

## One-pool model for $\text{Ca}^{2+}$ oscillations involving $\text{Ca}^{2+}$ and inositol 1,4,5-trisphosphate as co-agonists for $\text{Ca}^{2+}$ release

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**Abstract** — Experimental observations indicate that  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) may underlie  $\text{Ca}^{2+}$  oscillations in a variety of cells. In its original version, a theoretical model for signal-induced  $\text{Ca}^{2+}$  oscillations based on CICR assumed the existence of two types of pools, one sensitive to inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ) and the other one sensitive to  $\text{Ca}^{2+}$ . Recent experiments indicate that  $\text{Ca}^{2+}$  channels may sometimes be sensitive to both  $\text{IP}_3$  and  $\text{Ca}^{2+}$ . Such a regulation may be viewed as  $\text{Ca}^{2+}$ -sensitized  $\text{IP}_3$ -induced  $\text{Ca}^{2+}$  release or, alternatively, as a form of  $\text{IP}_3$ -sensitized CICR. We show that sustained oscillations can still occur in a one-pool model, provided that the same  $\text{Ca}^{2+}$  channels are sensitive to both  $\text{Ca}^{2+}$  and  $\text{IP}_3$  behaving as co-agonists. This model and the two-pool model based on CICR both account for a number of experimental observations but differ in some respects. Thus, while in the two-pool model the latency and period of  $\text{Ca}^{2+}$  oscillations are of the same order of magnitude and correlate in a roughly linear manner, latency in the one-pool model is always brief and remains much shorter than the period of oscillations. Moreover, the first  $\text{Ca}^{2+}$  spike is much larger than the following ones in the one-pool model. These distinctive properties might provide an explanation for the differences in  $\text{Ca}^{2+}$  oscillations observed in various cell types.

In a variety of cells, stimulation by an extracellular signal leads to the onset of repetitive  $\text{Ca}^{2+}$  spikes [1–4]. During the last few years, the molecular mechanism of  $\text{Ca}^{2+}$  oscillations has been increasingly investigated. Some theoretical models for the oscillatory phenomenon have also been proposed [5–8]. One of the various mechanisms that has been mathematically formulated [5] is based on the so-called ‘ $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release’ (CICR) process: cytosolic  $\text{Ca}^{2+}$  is assumed to activate its own release from intracellular stores, after an initial

$\text{Ca}^{2+}$  increase elicited by inositol 1,4,5-trisphosphate ( $\text{IP}_3$ )-induced  $\text{Ca}^{2+}$  release (IICR);  $\text{IP}_3$  is synthesized in response to external stimulation. Experimental evidence in favour of CICR, initially obtained for muscle [9] and cardiac cells [10], has since been provided in oocytes [11], chromaffin cells [12], pancreatic acinar cells [13], and hepatocytes [14].

Recent experimental investigations have also focused on the intracellular  $\text{Ca}^{2+}$  pools involved in the generation of repetitive  $\text{Ca}^{2+}$  spikes. In its initial

version [5], the CICR model (*see* Fig. 1) assumed the existence of two types of  $\text{Ca}^{2+}$  pool [1]: one sensitive to  $\text{IP}_3$  and one insensitive to  $\text{IP}_3$  but sensitive to cytosolic  $\text{Ca}^{2+}$ . The channels present in the membrane of the latter  $\text{Ca}^{2+}$  store are responsible for CICR; these channels are experimentally characterized by their sensitivity to ryanodine and caffeine [3].  $\text{Ca}^{2+}$  channels involved in CICR may, however, be more widespread than previously considered, given the recent characterization of receptors/ $\text{Ca}^{2+}$  channels sensitive to ryanodine but not to caffeine [15]. The distinction between  $\text{IP}_3$ -sensitive and caffeine-sensitive stores has been made clear in adrenal chromaffin cells [16–18], pituitary cells [19], Purkinje neurons [20], acinar cells [21] and smooth muscle cells [22]. The various stores, characterized by different  $\text{Ca}^{2+}$  release properties, have generally distinct cellular locations, though they are both thought to be part of the endoplasmic (ER) or sarcoplasmic reticulum (SR). It has also been suggested that in non-muscle cells the  $\text{IP}_3$ -sensitive store could be a specialized  $\text{Ca}^{2+}$ -storing organelle, called 'calciosome' [23].

In other cells, however, the existence of a unique type of non-mitochondrial  $\text{Ca}^{2+}$  pool has been demonstrated. Thus, in the neurosecretory cell line PC12,  $\text{Ca}^{2+}$  release evoked by caffeine/ryanodine or  $\text{IP}_3$  originates from the same  $\text{Ca}^{2+}$  pool [24]. On the other hand, rapid release of  $\text{Ca}^{2+}$  by purified  $\text{IP}_3$  receptors isolated from mammalian brain and reconstituted into vesicles requires cytosolic  $\text{Ca}^{2+}$  as well as  $\text{IP}_3$  [25]: there,  $\text{IP}_3$  and  $\text{Ca}^{2+}$  behave as co-agonists for  $\text{Ca}^{2+}$  release. The same property characterizes the  $\text{IP}_3$  receptor from Purkinje cells of canine cerebellum [26] and hamster egg [27]. When  $\text{Ca}^{2+}$  and  $\text{IP}_3$  behave as co-agonists for the induction of  $\text{Ca}^{2+}$  release, the regulatory process may be viewed either as  $\text{Ca}^{2+}$ -sensitized IICR [27] or as  $\text{IP}_3$ -sensitized CICR. With regard to the mechanism of  $\text{Ca}^{2+}$  oscillations, referring to the action of  $\text{Ca}^{2+}$  on IICR as a form of CICR emphasizes the prominent role of the positive feedback exerted by cytosolic  $\text{Ca}^{2+}$  on its release from intracellular stores. Therefore, in this paper devoted to the modelling of  $\text{Ca}^{2+}$  oscillations, we elect to refer to the ( $\text{IP}_3$ -independent) CICR and  $\text{Ca}^{2+}$ -sensitized IICR as  $\text{IP}_3$ -insensitive and  $\text{IP}_3$ -sensitive forms of CICR, respectively.

The question arises as to the possibility of  $\text{Ca}^{2+}$  oscillations in a one-pool model based on CICR. One might argue, indeed, that in a model with a single pool sensitive to both  $\text{IP}_3$  and  $\text{Ca}^{2+}$ , the rise in  $\text{IP}_3$  after stimulation could prevent oscillations by inducing the depletion of the  $\text{Ca}^{2+}$  pool, which would annihilate the destabilizing effect of CICR. In the following study, we investigate the possibility of sustained  $\text{Ca}^{2+}$  oscillations in two modified versions of the original CICR model [5], both containing a single  $\text{Ca}^{2+}$  pool. In the first version of the one-pool model, the same  $\text{Ca}^{2+}$  channel is assumed to be sensitive to both  $\text{IP}_3$  and  $\text{Ca}^{2+}$  behaving as co-agonists [25]; in the second version, two distinct  $\text{Ca}^{2+}$  channels, sensitive to  $\text{Ca}^{2+}$  or  $\text{IP}_3$ , are envisaged. We show that only the first version of the one-pool model readily gives rise to  $\text{Ca}^{2+}$  oscillations, and compare its predictions with those of the two-pool model based on CICR. Besides a number of common properties, the one- and two-pool models based, respectively, on  $\text{IP}_3$ -sensitive and  $\text{IP}_3$ -insensitive CICR lead to distinctive predictions which might provide an explanation for differences in  $\text{Ca}^{2+}$  oscillations observed in various cell types. In the following, for the sake of brevity, we shall sometimes refer to these models simply as one- and two-pool models for  $\text{Ca}^{2+}$  oscillations.

#### *Two-pool model based on CICR*

The original version of the CICR model for signal-induced  $\text{Ca}^{2+}$  oscillations is schematized in Figure 1. After external stimulation,  $\text{IP}_3$  is synthesized and binds to receptors located on the ER (or SR) membrane, provoking the liberation of  $\text{Ca}^{2+}$  into the cytosol. Through CICR, the latter increase triggers the release of  $\text{Ca}^{2+}$  from another,  $\text{Ca}^{2+}$ -sensitive store, leading to the rising part of the  $\text{Ca}^{2+}$  peak. Cytosolic  $\text{Ca}^{2+}$  decreases due to pumping into the  $\text{Ca}^{2+}$ -sensitive store and extrusion from the cell. As the  $\text{IP}_3$  level is assumed to remain constant during stimulation, cytosolic  $\text{Ca}^{2+}$  again accumulates owing to the  $\text{IP}_3$ -elicited rise in  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$  entry from the extracellular medium (part of the latter influx could be triggered by stimulation); a new cycle of the oscillations begins as soon as the level of cytosolic  $\text{Ca}^{2+}$  reaches the

threshold for CICR.

This scheme was formulated into two evolution equations for the two variables, namely cytosolic  $Ca^{2+}$  ( $Z$ ) and  $Ca^{2+}$  in the  $IP_3$ -insensitive store ( $Y$ ):

$$\begin{aligned} \frac{dZ}{dt} &= V_{in} - V_2 + V_3 + k_f Y - kZ \\ \frac{dY}{dt} &= V_2 - V_3 - k_f Y \end{aligned} \quad \text{Eq. 1}$$

with:

$$V_{in} = v_0 + v_1 \beta \quad \text{Eq. 2a}$$

$$V_2 = V_{M2} \frac{Z^n}{K_2^n + Z^n} \quad \text{Eq. 3}$$

$$V_3 = V_{M3} \frac{Y^m}{K_R^m + Y^m} \frac{Z^p}{K_A^p + Z^p} \quad \text{Eq. 4a}$$

In these equations,  $V_{in}$  represents the total constant entry of  $Ca^{2+}$  into the cytosol; it includes the influx  $v_0$  from the extracellular medium and the  $IP_3$ -stimulated  $Ca^{2+}$  release  $v_1 \beta$  ( $\beta$  is the degree of saturation of the  $IP_3$  receptor);  $V_2$  and  $V_3$  are, respectively, the rates of pumping into and release from the  $Ca^{2+}$ -sensitive store with  $V_{M2}$  and  $V_{M3}$  denoting the maximum rates of these processes;  $K_2$ ,  $K_R$ , and  $K_A$  are the threshold constants for pumping,

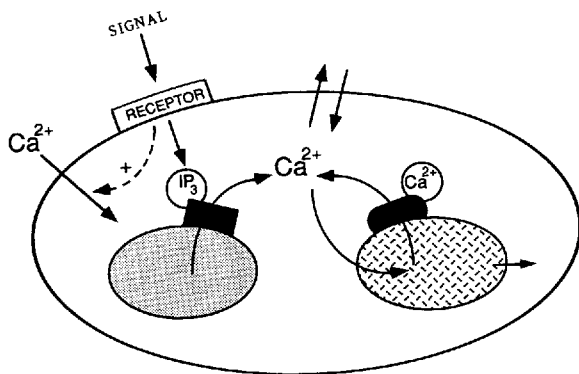


Fig. 1 Schematic representation of the two-pool model for signal-induced  $Ca^{2+}$  oscillations based on  $Ca^{2+}$ -induced  $Ca^{2+}$  release (see text and [5] for details).

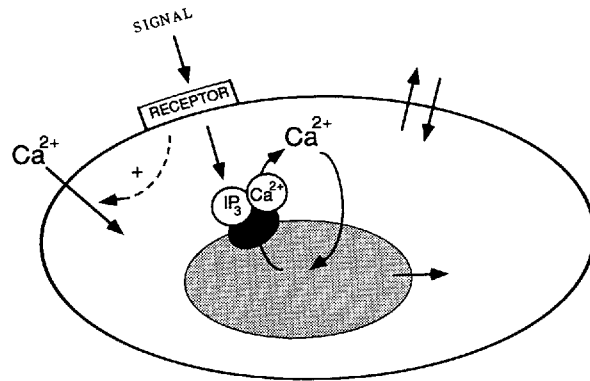


Fig. 2 Schematic representation of a one-pool model for signal-induced  $Ca^{2+}$  oscillations. It is assumed that a single type of  $Ca^{2+}$  channel is activated both by  $IP_3$  and  $Ca^{2+}$  behaving as co-agonists (see text for details).

release and activation while  $n$ ,  $m$  and  $p$  are the Hill coefficients characterizing the latter processes;  $k_f Y$  and  $kZ$  refer to the passive efflux from the  $Ca^{2+}$ -sensitive store and from the cytosol; all concentrations, including the intravesicular  $Ca^{2+}$  concentration, are defined with respect to the total cell volume (for further details about the equations, see [5, 28]).

Numerical simulations of this simple model lead to sustained oscillations in a certain range of stimulation bounded by two critical values of parameter  $\beta$ . Other theoretical predictions in agreement with experimental observations relate to the increase in frequency with the level of stimulation and with extracellular  $Ca^{2+}$  [5, 28], the correlation between period and latency [29], or the generation of  $Ca^{2+}$  waves when diffusion of cytosolic  $Ca^{2+}$  is taken into account [8, 30].

*One-pool model:  $Ca^{2+}$  and  $IP_3$  as co-agonists for induction of  $Ca^{2+}$  release*

The question arises as to whether  $Ca^{2+}$  oscillations can still arise in a model containing a single pool sensitive to  $Ca^{2+}$  as well as  $IP_3$ . When considering one type of pool possessing channels activated by both  $IP_3$  and  $Ca^{2+}$ , the  $Ca^{2+}$  exchange processes are still globally represented by Equation 1 but the

detailed nature of some of the processes has to be modified (compare Fig. 1 and Fig. 2). Pumping into the unique IP<sub>3</sub>- and Ca<sup>2+</sup>-sensitive store is still given by Equation 3 while the release of Ca<sup>2+</sup> into the cytosol now takes the form:

$$V_3 = \beta V_{M3} \frac{Y^m}{K_R^m + Y^m} \frac{Z^p}{K_A^p + Z^p} \quad \text{Eq. 4b}$$

where  $\beta$  represents the degree of saturation by IP<sub>3</sub> of this 'bi-activated' receptor and  $V_{M3}$  the maximum rate of release. As in the two-pool version (Eq. 4a) the last factor reflects the assumption that Ca<sup>2+</sup> release is activated by cytosolic Ca<sup>2+</sup>. Though the activation of the IP<sub>3</sub> receptor by cytosolic Ca<sup>2+</sup> has in some cases been shown to be followed by an inhibition of the same receptor at higher cytosolic Ca<sup>2+</sup> concentration [25, 26], the model predicts that, even if present, this inhibition plays no significant role in the generation of sustained Ca<sup>2+</sup> oscillations.

In the two-pool model, the term  $v_1\beta$  (Eq. 2a) denotes the constant influx of Ca<sup>2+</sup> from the IP<sub>3</sub>-sensitive pool. If such a term is suppressed in the one-pool model – because the effect of IP<sub>3</sub> on Ca<sup>2+</sup> release is then expressed by Equation 4b – and

if one only considers a constant Ca<sup>2+</sup> influx,  $v_0$ , from the extracellular medium, one loses an important property of the CICR model, namely, that the mean cytosolic Ca<sup>2+</sup> concentration rises with the stimulation level. In most cell types [1–4], indeed, a low (high) concentration of agonist generates a constant low (high) level of cytosolic Ca<sup>2+</sup>, when the stimulus is outside the range leading to sustained oscillations. One way to obviate this shortcoming is to assume that the stimulation, besides inducing IP<sub>3</sub> synthesis, also leads to a direct activation of Ca<sup>2+</sup> entry from the extracellular medium into the cytosol (see Fig. 2). Such a stimulus-activated Ca<sup>2+</sup> entry has been reported in some cell types and could be triggered by depletion of the intracellular stores [31–34].

For the present purpose, we retain the simplest assumption that the influx from the extracellular medium triggered by external stimulation is proportional to parameter  $\beta$ , much as the IP<sub>3</sub>-regulated release from the Ca<sup>2+</sup> pool. The influx  $V_{in}$  from the extracellular medium is thus given here by Equation 2b formally similar to Equation 2a:

$$V_{in} = v_0 + v_1\beta \quad \text{Eq. 2b}$$

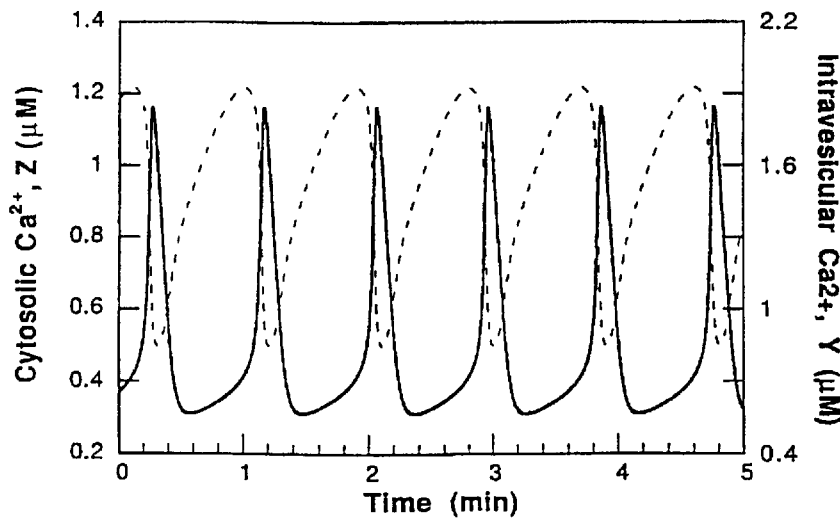


Fig. 3 Typical Ca<sup>2+</sup> oscillations generated by the one-pool model based on IP<sub>3</sub>-sensitive Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release schematized in Figure 2. The solid and dotted lines represent the evolution of cytosolic and intravesicular Ca<sup>2+</sup>, respectively. Curves are obtained by numerical integration of Equations 1, 2b, 3 and 4b with  $\beta = 0.4$ ,  $v_0 = v_1 = 3.4 \mu\text{M}\cdot\text{min}^{-1}$ ,  $V_{M2} = 50 \mu\text{M}\cdot\text{min}^{-1}$ ,  $V_{M3} = 650 \mu\text{M}\cdot\text{min}^{-1}$ ,  $K_2 = 1 \mu\text{M}$ ,  $K_R = 2 \mu\text{M}$ ,  $K_A = 0.9 \mu\text{M}$ ,  $k = 10 \text{ min}^{-1}$ ,  $k_f = 1 \text{ min}^{-1}$ ,  $n = m = 2$  and  $p = 4$ . Initial conditions are  $Y = 1.87 \mu\text{M}$ ,  $Z = 0.37 \mu\text{M}$ .

where  $v_0$  still denotes the constant rate of  $\text{Ca}^{2+}$  influx in the absence of stimulation while  $v_1$  is now the maximum rate of stimulus-induced influx from the extracellular medium into the cytosol.

Although based on distinct assumptions, the model where  $\text{Ca}^{2+}$  and  $\text{IP}_3$  behave as co-agonists is mathematically similar to the two-pool model, the only difference being that  $V_{M3}$  in Equation 4a is replaced by  $\beta V_{M3}$  in Equation 4b. Oscillations of  $\text{Ca}^{2+}$  therefore readily arise in this one-pool model, as shown in Figure 3 where cytosolic  $\text{Ca}^{2+}$  oscillations obtained by numerical simulations are represented together with the variation of the  $\text{Ca}^{2+}$  content of the pool sensitive to  $\text{IP}_3$  and  $\text{Ca}^{2+}$ .

Compared in Figure 4 are the steady-state level ( $Z_0$ ) and the envelope of the oscillations of cytosolic  $\text{Ca}^{2+}$  in the one- and two-pool versions of the model. The solid line indicates the steady-state level of cytosolic  $\text{Ca}^{2+}$  and, for intermediate stimuli for which sustained oscillations occur, the maximum and minimum values reached by the  $\text{Ca}^{2+}$  concentration in the cytosol; the dashed line represents the unstable steady state. In both cases the model exhibits the property that cytosolic  $\text{Ca}^{2+}$  progressively rises with the level of stimulation. While the amplitude of the oscillations in the two-pool model decreases only slightly as the system passes through the oscillatory domain, it decreases more significantly in the one-pool model.

Common to the one- and two-pool models analyzed here is the role of intravesicular  $\text{Ca}^{2+}$  as a counterpoise to the increase in cytosolic  $\text{Ca}^{2+}$  in the course of oscillations. The rise in cytosolic  $\text{Ca}^{2+}$  is indeed accompanied by a concomitant decrease in the  $\text{Ca}^{2+}$  level in the  $\text{Ca}^{2+}$ -sensitive pool (see dashed line in Fig. 3 for oscillations of intravesicular  $\text{Ca}^{2+}$  in the one-pool model, and Fig. 2 in [28] for the corresponding curve in the two-pool model). The level of cytosolic  $\text{Ca}^{2+}$  drops thereafter as a result of the decreased rate of release from the pool and of the extrusion of  $\text{Ca}^{2+}$  from the cell. The level of  $\text{Ca}^{2+}$  in the store begins to rise again as soon as the rate of pumping from the cytosol exceeds the rate of  $\text{IP}_3$ -sensitive or  $\text{IP}_3$ -insensitive CICR. Since the level of intravesicular  $\text{Ca}^{2+}$  begins to rise well before complete depletion of the pool, this pool is never empty.

A noticeable difference between the one- and

two-pool models based on  $\text{IP}_3$ -sensitive and  $\text{IP}_3$ -insensitive CICR pertains to the concentration of intravesicular  $\text{Ca}^{2+}$  as a function of the stimulation

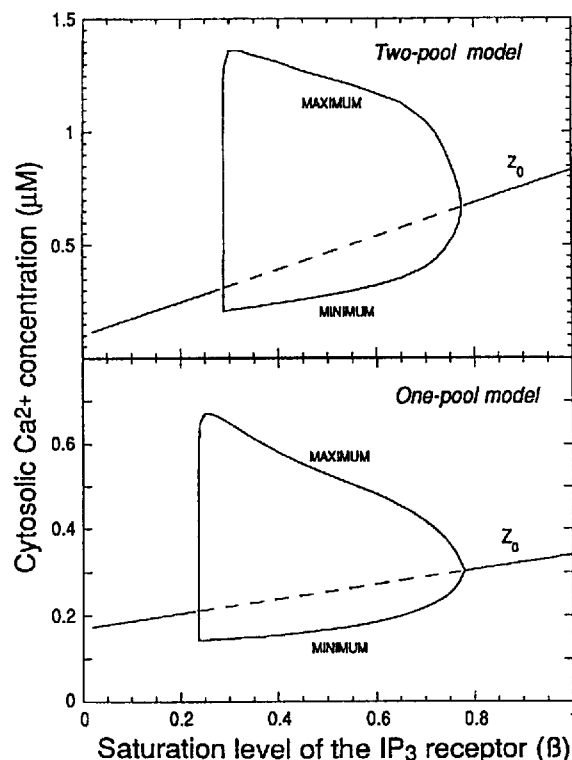


Fig. 4 Concentration of cytosolic  $\text{Ca}^{2+}$  as a function of the stimulation level ( $\beta$ ) in the one- and two-pool models based, respectively, on the  $\text{IP}_3$ -sensitive and  $\text{IP}_3$ -insensitive  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. The solid lines represent the stable level of cytosolic  $\text{Ca}^{2+}$  or the maximum and minimum cytosolic  $\text{Ca}^{2+}$  concentration reached during oscillations; the dashed line indicates the steady-state level of cytosolic  $\text{Ca}^{2+}$  in the domain of  $\beta$  values where this state is unstable and oscillations occur. Parameter values are  $k = 10 \text{ min}^{-1}$ ,  $k_f = 1 \text{ min}^{-1}$ ,  $n = m = 2$  and  $p = 4$ . Moreover, for the upper (lower) panel,  $v_0 = 1$  ( $1.7$ )  $\mu\text{M}\cdot\text{min}^{-1}$ ,  $v_1 = 7.3$  ( $1.7$ )  $\mu\text{M}\cdot\text{min}^{-1}$ ,  $V_{M2} = 65$  ( $25$ )  $\mu\text{M}\cdot\text{min}^{-1}$ ,  $V_{M3} = 500$  ( $325$ )  $\mu\text{M}\cdot\text{min}^{-1}$ ,  $K_2 = 1$  ( $0.5$ )  $\mu\text{M}$ ,  $K_R = 2$  ( $1$ )  $\mu\text{M}$ ,  $K_A = 0.9$  ( $0.45$ )  $\mu\text{M}$ . The lower values considered for some parameters in the one-pool model have been adjusted so as to limit the amplitude of the first  $\text{Ca}^{2+}$  spike to the  $1\text{--}2 \mu\text{M}$  range. The concentrations of intravesicular and cytosolic  $\text{Ca}^{2+}$  are defined with respect to the total cell volume; the actual intravesicular  $\text{Ca}^{2+}$  concentration is therefore larger than on the given scale. The curves are established by linear stability analysis and numerical integration of Equations 1, 2a, 3 and 4a for the two-pool model, and Equations 1, 2b, 3 and 4b for the one-pool model.

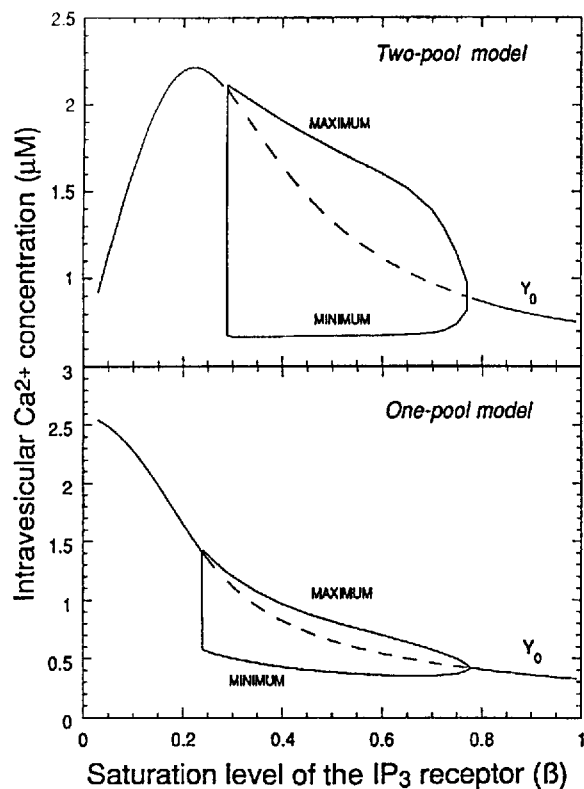


Fig. 5 Concentration of intravesicular  $\text{Ca}^{2+}$  as a function of the stimulation level in the one- and two-pool models based on the two versions of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. The curves are established as described in Figure 4, where solid and dashed lines have been defined.

level for various agonist concentrations (see Fig. 5). In the two-pool model, the steady-state level of intravesicular  $\text{Ca}^{2+}$  ( $Y_0$ ) first rises and then decreases when stimulation increases; this behaviour reflects the fact that cytosolic  $\text{Ca}^{2+}$  – whose level increases with stimulation – at first replenishes the  $\text{Ca}^{2+}$ -sensitive pool but later favours its depletion once CICR becomes significant. In contrast, in the one-pool model,  $Y_0$  decreases over the whole range of stimulation, because the predominant  $\text{Ca}^{2+}$  efflux from the single pool is directly proportional to the stimulation level reflected by  $\beta$  (see Eq. 4b).

The fact that  $Y_0$  has a large value in the absence

of stimulation in the one-pool model, in contrast to the two-pool model (see Fig. 5), has consequences that might help to distinguish experimentally between the two situations. When increasing in a stepwise manner  $\beta$  from zero up to a finite value in the oscillatory range, the first  $\text{Ca}^{2+}$  spike in the one-pool model occurs immediately regardless of the final value of  $\beta$  (Fig. 6); the time between the stimulus and the first  $\text{Ca}^{2+}$  spike – i.e. the latency –

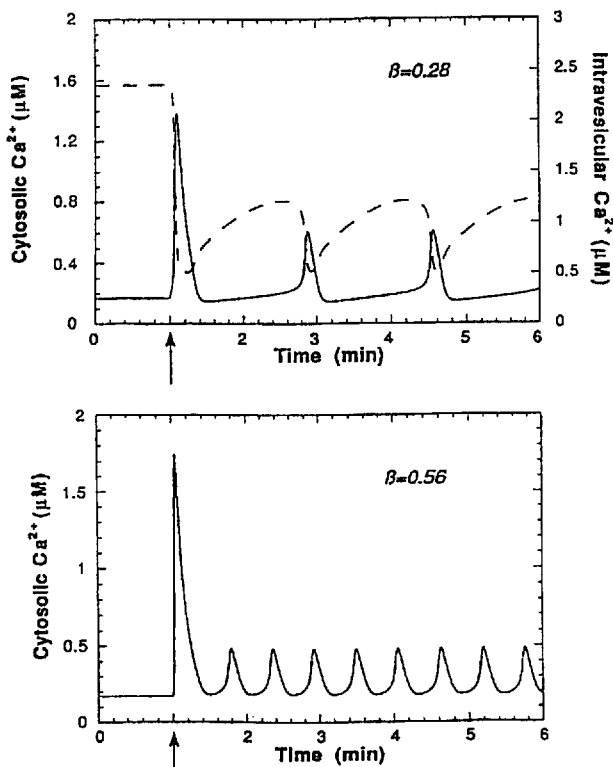


Fig. 6 Oscillations in cytosolic  $\text{Ca}^{2+}$  ( $Z$ , solid line) triggered by two stimuli of increasing magnitude in the one-pool model schematized in Figure 2, where  $\text{Ca}^{2+}$  and  $\text{IP}_3$  behave as co-agonists. After 1 min (arrow), the value of parameter  $\beta$  measuring stimulation is increased instantaneously from zero up to 0.28 (upper panel) or 0.56 (lower panel). Notice that the first  $\text{Ca}^{2+}$  spike each time is much larger than the following ones and occurs with negligible latency. The variation of intravesicular  $\text{Ca}^{2+}$  ( $Y$ , dashed line) is also indicated in the upper panel. The curves are obtained by integration of Equations 1, 2b, 3 and 4b for the parameter values of Figures 4 and 5 (lower panels), with  $k_f = 1.1 \text{ min}^{-1}$ . Initial conditions correspond to the stable steady state in the absence of stimulation ( $\beta = 0$ ):  $Y = 2.355 \text{ } \mu\text{M}$  and  $Z = 0.17 \text{ } \mu\text{M}$ .

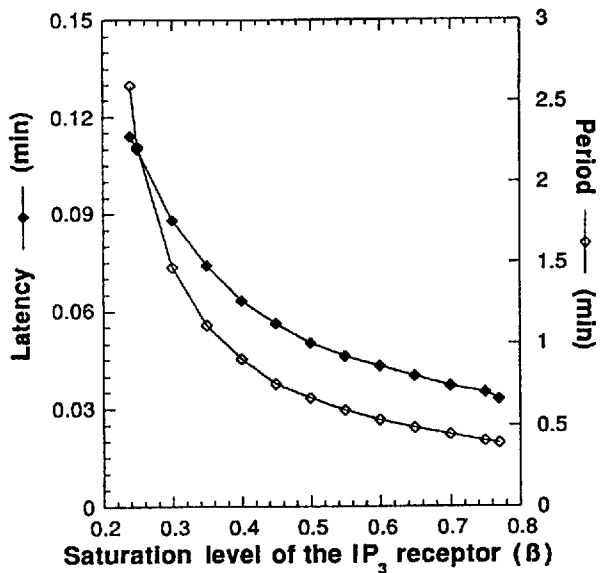


Fig. 7 Period and latency of oscillations in the one-pool model based on  $\text{IP}_3$ -sensitive CICR (Fig. 2) as a function of the magnitude of stimulation measured by the saturation function of the receptor ( $\beta$ ). The time of the peak of the first  $\text{Ca}^{2+}$  spike, i.e. latency, and the period of oscillations are determined for increasing values of parameter  $\beta$  which is raised instantaneously from zero up to the final value indicated. Data are obtained as described in Figure 6, for  $k_f = 1 \text{ min}^{-1}$ .

is therefore very short compared with the period of oscillations. The reason is that the pool, filled to capacity before stimulation, discharges its content as soon as  $\beta$  rises, as expected from Equation 4b; latency nevertheless slightly decreases as the value of  $\beta$  increases (compare the two panels of Fig. 6 and see also Fig. 7). A roughly linear correlation between period and latency therefore holds in the one-pool model, but the time scales for period and latency are widely different (Fig. 7), in contrast with the predictions of the two-pool model [28, 29] (see Fig. 8) and with experimental observations in hepatocytes [35]. Comparing the period-latency relationship in the one- and two-pool models based, respectively, on  $\text{IP}_3$ -sensitive and  $\text{IP}_3$ -insensitive CICR shows (Fig. 9) that only the two-pool version yields satisfactory agreement with data obtained for hepatocytes [35].

Another difference between the one- and two-pool models based on CICR pertains to the magnitude of the first  $\text{Ca}^{2+}$  spike that follows stimulation. As shown in Figure 6, the first spike triggered by the increase in  $\beta$  is significantly larger than the following spikes whose amplitude settles to a reduced level. In contrast, the first spike in the two-pool model has the same magnitude as the following ones (see Fig. 8). The difference in behaviour again originates from the fact that in the two-pool model, the  $\text{Ca}^{2+}$  level in the  $\text{Ca}^{2+}$  store is initially low and progressively increases after stimulation; the level it reaches just before the first

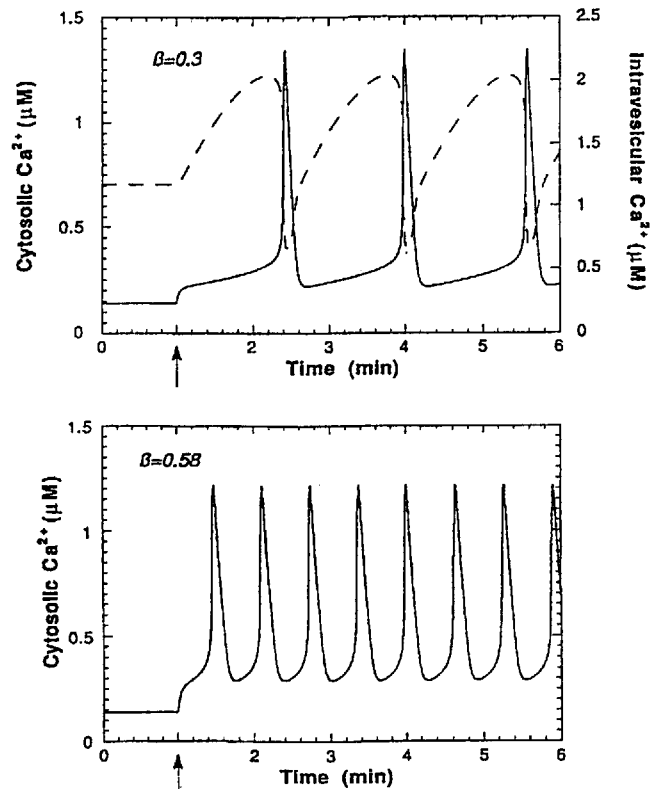


Fig. 8 Oscillations in cytosolic  $\text{Ca}^{2+}$  triggered by two stimuli of increasing magnitude in the two-pool model based on CICR, schematized in Figure 1. Notice that the first  $\text{Ca}^{2+}$  spike has the same magnitude as the following ones and occurs after a finite latency. The curves are obtained as described in Figure 6, by integration of Equations 1, 2a, 3 and 4a for the parameter values of Figures 4 and 5 (upper panels). The initial conditions correspond to the stable steady state in the absence of stimulation ( $\beta = 0$ ):  $Y = 1.1745 \mu\text{M}$ ,  $Z = 0.14 \mu\text{M}$ .

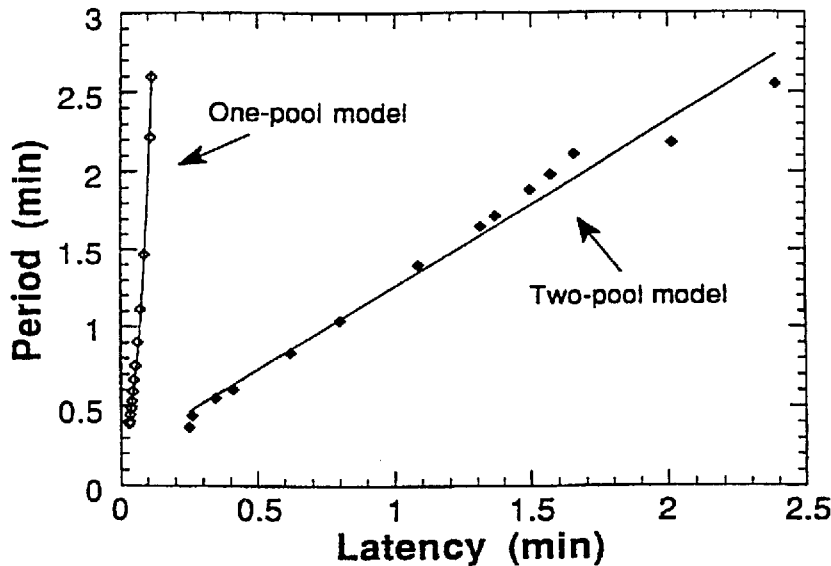


Fig. 9 Period versus latency relationship predicted by the one- and two-pool models based, respectively, on the  $\text{IP}_3$ -sensitive and  $\text{IP}_3$ -insensitive CICR. Parameter values are those of Figures 6 and 8 for the one- and two-pool models, respectively.

discharge is dictated by the threshold for CICR in the cytosol and is therefore the same as for the next spikes (Fig. 8, top panel). In the one-pool model, in contrast, the analysis of the model shows that regardless of parameter values, the  $\text{Ca}^{2+}$  store is initially filled to capacity; because the store is so much charged and begins to release its content immediately as a result of the rise in  $\text{IP}_3$  that follows stimulation, the threshold for CICR in the cytosol is exceeded rapidly and a large spike occurs with negligible latency. For the next spikes, when the threshold for CICR is reached in the cytosol, the level of  $\text{Ca}^{2+}$  in the store is below its maximum capacity so that the spikes will have a reduced magnitude (Fig. 6, top panel).

From an experimental point of view, it is of interest to note that the two situations appear to be encountered, depending on the cell type. Thus, while the first  $\text{Ca}^{2+}$  spike has often the same magnitude as the successive ones and occurs after a finite latency in hepatocytes (*see* [35] and also Fig. 3 in [36]), as predicted by the two-pool model, the first spike is larger and occurs immediately upon stimulation in fibroblasts [37, 38], in accordance

with the predictions of the one-pool model based on CICR. In the latter cells, however, experimental observations [38] can also be accounted for in terms of a mechanism involving the cross-activation of  $\text{Ca}^{2+}$  and  $\text{IP}_3$  [6].

It should be noted that the response of hepatocytes to high levels of certain agonists sometimes appears to be more complex: a high initial spike is followed by a brief silent phase and by a train of  $\text{Ca}^{2+}$  spikes of smaller magnitude (*see* Fig. 10 in [36]). Such a behaviour, however, does not contradict the predictions of the two-pool model based on CICR. On the contrary, a similar time course, also observed in oocytes [28], can be accounted for by the model when the initial stimulation is so high that it brings the system transiently above the oscillatory range of parameter  $\beta$  (*see* Fig. 4 in [28]).

While the above theoretical results and some experimental studies [14, 35] point to CICR as being involved in the mechanism of  $\text{Ca}^{2+}$  oscillations in hepatocytes, other experimental observations can apparently not be reconciled with this hypothesis. Thus, the observation that ryanodine fails to suppress  $\text{Ca}^{2+}$  oscillations in these cells does



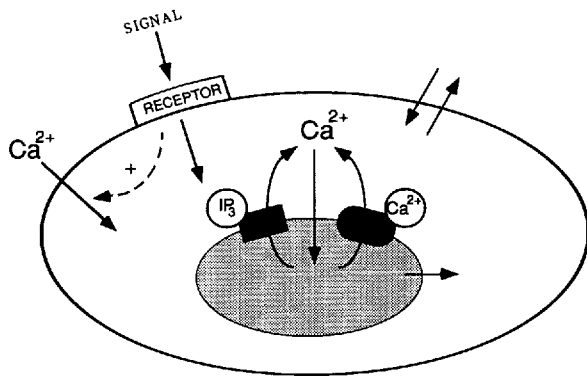


Fig. 10 Schematic representation of a one-pool model for signal-induced  $\text{Ca}^{2+}$  oscillations based on CICR in which two distinct  $\text{Ca}^{2+}$  channels sensitive either to  $\text{IP}_3$  or  $\text{Ca}^{2+}$  are considered. Unless the  $\text{IP}_3$ -activated efflux is much smaller than  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release, this model does not produce sustained  $\text{Ca}^{2+}$  oscillations.

not hold with a mechanism involving CICR through the ryanodine-sensitive  $\text{Ca}^{2+}$  channel [39].

#### One-pool model with distinct receptors for $\text{Ca}^{2+}$ and $\text{IP}_3$

The model with a single  $\text{Ca}^{2+}$  pool possessing distinct receptors sensitive to  $\text{Ca}^{2+}$  or  $\text{IP}_3$  is schematized in Figure 10. The temporal evolution corresponding to such a situation is modelled by Equations 1, 2b, 3 and by a new term for the release of  $\text{Ca}^{2+}$  from internal stores, consisting of two distinct contributions representing  $\text{Ca}^{2+}$ -activated and  $\text{IP}_3$ -mediated release, respectively:

$$V_3 = \left( V_{M3} \frac{Z^p}{K_A^p + Z^p} + \beta V'_{M3} \right) \frac{Y^m}{K_R^m + Y^m} \quad \text{Eq. 4c}$$

where  $V_{M3}$  denotes the maximum rate of CICR and  $V'_{M3}$  stands for the maximal rate of  $\text{Ca}^{2+}$  release through the  $\text{IP}_3$ -sensitive channel.

Sustained oscillations can arise in this model only if the  $\text{IP}_3$ -stimulated release (i.e.  $V'_{M3}$ ) is much smaller than the  $\text{Ca}^{2+}$ -activated  $\text{Ca}^{2+}$  release (i.e.  $V_{M3}$ ). As shown by the stability diagram established as a function of  $V_{M3}$  and  $V'_{M3}$ , the steady state is

indeed unstable – and  $\text{Ca}^{2+}$  oscillations develop – only when  $V'_{M3}$  is much smaller than  $V_{M3}$  (Fig. 11). However, in view of experimental observations [1], it is unlikely that such a large difference exists between the relative amounts of  $\text{Ca}^{2+}$  released via the  $\text{IP}_3$ - and  $\text{Ca}^{2+}$ -regulated channels. Clearly, in this version of the one-pool model, if a constant level of  $\text{IP}_3$  elicits a continuous, significant efflux of  $\text{Ca}^{2+}$  from the store, the role of CICR will be bypassed; this would attenuate the importance of the main process responsible for oscillations. The theoretical analysis thus predicts that two distinct  $\text{Ca}^{2+}$  channels located on the same pool do not favour the occurrence of  $\text{Ca}^{2+}$  oscillations. When  $V'_{M3}$  is much smaller than  $V_{M3}$ , the oscillations obtained in this version of the one-pool model are similar to those obtained with the two-pool model as Equation 4c becomes identical to Equation 4a. The assumption of a stimulus-induced influx of  $\text{Ca}^{2+}$  into the cytosol (Eq. 2b) still ensures that the mean level of cytosolic  $\text{Ca}^{2+}$  progressively rises with the degree of stimulation.

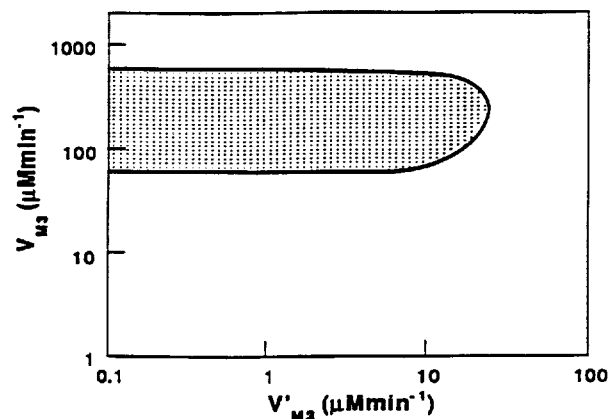


Fig. 11 Domain of sustained  $\text{Ca}^{2+}$  oscillations in the one-pool model based on CICR schematized in Figure 10, where two channels sensitive either to  $\text{Ca}^{2+}$  or  $\text{IP}_3$  coexist. Sustained oscillations occur in the dotted domain where the steady state is unstable. Repetitive  $\text{Ca}^{2+}$  spiking occurs only when the maximum rate of CICR ( $V_{M3}$ ) largely exceeds the maximum rate of  $\text{IP}_3$ -induced release ( $V'_{M3}$ ) from the store. The instability of the unique steady state is determined by linear stability analysis of Equations 1, 2b, 3 and 4c. Parameter values are  $\beta = 0.6$ ,  $\nu_0 = \nu_1 = 4 \mu\text{M}\cdot\text{min}^{-1}$ ,  $V_{M2} = 65 \mu\text{M}\cdot\text{min}^{-1}$ ,  $K_2 = 1 \mu\text{M}$ ,  $K_R = 2 \mu\text{M}$ ,  $K_A = 0.9 \mu\text{M}$ ,  $k = 10 \text{ min}^{-1}$ ,  $k_f = 1 \text{ min}^{-1}$ ,  $n = m = 2$  and  $p = 4$ .

## Discussion

We have shown that cytosolic  $\text{Ca}^{2+}$  oscillations can occur in a one-pool model when  $\text{Ca}^{2+}$  and  $\text{IP}_3$  together induce  $\text{Ca}^{2+}$  release from that pool into the cytosol. Repetitive  $\text{Ca}^{2+}$  spikes obtained in that model resemble those previously obtained with the two-pool model based on CICR. The theoretical analysis indicates that the single pool has to possess  $\text{Ca}^{2+}$  channels sensitive to both  $\text{IP}_3$  and  $\text{Ca}^{2+}$  behaving as co-agonists [25]; if a single pool with two different types of channel is considered (one sensitive to  $\text{Ca}^{2+}$  and the other to  $\text{IP}_3$ ), no oscillations occur unless the contribution of the  $\text{IP}_3$ -controlled channel is negligible with respect to that of the channel involved in CICR. With respect to the propagation of  $\text{Ca}^{2+}$  waves within the cytosol, a mechanism involving  $\text{Ca}^{2+}$  and  $\text{IP}_3$  as co-agonists for  $\text{Ca}^{2+}$  release has also been invoked [40–42]. In view of the results presented here for oscillations, the results obtained for  $\text{Ca}^{2+}$  wave propagation by means of the two-pool model based on CICR [8, 28, 30] would likely be recovered with the one-pool model involving  $\text{Ca}^{2+}$  and  $\text{IP}_3$  as co-agonists for  $\text{Ca}^{2+}$  release.

In order for the one-pool model to retain the experimentally observed property that the mean cytosolic  $\text{Ca}^{2+}$  concentration increases with the stimulation level, it is necessary to assume the existence of a stimulus-activated  $\text{Ca}^{2+}$  entry into the cytosol. Such an assumption seems most plausible and can be viewed as reflecting the fact that the depletion of the store triggers  $\text{Ca}^{2+}$  entry into the cytosol [31–34]; similar results would be obtained with a stimulus-activated inhibition of  $\text{Ca}^{2+}$  efflux which has been proposed by some authors [35]. It should be noted that the constant term  $v_1\beta$  in the two-pool model might represent stimulus-enhanced  $\text{Ca}^{2+}$  entry from the extracellular medium into the cytosol (*see* dashed line in Fig. 1) in addition to the usual  $\text{IP}_3$ -mediated  $\text{Ca}^{2+}$  mobilization from the  $\text{IP}_3$ -sensitive store.

The distinction between one- and two-pool models has been made here on the basis of whether the channels involved in CICR are sensitive to  $\text{Ca}^{2+}$  only or to  $\text{Ca}^{2+}$  and  $\text{IP}_3$  behaving as co-agonists. It is possible, however, to distinguish between  $\text{Ca}^{2+}$  pools in another manner if one considers the spatial

distribution of  $\text{IP}_3$  within the cell. Thus it has been argued [43] that the  $\text{IP}_3$ -sensitive pool near the plasma membrane may discharge its content in response to an elevated level of  $\text{IP}_3$ , while deeper inside the cell, the lower level of  $\text{IP}_3$  would not *per se* deplete the  $\text{IP}_3$ -sensitive  $\text{Ca}^{2+}$  pool and CICR would be fully active. In such a case the cell would effectively contain two types of  $\text{Ca}^{2+}$  pool, even if these possess the same type of  $\text{Ca}^{2+}$  channel activated by  $\text{Ca}^{2+}$  and  $\text{IP}_3$ : one pool, near the membrane, would provide a  $\text{Ca}^{2+}$  influx in response to high levels of  $\text{IP}_3$  established upon stimulation; this  $\text{Ca}^{2+}$  influx would prime the other pool, further away from the membrane, for oscillations based on CICR [43]. Such a situation is encompassed in our treatment of the one-pool model if one considers that the  $\text{Ca}^{2+}$  influx  $v_1\beta$  triggered by the stimulus includes the release of  $\text{Ca}^{2+}$  from the  $\text{IP}_3$ -sensitive store located near the membrane.

Stimulus-activated  $\text{Ca}^{2+}$  entry from the extracellular medium was not considered in another one-pool model based on CICR [44]; in that model, the mean cytosolic  $\text{Ca}^{2+}$  concentration does not show any dependence on the stimulation level. Besides the latter shortcoming, the model is analogous to the one here schematized in Figure 2, though based on less realistic biochemical kinetics. On the other hand, the first mathematical model based on CICR [45] also envisaged only one pool; however, the role of  $\text{IP}_3$ , unknown at that time, was not taken into account. The other theoretical models for repetitive  $\text{Ca}^{2+}$  spikes, based on mechanisms other than CICR, also rely on the existence of a single  $\text{Ca}^{2+}$  pool [6, 7, 46]. These latter models do not assign any role to the caffeine and/or ryanodine sensitive  $\text{Ca}^{2+}$  channels in the generation of oscillations, in spite of the fact that such a role is well established for a number of cell types.

The comparison of the two versions of the one-pool model based on CICR (*see* Figs 2, 10) showed that the existence of a single  $\text{Ca}^{2+}$  channel sensitive to both  $\text{IP}_3$  and  $\text{Ca}^{2+}$  favours oscillations. This result can be related to the intriguing observation that while channels sensitive to both  $\text{IP}_3$  and  $\text{Ca}^{2+}$  have been characterized in different types of nerve cells where  $\text{Ca}^{2+}$  oscillations occur [25, 26], repetitive  $\text{Ca}^{2+}$  spikes have never been detected in PC12 cells where two distinct receptors on the membrane of the

same pool have been reported [24].

In other cell types, however, the distinction between  $\text{IP}_3$ - or  $\text{Ca}^{2+}$ -sensitive pools is not so clear-cut; in some cells, indeed, the  $\text{Ca}^{2+}$  pools appear to be sensitive to the two messengers to various degrees [18, 47, 48]. Modeling such a complex network of pools and regulations would lead to intermediate behaviours in comparison with the extreme cases analyzed here. The present study thus demonstrates that CICR, sensitized or not by  $\text{IP}_3$ , keeps providing a plausible, robust mechanism to account for experimental observations about  $\text{Ca}^{2+}$  oscillations in a number of cell types regardless of whether the cell possesses a single or two distinct types of  $\text{Ca}^{2+}$  pool.

Of possible help in distinguishing between the two situations is the result that the one- and two-pool models based, respectively, on  $\text{IP}_3$ -sensitive and  $\text{IP}_3$ -insensitive CICR, lead to different predictions with respect to the magnitude of the first spike (Figs 6 and 8) and the relationship between the latency and period of  $\text{Ca}^{2+}$  oscillations obtained at different levels of stimulation (Fig. 9). While in the two-pool model period and latency are often of the same order of magnitude and correlate in a roughly linear manner as generally observed in hepatocytes [35], the one-pool model predicts that latency should always be negligible with respect to the period of  $\text{Ca}^{2+}$  oscillations. Furthermore, the theoretical analysis shows that the magnitude of the first spike and its latency are closely linked, as observed, for example, in fibroblasts and hepatocytes. Thus, one obtains either a large initial spike appearing without latency (one-pool model), or an initial spike of the same magnitude as the following ones, appearing after a time lag (two-pool model). Given that the first situation occurs in fibroblasts [37] while the second is encountered in hepatocytes at moderate stimulation levels, [35, 36],  $\text{Ca}^{2+}$  oscillations in these cell types could originate from a mechanism involving either a single pool sensitive to both  $\text{Ca}^{2+}$  and  $\text{IP}_3$  behaving as co-agonists, or two pools sensitive to  $\text{IP}_3$  or  $\text{Ca}^{2+}$ , respectively.

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#### Note added in proof

While this paper was in press, a related study of a one-pool model with activation and inhibition of the  $\text{IP}_3$ -receptor by cytosolic  $\text{Ca}^{2+}$  has been published (De Young GW. Keizer J. (1992) Proc. Natl Acad. Sci. USA, 89, 9895-9899).

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