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Latency correlates with period in a model for signal-induced Ca^{2+} oscillations based on Ca^{2+}-induced Ca^{2+} release

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Oscillations in cytosolic Ca^{2+} develop in a variety of cells after an induction phase, called *latency*, the duration of which depends on the magnitude of external stimulation. Experiments in hepatocytes indicate that the period and latency of Ca^{2+} oscillations both decrease as the level of the stimulus increases. We analyze the correlation between period and latency in a model recently proposed for signal-induced Ca^{2+} oscillations. We show that the linear relationship between period and latency observed in the experiments arises naturally in this model as a result of the mechanism of Ca^{2+}-induced Ca^{2+} release on which it is based.

Introduction

Oscillations of intracellular Ca^{2+} occur in a wide variety of cells, either spontaneously or as a result of stimulation by a hormone or neurotransmitter, with periods generally ranging from seconds to minutes (for recent reviews, see Berridge *et al.*, 1988; Berridge and Galione, 1988; Berridge, 1990; Cuthbertson, 1989; Rink and Jacob, 1989; Jacob, 1990a). The effect of external signals appears to be mediated by inositol-1,4,5-trisphosphate ($InsP_3$) (Berridge and Irvine, 1989). The origin of repetitive Ca^{2+} transients has been discussed in terms of various mechanisms such as feedback regulation of $InsP_3$ production (Woods *et al.*, 1987; Berridge *et al.*, 1988; Cuthbertson, 1989) or Ca^{2+} cycling between the cytosol and an $InsP_3$-insensitive Ca^{2+} pool (Berridge, 1988, 1990; Berridge *et al.*, 1988; Berridge and Galione, 1988; Berridge and Irvine, 1989).

Theoretical models proposed for signal-induced Ca^{2+} oscillations rely either on the cross-activation of $InsP_3$ synthesis and cytosolic Ca^{2+} mobilization (Meyer and Stryer, 1988) or on the self-amplified process of Ca^{2+}-induced Ca^{2+} release. The latter phenomenon, observed in excitable as well as nonexcitable cells (Endo *et al.*, 1970; Fabiato and Fabiato, 1975; Busa *et al.*, 1985; Osipchuk *et al.*, 1990), is at the core of a model proposed by Kuba and Takeshita (1981) for Ca^{2+} oscillations induced by caffeine in sympathetic neurons, and of a more general model for Ca^{2+} oscillations that takes into account the triggering role of $InsP_3$ (Dupont and Goldbeter, 1989; Goldbeter *et al.*, 1990). The latter, minimal model predicts that Ca^{2+} oscillations can occur in the absence of a concomitant, periodic variation in $InsP_3$, in agreement with a number of experimental observations (Capiod *et al.*, 1987; Wakui *et al.*, 1989; Osipchuk *et al.*, 1990).

Previous analysis of the minimal model based on Ca^{2+}-induced Ca^{2+} release has shown how this mechanism accounts for the observation that the frequency of Ca^{2+} transients increases with the magnitude of external stimulation. Building on previous work by Woods *et al.* (1987), Rooney *et al.* (1989) have shown, moreover, that in hepatocytes the period of Ca^{2+} oscillations correlates with the time required for observing the first peak in Ca^{2+} after the onset of stimulation. Specifically, this time interval, called *latency*, increases in a roughly linear manner with the period of Ca^{2+} oscillations as stimulation decreases. Given that this observation brings further insight into the mechanism of signal-induced Ca^{2+} mobilization and provides an additional test for any theoretical explanation of the oscillatory phenomenon, we examine here the relationship between period and latency of Ca^{2+} transients in the model based on Ca^{2+}-induced Ca^{2+} release. We show that the existence of an approximately linear correlation between

period and latency is a natural consequence of this mechanism of Ca^{2+} oscillations.

Minimal model for signal-induced Ca^{2+} oscillations based on Ca^{2+}-induced Ca^{2+} release

The model considered (Dupont and Goldbeter, 1989; Goldbeter *et al.*, 1990), schematized in Figure 1, relies on the hypothesis (Berridge and Galione, 1988; Berridge and Irvine, 1989) that an external stimulus triggers the synthesis of a certain amount of $InsP_3$ that induces the release of Ca^{2+} from an $InsP_3$-sensitive pool; the amount of Ca^{2+} thus released is controlled by the level of the stimulus through modulation of the saturation function (β) of the $InsP_3$ receptor. It is assumed that the Ca^{2+} concentration in the $InsP_3$-sensitive pool remains constant, owing to fast replenishment that could involve regulation of the uptake of external Ca^{2+}, as proposed in the capacitative model of Ca^{2+} entry (Putney, 1986; Berridge, 1990). Cytosolic Ca^{2+} is pumped into an $InsP_3$-insensitive compartment; Ca^{2+} in this compartment is released into the cytosol in a process activated by cytosolic Ca^{2+}. This model is minimal because it contains only two variables, the time evolution of which is governed by the following kinetic equations (Dupont and Goldbeter, 1989; Goldbeter *et al.*, 1990)

$$\frac{dZ}{dt} = v_0 + v_1\beta - v_2 + v_3 + k_f Y - kZ$$

$$\frac{dY}{dt} = v_2 - v_3 - k_f Y \qquad (1)$$

with

$$v_2 = V_{M2}\frac{Z^n}{K_2^n + Z^n}$$

$$v_3 = V_{M3}\frac{Y^m}{K_R^m + Y^m}\cdot\frac{Z^p}{K_A^p + Z^p} \qquad (2)$$

In these equations, Z and Y denote the concentration of free Ca^{2+} in the cytosol and in the $InsP_3$-insensitive pool; v_0 refers to a constant input of Ca^{2+} from the extracellular medium; $v_1\beta$ denotes the $InsP_3$-modulated input from the $InsP_3$-sensitive store. The rates v_2 and v_3 refer, respectively, to the pumping of Ca^{2+} into the $InsP_3$-insensitive store and to the release of Ca^{2+} from that store into the cytosol in a process activated by cytosolic Ca^{2+}; V_{M2} and V_{M3} denote the maximum values of these rates. Parameters K_2, K_R, and K_A are threshold constants for pumping, release, and activation; k_f is a rate constant measuring the passive, linear leak of Y into Z; k relates to the assumed linear transport of cytosolic Ca^{2+} into the extracellular medium. The equations in *2* allow for cooperativity in pumping, release, and activation; n, m, and p denote the Hill coefficients characterizing these processes. In the above equations, all parameters and concentrations are defined with respect to the total cell volume.

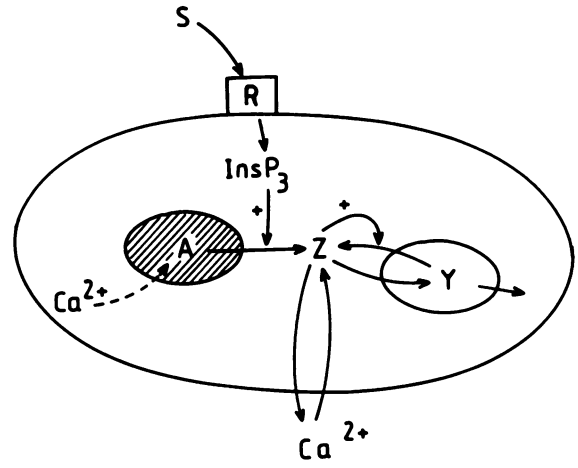

***Figure 1.* Minimal model for signal-induced Ca^{2+} oscillations based on the self-amplified release of Ca^{2+} from intracellular stores.** The external signal (S) binds to a membrane receptor (R) and thereby triggers the synthesis of $InsP_3$; the latter messenger elicits the release of Ca^{2+} from an $InsP_3$-sensitive store (▨), the Ca^{2+} content (A) of which is then assumed to produce a constant, net input of cytosolic Ca^{2+} (Z), proportional to the saturation function of the $InsP_3$-receptor (β). Cytosolic Ca^{2+} is pumped into an $InsP_3$-insensitive store; Ca^{2+} in this store (Y) is transported into the cytosol in a process activated by cytosolic Ca^{2+}. Other solid arrows refer to the passive leak of Y into Z and to calcium influx into and extrusion from the cell. The dashed arrow refers to replenishment of the $InsP_3$-sensitive Ca^{2+} pool, the content of which is assumed to remain constant (see Goldbeter *et al.*, 1990 for further details).

Not considered in the model is the fact that Ca^{2+} oscillations are often associated with Ca^{2+} propagating waves, as observed in fertilized eggs (Gilkey *et al.*, 1978; Busa and Nuccitelli, 1985) and some other cells (Cornell-Bell *et al.*, 1990; Jacob, 1990c; Rooney *et al.*, 1990; Takamatsu and Wier, 1990). Much like the oscillations, the wavelike phenomenon has been attributed to the propagation of the Ca^{2+} spike through Ca^{2+}-induced Ca^{2+} release (Jaffe, 1983; Busa and Nuccitelli, 1985; Berridge, 1990; Takamatsu and Wier, 1990). Incorporation of Ca^{2+} diffusion into Eq. *2*, together with a spatial distribution of Ca^{2+} intracellular pools, should produce Ca^{2+} waves, as shown by theoretical

studies based on empirical representations of Ca^{2+}-induced Ca^{2+} release for fertilized eggs (Cheer *et al.,* 1987) and cardiac cells (Backx *et al.,* 1989). The spatial aspects of Ca^{2+} signaling have not been considered in the present study, given that latency and period can be regarded as local properties of the oscillations in a particular region of the cell.

Previous analysis of Eq. *2* has shown (Dupont and Goldbeter, 1989; Goldbeter *et al.,* 1990) that parameter β governs the dynamic behavior in response to stimulation: this parameter indeed measures saturation of the $InsP_3$ receptor, which rises with the level of the stimulus. At low values of β, the steady state is stable and corresponds to a low level of cytosolic Ca^{2+}. Then, as β increases, the steady state becomes unstable and oscillations occur, with a period that decreases as β further augments. Above a second critical value of β, oscillations disappear as the system reaches a stable steady state characterized by a high level of cytosolic Ca^{2+}. Such qualitative behavior is in agreement with experimental observations on signal-induced Ca^{2+} oscillations in a variety of cells (Berridge *et al.,* 1988; Berridge and Galione, 1988; Jacob *et al.,* 1988; Cuthbertson, 1989; Harootunian *et al.,* 1989; Rink and Jacob, 1989; Rooney *et al.,* 1989).

For a given set of parameter values, Ca^{2+} oscillations thus occur whenever parameter β lies in a range bounded by two critical values, i.e., when Condition *3* holds

$$\beta_{1c} < \beta < \beta_{2c} \qquad (3)$$

The critical values β_{1c} and β_{2c} depend on other parameters and, in particular, on the choice of v_0 and v_1.

In fact, the parameter that actually controls the oscillations is the sum $v_0 + v_1\beta$: oscillations can thus result either from an increase in stimulation leading to enhanced Ca^{2+} release from the $InsP_3$-sensitive store ($v_1\beta$) or from an increase in the influx of extracellular Ca^{2+} (v_0) (Goldbeter *et al.,* 1990). We shall focus here on the first situation, i.e., the induction of oscillations by a signal-induced rise in $InsP_3$ corresponding to an increase in β.

Initiation of Ca^{2+} oscillations and latency after stimulation

As soon as β is raised from a low initial value corresponding to a stable steady state up to a value in the range defined by Condition *3,* sustained Ca^{2+} oscillations develop. To determine the effect of a rise in β on the time required for inducing oscillations, we consider two situations: one in which the rise in β is so fast that it corresponds to a step increase up to a final value β_f (Figure 2A), and one in which β increases up to β_f according to Eq. *4* with a characteristic time t_c, to account for an exponential rise in $InsP_3$ (Miledi and Parker, 1989) (Figure 2B)

$$\beta = \beta_f(1 - e^{-t/t_c}) \qquad (4)$$

Although there could be a decline in the level of $InsP_3$ (and hence in β) in the continuous presence of the stimulus, owing to some form of desensitization, such a biphasic time course is not likely to occur at moderate levels of stimulation, which are known to produce oscillations. Moreover, oscillations have been shown to occur under continuous perfusion with $InsP_3$ or with a nonmetabolizable analogue (Capiod *et al.,* 1987; Wakui *et al.,* 1989). Because we are concerned with the first peaks of oscillations, it seems appropriate to consider the simplest situation where β is increased in a stepwise or exponential manner up to a higher, constant level. A significant decline in β in the course of time would result in a progressive increase in the period of Ca^{2+} oscillations and might eventually lead to their disappearance.

We define latency, L, as the time needed to reach the first Ca^{2+} spike after the onset of the increase in β (see Figure 2, A and B). A progressive increase in stimulation, measured by the final value β_f, results in a decrease in latency toward a plateau value. This is shown in Figure 2C for the case of a step increase in β and in Figure 2D for the situation where β increases exponentially for three different values of the rise time t_c. The results in both cases agree with experimental observations showing that latency declines toward a constant minimal value as agonist concentration increases in blood platelets (Sage and Rink, 1987), blowfly salivary gland (Berridge *et al.,* 1988), adrenal glomerulosa cells (Quinn *et al.,* 1988), and hepatocytes (Rooney *et al.,* 1989).

The step increase is in fact the limiting case where the exponential rise in β is infinitely rapid ($t_c \rightarrow 0$) (The curve in Figure 2C, however, is not the limit of the curves in Figure 2D, owing to the use of different values for v_0 and v_1.) The approximation of a step increase in β should be appropriate when the synthesis of $InsP_3$ is a fast process, as is often observed (see, e.g., Downes *et al.,* 1989). For very large values of t_c, latency will largely result from the time required to reach

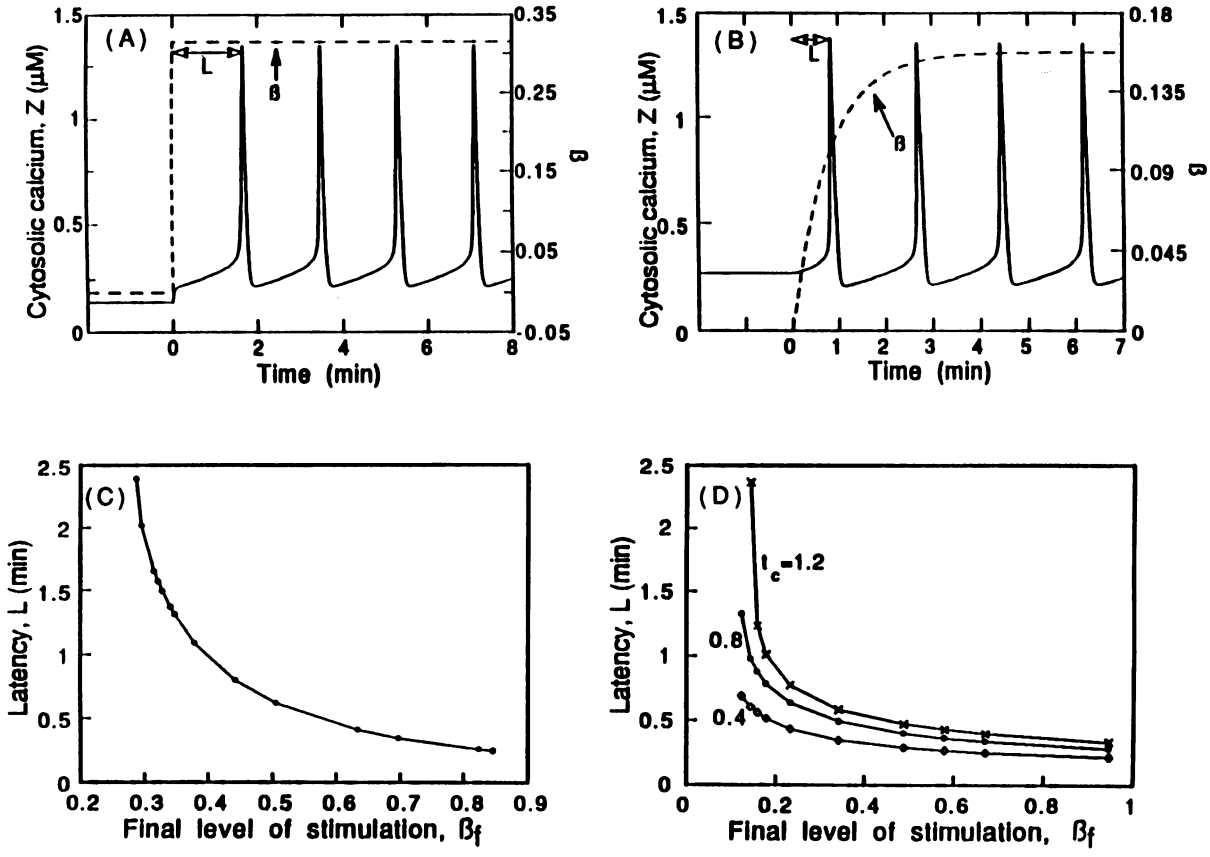

***Figure 2.* Initiation of Ca^{2+} oscillations and latency after stimulation.** The upper panels (A and B) show the development of oscillations in cytosolic Ca^{2+} (Z, ——) when parameter β (– – –), which measures the extent of stimulation, is increased in a quasi-instantaneous (A) or exponential (B) manner from zero up to the final values 0.315 and 0.157, respectively; these values of β_f are in the oscillatory range defined by Condition *3*. The critical range of β for oscillations is 0.286–0.846 for A and 0.122–0.944 for B. The time evolution of Z is obtained by numerical integration of Eqs. *1* and *2*. Latency, L, is defined as the time between the onset of stimulation at time 0 and the first peak in Z. Lower panels (C and D) show the variation of latency as a function of the final values β_f in situations corresponding to A and B, respectively. Points denote results of numerical simulations. The curves in D are established for three different values of the characteristic time t_c (in minutes) for the exponential increase in β given by Eq. *4*. The curve in B is obtained for t_c = 0.8 min. Parameter values are V_{M2} = 65 $\mu M \cdot min^{-1}$, V_{M3} = 500 $\mu M \cdot min^{-1}$, K_2 = 1 μM, K_R = 2 μM, K_A = 0.9 μM, m = n = 2, p = 4, k_f = 1 min^{-1}, and k = 10 min^{-1}. Moreover, v_0 = 1.4 $\mu M \cdot min^{-1}$ and v_1 = 6 $\mu M \cdot min^{-1}$ for A and C, whereas those parameters are, respectively, equal to 2.7 $\mu M \cdot min^{-1}$ and 4 $\mu M \cdot min^{-1}$ for B and D.

the value of β leading to oscillations. Such a situation is not considered in the subsequent simulations, because we wish to determine whether the experimentally observed relationship between latency and period of Ca^{2+} oscillations can result from the mechanism of Ca^{2+}-induced Ca^{2+} release.

The possibility that latency originates primarily from a delay in $InsP_3$ synthesis has been considered by Miledi and Parker (1989) and by Marty *et al.* (1989; see also Neher *et al.*, 1988), who showed theoretically that such a mechanism can account for the linear relationship between latency and the inverse of the agonist concentration in cells responding to muscarinic stimulation.

The values of the parameters in Figure 2 were taken so as to obtain periods of the order of minutes, as in the experiments of Rooney *et al.* (1989). Another choice of parameter values yields periods in the range of seconds (Dupont and Goldbeter, 1989; Goldbeter *et al.*, 1990), but the relationship between period and latency, to be described below, remains unchanged.

Correlation of latency with period of Ca^{2+} oscillations

We have previously shown (Dupont and Goldbeter, 1989; Goldbeter *et al.*, 1990) that the pe-

riod of Ca^{2+} oscillations diminishes when the extent of stimulation measured by β increases. To compare the predictions of the model with the experimental results (see Figure 4 in Rooney *et al.*, 1989), we have plotted period versus latency for different final values of β. Such theoretical curves are shown in Figure 3 in the cases where β increases in a stepwise manner (A) or exponentially (B). In both conditions, period correlates with latency in a manner that is, to a good approximation, linear. The slope of the period-versus-latency line in Figure 3, A and B, is close to unity, whereas the ordinate at the origin is close to the smallest value obtained for the period. These results are in good agreement with the experimental observations, which also indicate an approximately linear relationship between period and latency, with a similar slope (Rooney *et al.*, 1989).

The comparison of Figure 3, A and B, indicates that the linear fit is better for the stepwise increase in β. When there is delay in $InsP_3$ synthesis, a slight curvature is indeed apparent at low stimulation levels (Figure 3B). Deciding which one of the two curves better fits the results of Rooney *et al.* (1989) is difficult because of the scattering of the experimental points.

The slope of the T-versus-L line predicted by the model markedly depends on the values of parameters such as v_0 and v_1, which govern the constant input of Ca^{2+} from the extracellular medium into the cell and the influx of Ca^{2+} ($v_1\beta$) from the $InsP_3$-sensitive store into the cytosol, respectively. In some conditions, the linear relationship between L and T ceases to hold. Thus, when the Ca^{2+} influx from the extracellular medium is so large that a slight increase in v_0 could destabilize the steady state and induce oscillations even in the absence of external signal, the initial content of the Ca^{2+}-sensitive pool (Y) is relatively high, and latency remains negligible at all values β_f established on stimulation. The decrease in latency at higher levels of extracellular Ca^{2+} (corresponding in the model to higher values of v_0) is also observed in the experiments (Sage and Rink, 1987; Rooney *et al.*, 1989).

Conversely, at very low values of v_0, latency becomes so large that it can exceed the period of Ca^{2+} oscillations. An example of such a situation is shown in Figure 4. Besides a possible delay due to $InsP_3$ accumulation, such a phenomenon could explain the very long latencies observed in some experiments, e.g., in adrenal glomerulosa cells (Quinn *et al.*, 1988) and *Xenopus* oocytes (Parker *et al.*, 1987; Miledi and Parker, 1989).

The slope of the T-versus-L line also changes with the value of the Hill coefficients n, m, and p, which measure the degree of cooperativity of Ca^{2+} pumping, release, and activation. Thus, oscillations do occur for m = n = p = 1 (Goldbeter and Dupont, 1990), but then the slope of the line and its ordinate at the origin do not match well the values obtained in the experiments.

To gain insight into the observed dependence of latency and period on the magnitude of external stimulation, it is useful to inspect the time course of the two variables, namely, the con-

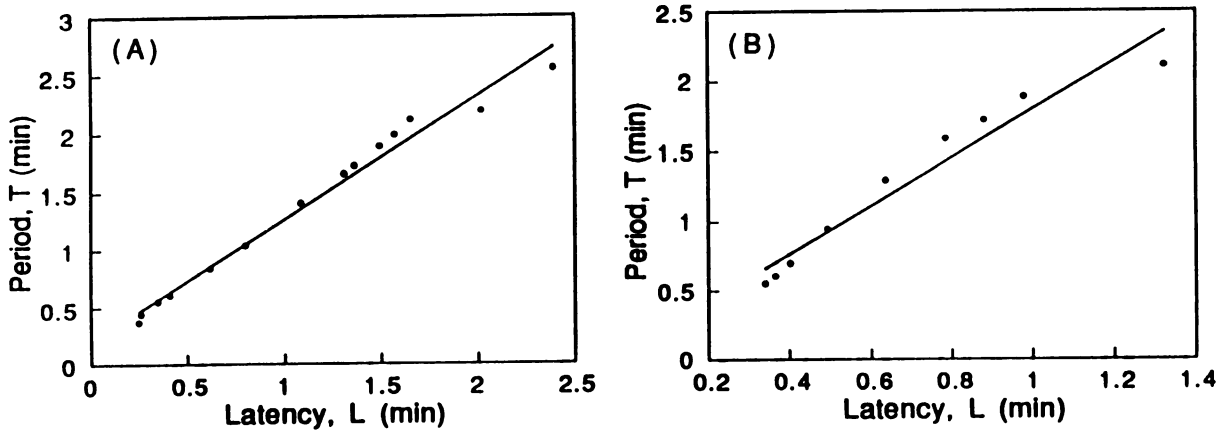

***Figure 3.* Correlation of latency with period of Ca^{2+} oscillations.** The approximately linear relationship is obtained, as indicated in Figure 2, by determining the latency and the period at different final values of the stimulus, measured by β_f, in the case of a quasi-instantaneous (A) or exponential (B) increase in β. The slope of the T-versus-L line drawn through the points obtained is equal to 1.06 in A and 1.72 in B. Parameter values for A and B are the same as in Figure 2, A and B, respectively.

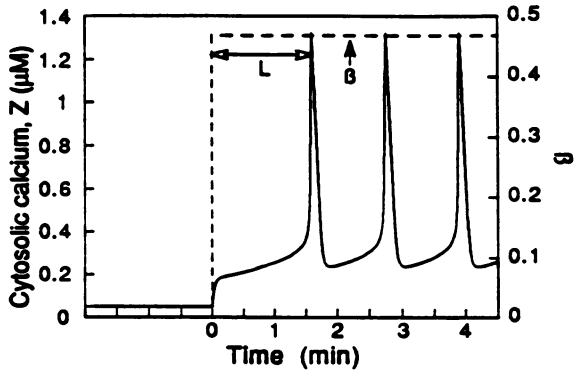

Figure 4. **Illustration of a case where latency exceeds the period of Ca^{2+} oscillations at very low values of the Ca^{2+} input from the extracellular medium, v_0.** The situation considered is that of Figure 2A, with $v_0 = 0.5\ \mu M \cdot min^{-1}$, $v_1 = 7\ \mu M \cdot min^{-1}$, and $\beta_f = 0.468$. The steady-state level of cytosolic Ca^{2+} before stimulation is 0.05 μM.

centration of free Ca^{2+} in the cytosol (Z) and in the $InsP_3$-insensitive intracellular store (Y). The two panels of Figure 5 show how the latency and period of Ca^{2+} spikes both decrease when the level of external stimulation rises. A and B represent the transition that occurs on increasing β up to the final values $\beta_f = 0.31$ and 0.51, respectively, in the situation described in Figure 2C, starting from the stable steady state obtained for $\beta = 0$, which corresponds to a low level of cytosolic Ca^{2+} equal to 0.14 μM.

As expected from the results of Figure 2C, the latency L, which separates the first peak in cytosolic Ca^{2+} from the onset of stimulation, decreases as β_f goes from 0.31 to 0.51. The data of Figure 5 provide an explanation for this phenomenon. In the two panels, the rise in β produces an increase in Y and Z, which both evolve to a new steady state. However, in each case, this steady state is unstable: as soon as Y accumulates sufficiently through Ca^{2+} pumping from the cytosol, and Z reaches a critical level where the self-amplified release of Y into Z becomes significant, a spike of cytosolic Ca^{2+} occurs. Because of the instability of the steady state, this process possesses a repetitive nature. The main difference between A and B is that the accumulation of Y and Z is more rapid at the larger value of β considered, because the accumulation of Z in that phase is primarily governed by the influx $v_1\beta$ from the $InsP_3$-sensitive store. Hence, the slope of the rise in Z before the spike is steeper, so that the threshold for self-amplified release is reached more rapidly and latency is diminished. For the same reason, the period of the oscillations is reduced when the value of β increases. It is therefore not surprising to find in this model a strong correlation between period and latency.

Discussion

We have shown that a linear correlation exists between the latency and the period of Ca^{2+} oscillations induced by a rise in $InsP_3$ triggered by an external signal, in a model where repetitive Ca^{2+} spikes originate from the self-amplified release of Ca^{2+} from intracellular stores. Similar results were obtained when we assumed that the rise in the saturation function of the $InsP_3$ receptor is quasi-instantaneous or occurs in an exponential manner with a characteristic time

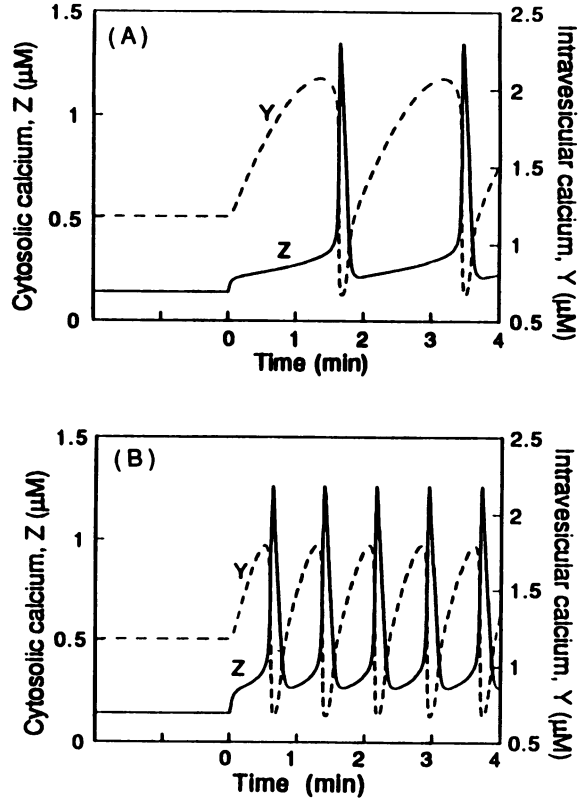

Figure 5. **Time course of Ca^{2+} in the cytosol (Z) and in the $InsP_3$-insensitive compartment (Y) after an increase in β from 0 up to 0.315 (A) and 0.506 (B) in the situation described in Figure 2A.** An increase in the slope of Z accumulation between spikes is noticeable at the largest value of β. Similar oscillations in Z can occur with higher values of Y, of the order of K_R, when the latter parameter is increased. Thus, Y oscillates in a range close to 8–10 μM when $K_R = 15\ \mu M$, $v_0 = 4\ \mu M \cdot min^{-1}$, $v_1 = 17\ \mu M \cdot min^{-1}$, $V_{M2} = 160\ \mu M \cdot min^{-1}$, $V_{M3} = 500\ \mu M \cdot min^{-1}$, $K_A = 0.8\ \mu M$, $K_2 = 1\ \mu M$, $k_f = 1.2\ min^{-1}$, $k = 20\ min^{-1}$, $n = m = 2$, and $p = 4$, with β in the range 0.31–0.97.

comparable with the period of Ca^{2+} transients. The main conclusion to emerge from this analysis is that exactly the same process occurs during latency as during the interval between spikes in the course of oscillations, i.e., an $InsP_3$-induced mobilization of Ca^{2+} charges up an $InsP_3$-insensitive Ca^{2+} store, which then mobilizes its Ca^{2+} by Ca^{2+}-induced Ca^{2+} release.

What is the generality of the mechanism of Ca^{2+}-induced Ca^{2+} release on which the two-pool model for oscillations is based? Evidence for the occurrence of such a process is widespread; it has been obtained for excitable cells (Endo *et al.,* 1970; Fabiato and Fabiato, 1975) and is beginning to appear for nonexcitable cells as well (Marty and Tan, 1989; Jacob, 1990b; Osipchuk *et al.,* 1990), including oocytes (Busa *et al.,* 1985).

The correlation between latency and period has been obtained over a wide range of parameter values, which can accommodate a variety of cellular conditions. In particular, the question arises about the range predicted by the model for the concentration of free Ca^{2+} in the Ca^{2+}-sensitive pool during oscillations. Precise calculations of this concentration are difficult to make. For the parameter values of Figures 2–5, the value of Y (defined with respect to the total cell volume) approaches 2 μM. If the total Ca^{2+} pool, e.g., the endoplasmic reticulum (ER), is of the order of 5% of the cell volume, this 2-μM level would correspond to a Ca^{2+} concentration of at least 40 μM in the $InsP_3$-insensitive store. Given that the latter represents only a part of the total ER Ca^{2+} store, the corresponding concentration of Ca^{2+} in the Ca^{2+}-sensitive store could be much higher. Moreover, Ca^{2+} oscillations with values of Y larger than those shown in Figure 5 occur when raising the value of the release constant K_R (see legend to Figure 5); the values of Y are found to be of the order of K_R. These two factors (the larger values of Y and the fact that the Ca^{2+}-sensitive pool is only a fraction of the total ER) could result in values of Ca^{2+} in the Ca^{2+}-sensitive pool as large as 1 mM. The correlation between period and latency continues to hold in such conditions.

Concluding from the latency-period relationship that the same process of Ca^{2+}-induced Ca^{2+} release is responsible for initiating not only the first but also all subsequent Ca^{2+} spikes implies that the initial spike should resemble those that follow, which is exactly what has been described in hepatocytes (Woods *et al.,* 1987) and endothelial cells (Jacob *et al.,* 1988). The mathematical analysis, therefore, can account not only for the effect of agonist concentration on latency but also for the constant amplitude of the individual spikes during oscillations.

An important prediction from this model is that the cytosolic Ca^{2+} concentration increases during the latent period before the onset of Ca^{2+} spiking. The existence of such a pacemaker elevation of Ca^{2+} is evident in some of the Ca^{2+} traces obtained from hepatocytes (Rooney *et al.,* 1989) and endothelial cells (Jacob *et al.,* 1988). Another prediction is that, for us to observe a linear correlation between period and latency, the Ca^{2+}-sensitive Ca^{2+} pool, the content of which varies in a sawtooth manner, should be at a relatively low level before stimulation (see Figure 5). The existence of the relationship between period and latency thus provides insights into the mechanism of $InsP_3$-directed mobilization of Ca^{2+} in response to extracellular signals.

In the alternative model proposed by Meyer and Stryer (1988), Ca^{2+} repetitive spikes are necessarily accompanied by periodic changes in $InsP_3$. Other differences with the present model are, first, that the steady state of cytosolic Ca^{2+} does not change with the level of external stimulation, whereas here this (stable or unstable) steady state increases with the external signal, in agreement with experimental observations (Sage and Rink, 1987; Berridge *et al.,* 1988; Berridge and Galione, 1988; Jacob *et al.,* 1988; Cuthbertson, 1989; Harootunian *et al.,* 1989; Rink and Jacob, 1989; Rooney *et al.,* 1989). Second, although the period of oscillations decreases with the level of external stimulation as in the experiments and in the present model, latency is close to zero for all stimuli tested. In the Meyer-Stryer model there is indeed no phase of gradual accumulation of Ca^{2+} in the intracellular store just after stimulation: the steady-state level of the single pool of Ca^{2+} is highest at low stimulation, so that an increase in stimulus produces a rise in $InsP_3$ and, subsequently, a quasi-instantaneous discharge of Ca^{2+} from that pool, leading to the first spike. Latency in that model could only occur through a delay due to the synthesis of $InsP_3$. Here, in contrast, besides a possible delay due to the production of $InsP_3$ (taken into account via Eq. *4*), the change in latency primarily originates from a change in the rate of Ca^{2+} accumulation in the cytosol and in the $InsP_3$-insensitive intracellular store.

A similarity of waveform of Ca^{2+} transients with that of the cardiac potential has been noted by Jacob *et al.* (1988) in their study of Ca^{2+} spikes

triggered by histamine in endothelial cells. This analogy can be further extended. Although the mechanism of cytosolic Ca^{2+} oscillations based on Ca^{2+}-induced Ca^{2+} release does not rely on the excitable properties of the membrane, the explanation that it provides for the change in latency and period of Ca^{2+} oscillations bears some resemblance to the manner by which adrenaline controls the frequency of the heartbeat through modulating the slope of the pacemaker potential in cardiac cells (Noble, 1979). In both systems, a rise in stimulation leads to the increase of the slope between successive spikes. This steepening of the pacemaker slope brings the system above the self-excitation threshold more rapidly and thereby accelerates the rhythm.

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