{2+} to account for a cytosolic Ca^{2+} oscillator, but cannot generate certain oscillatory patterns which are experimentally observed.

It has been repeatedly reported that each cell of the same population is characterized by a unique and reproductible pattern of oscillations described by their amplitude, frequency and shape (Kawanishi *et al.*, 1989; Rooney *et al.*, 1989; Tsunoda *et al.*, 1990). This observed variability is probably a consequence of a high sensitivity of the Ca^{2+} response to intercellular variations of the value of kinetic parameters, which could be due to different cellular contents of certain effectors. Such a high sensitivity is present in control processes exhibiting positive co-operativity. It has to be noted that positive co-operativity has been introduced in both models previously proposed to account for Ca^{2+} oscillations (Meyer & Stryer, 1988; Goldbeter *et al.*, 1990). Interestingly, both IP_3 binding to the Ca^{2+}-channel-linked receptor and Ca^{2+} efflux exhibit a positively co-operative dependence on IP_3 concentration (Joseph *et al.*, 1989; Willems *et al.*, 1989; Meyer *et al.*, 1990). The Hill coefficients deduced from these data are close to 2. [Incidentally, the Hill coefficient of 4 reported by Meyer *et al.* (1990) was not obtained by means of the Hill equation.] We have thus investigated how the oscillatory pattern is modulated by the introduction of positive co-operativity in different reactions of the model. In summary, positively co-operative inhibition of Ca^{2+} flux by intravesicular Ca^{2+} leads to an abrupt switch between a phase of slow Ca^{2+} increase and the sharp Ca^{2+} spike. The dependence of oscillation frequency on IP_3 production rate

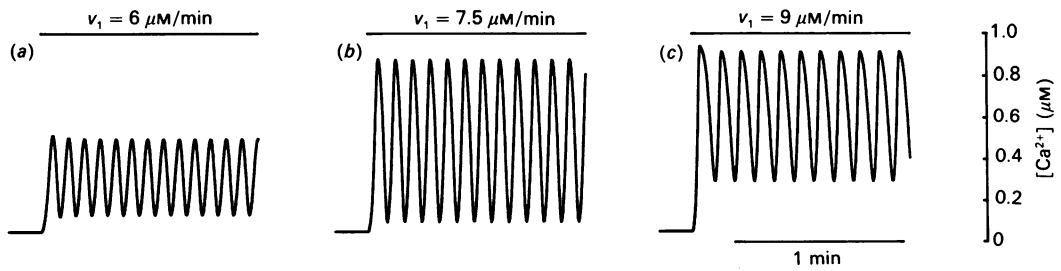

Fig. 1. Oscillatory patterns of cytosolic Ca^{2+} obtained with the model defined by eqns. (1) and (3′)

The kinetic equations were numerically integrated (fourth-order Runge–Kutta method with a step size of 0.001 min) using the following parameter values: $[Ca^{2+}]_{tot.} = 1$ μM; basal $v_1 = 1.75$ μM/min; $v_2 = 37.5$ μM/min; $K_2 = 25$ μM; $v_3 = 45$ μM/min; $K_3 = 0.6$ μM; $K_4 = 1$ μM; $v_5 = 1200$ μM/min; $K_5 = 0.01$ μM; $K_6 = 1.5$ μM; $K_7 = 5$ μM; $v_8 = 300$ μM/min; $K_8 = 0.01$ μM. All the Hill coefficients (h_i) are equal to 1. The rate of IP_3 production was set to three different stimulated levels (*a*–*c*) during the indicated period.

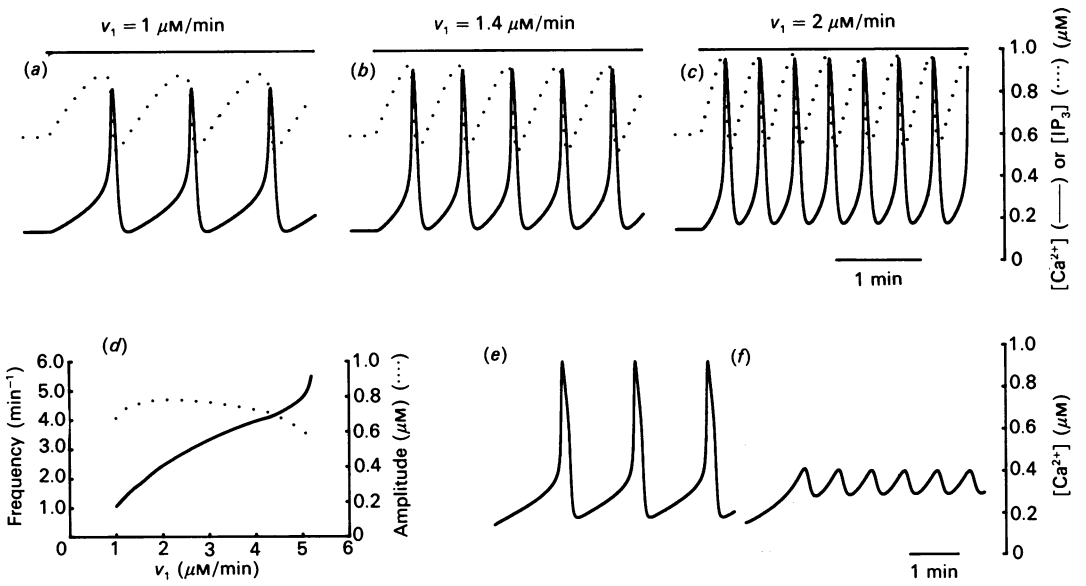

Fig. 2. Oscillatory patterns of cytosolic Ca^{2+} and IP_3 obtained with the model defined by eqns. (1) and (3′)

The kinetic equations were numerically integrated (fourth-order Runge–Kutta method with a step size of 0.002 min) using the following parameter values: $[Ca^{2+}]_{tot.}$ = 1 μM; basal v_1 = 0.36 μM/min; v_2 = 15 μM/min; K_2 = 25 μM; v_3 = 60 μM/min; K_3 = 1 μM; h_4 = 4; v_5 = 240 μM/min; K_5 = 0.01 μM; K_6 = 1.5 μM; h_6 = 2; K_7 = 0.5 μM; h_7 = 4; v_8 = 60 μM/min; K_8 = 0.6 μM; h_8 = 2. All the other Hill coefficients (h_i) are equal to 1. The rate of IP_3 production was set to three different stimulated levels (*a–c*) during the indicated period. (*d*) Frequency and amplitude of the Ca^{2+} oscillations as functions of v_1. (*e*) Simulation of the ISP_3 action in the basal condition (v_1 = 0.36 μM/min), assuming that ISP_3 (0.6 μM) activates the Ca^{2+} channel with the same ED_{50} as IP_3. (*f*) Same as (*e*), except that ISP_3 does not activate the Ca^{2+} channel, but completely inhibits the 5-phosphatase (v_2 = 0 μM/min).

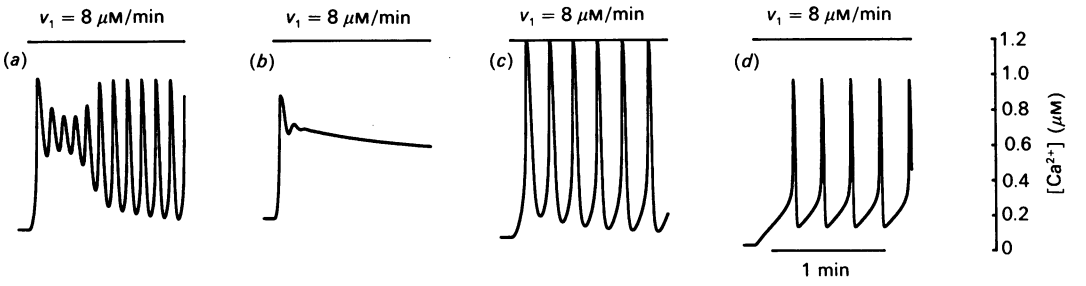

Fig. 3. Oscillatory patterns of cytosolic Ca^{2+} obtained with the model defined by eqns. (1) and (3″)

The kinetic equations were numerically integrated (fourth-order Runge–Kutta method with a step size of 0.0001 min) using the following parameter values: $[Ca^{2+}]_{tot.}$ = 1 μM, basal v_1 = 1 μM/min; v_2 = 75 μM/min; K_2 = 25 μM; v_3 = 500 μM/min; K_3 = 5 μM; K_4 = 1 μM; h_4 = 4; v_5 = 1200 μM/min; K_5 = 0.01 μM; K_6 = 2.5 μM; h_6 = 2; K_7 = 0.5 μM; h_7 = 4; v_8 = 300 μM/min; K_8 = 0.5 μM; h_8 = 2; v_9 = 7.5 μM/min; K_9 = 5 μM; K_{10} = 10 μM; h_{10} = 2; b_{10} = 0.1. All the other Hill coefficients (h_i) are equal to 1. (*a*) The rate of IP_3 production was set to 8 μM/min during the indicated period. (*b–d*) Same as (*a*), except that $[Ca^{2+}]_{tot.}$ = 0.9 μM (*b*), $[Ca^{2+}]_{tot.}$ = 1.2 μM (*c*), and b_{10} = 1 (*d*).

without enlargement of the peak can be obtained if the activation of 3-kinase by the calcium–calmodulin complex is positively co-operative. Finally, the basal concentration of Ca^{2+} is more easily controlled if the activity of the ATP-driven pump exhibits positive co-operativity, as experimentally observed in sarcoplasmic-reticulum vesicles (Inesi *et al.*, 1980). Positive co-operativity has been simultaneously introduced in reactions 4, 6, 7 and 8 of the model (Scheme 1). The increase of v_1 within the range of values for which Ca^{2+} oscillations are observed leads to a marked increase of frequency and to moderate changes of amplitude (Figs. 2*a–d*). Fig. 2 also shows IP_3 oscillations, which occur because of the cross-coupling between Ca^{2+} and IP_3. Wakui *et al.* (1989) recently reported that the injection of the non-metabolizable IP_3 analogue inositol trisphosphothioate (ISP_3) in a single cell may evoke regular spikes of Ca^{2+}, suggesting that Ca^{2+} oscillation does not depend on fluctuations in IP_3 concentration. However, this interpretation does not consider the possibility that, although the concentration of ISP_3 is constant, endogenous IP_3 may accumulate and oscillate because ISP_3 is a potent inhibitor of 5-phosphatase (Cooke *et al.*, 1989), but not of 3-kinase (Taylor *et al.*, 1989). This interpretation is supported by simulation results, showing the oscillatory patterns obtained in the presence of ISP_3 and with a basal rate of IP_3 production. In Fig. 2(*e*), ISP_3 was assumed to activate the Ca^{2+} channel without any significant inhibition of 5-phosphatase, whereas, in Fig. 2(*f*), ISP_3 was not capable of activating the Ca^{2+} channel but was considered as a full inhibitor of the 5-phosphatase.

Recently, Hill & Boynton (1990) showed that the rate of Ca^{2+} sequestration in IP_3-sensitive stores is stimulated by IP_4. This control is introduced in the model by replacing eqn. (3′) by:

$$\frac{d[Ca^{2+}]}{dt} = v_5 f_6/f_7 - v_8(f_{10}+b_{10}) \qquad (3'')$$

where b_{10} is the IP_4-independent contribution of the ATP-driven Ca^{2+} pump. Fig. 3(*a*) shows that this model may generate a first

high transient Ca^{2+} peak followed by oscillations with smaller, but increasing, amplitudes. This is due to the delayed accumulation if IP_4, allowing a first complete discharge of Ca^{2+} because of the low rate of Ca^{2+} re-uptake. Such a pattern resembles the Ca^{2+} oscillations induced by phenylephrine in hepatocytes (Kawanishi *et al.*, 1989). Variability of intercellular oscillatory patterns have been tentatively simulated by varying some parameters of the model. A slight decrease (Fig. 3*b*) or increase (Fig. 3*c*) of the cellular Ca^{2+} content may cause obvious modifications in the Ca^{2+} response. Fig. 3(*d*) shows the pattern when the IP_4-independent activity of the Ca^{2+} pump is increased.

DISCUSSION

So far, only two models have been demonstrated to contain a cytosolic Ca^{2+} oscillator. In the model of Meyer & Stryer (1988), Ca^{2+} oscillations are essentially the consequence of PIC activation by free Ca^{2+} and of the existence of an infinite Ca^{2+} reservoir insensitive to IP_3. However, numerical simulations may show that the baseline of the Ca^{2+} spikes is lower than the basal Ca^{2+} concentration obtained in the absence of extracellular stimulus. Moreover, above a certain stimulation level, the oscillations merge into a stationary state equal to the basal level. Thus very low and very high stimulation levels lead to identical stable Ca^{2+} concentrations, whereas for intermediate stimulation levels Ca^{2+} concentrations oscillate around the basal concentration. In this respect the model of Goldbeter *et al.* (1990) generates more realistic patterns of Ca^{2+} oscillations. The key feature of this latter model is the existence of an infinite extracellular Ca^{2+} reservoir and of a Ca^{2+}-induced Ca^{2+} release from IP_3-insensitive stores. Thus this model cannot describe those systems which exhibit Ca^{2+} oscillations in the absence of extracellular Ca^{2+} (Yule & Gallacher, 1988; Tsunoda *et al.*, 1990). Interestingly, it seems that Ca^{2+} would have opposite actions on its release from stores sensitive or insensitive to IP_3 respectively. As proposed in our model, the negative control of Ca^{2+} efflux from IP_3-sensitive stores would be better explained by the inhibiting action of intravesicular Ca^{2+}. This hypothesis seems to be also supported by the observed delayed action of Ca^{2+} injection in oocytes on IP_3-induced membrane currents (Parker & Ivorra, 1990). This delay could be accounted for by the kinetics of Ca^{2+} uptake by the vesicle. However, direct experimental evidence in favour of the proposed mode of Ca^{2+} action is still lacking.

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