Quantal Release, Incremental Detection, and Long-Period Ca^{2+} Oscillations in a Model Based on Regulatory Ca^{2+}-Binding Sites Along the Permeation Pathway

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ABSTRACT Quantal release, incremental detection, and oscillations are three types of Ca^{2+} responses that can be obtained in different conditions, after stimulation of the intracellular Ca^{2+} stores by submaximum concentrations of inositol 1,4,5-trisphosphate ($InsP_3$). All three phenomena are thought to occur through the regulatory properties of the $InsP_3$ receptor/Ca^{2+} channel. In the present study, we perform further analysis of the model (Swillens et al., 1994, *Proc. Natl. Acad. Sci. USA.* 91:10074–10078) previously proposed for transient $InsP_3$-induced Ca^{2+} release, based on the bell-shaped dependence of the $InsP_3$ receptor activity on the Ca^{2+} level and on the existence of an intermediate Ca^{2+} domain located around the mouth of the channel. We show that Ca^{2+} oscillations also arise in the latter model. Conditions for the occurrence of the various behaviors are investigated. Numerical simulations also show that the existence of an intermediate Ca^{2+} domain can markedly increase the period of oscillations. Periods on the order of 1 min can indeed be accounted for by the model when one assigns realistic values to the kinetic constants of the $InsP_3$ receptor, which, in the absence of a domain, lead to oscillations with periods of a few seconds. Finally, theoretical support in favor of a positive cooperativity in the regulation of the $InsP_3$ receptor by Ca^{2+} is presented.

INTRODUCTION

Oscillations of cytosolic Ca^{2+} resulting from extracellular stimulation by a hormone or a neurotransmitter have been observed in single intact cells of very different types (Tsien and Tsien, 1990; Meyer and Stryer, 1991; Berridge, 1993; Berridge and Dupont, 1994; Sneyd et al., 1995). Because this phenomenon is widespread and because it is expected to play a primary physiological role, many experimental and theoretical avenues have been explored to gain insight into the mechanisms of Ca^{2+} oscillations and of their spatial counterpart, propagating Ca^{2+} waves. A key feature for understanding these mechanisms is the so-called Ca^{2+}-induced Ca^{2+} release (CICR) regulation, through which Ca^{2+} release from intracellular stores is enhanced by cytosolic Ca^{2+} itself (Fabiato and Fabiato, 1975; Endo and Iino, 1990). Initial models predicted that this regulation, acting at the level of the ryanodine receptors, could underly the oscillatory behavior (Kuba and Takeshita, 1981; Berridge and Galione, 1988; Goldbeter et al., 1990). However, in many cell types, Ca^{2+} oscillations occur through Ca^{2+} mobilization from internal pools sensitive to inositol 1,4,5-trisphosphate ($InsP_3$). The most convincing evidence that CICR, first observed at the level of the ryanodine receptor, is a general regulatory element leading to oscillations came from studies on membrane preparations, showing that the Ca^{2+} channel activity of the $InsP_3$ receptor presents a bell-shaped dependence on cytosolic Ca^{2+} concentration (Bezprozvanny et al., 1991; Finch et al., 1991). A detailed description of successive activation and inhibition of Ca^{2+} release by cytosolic Ca^{2+} can qualitatively account for the existence of Ca^{2+} oscillations (De Young and Keizer, 1992; Atri et al., 1993; Tang et al., 1996); however, quantitative agreement between models and experiments is still lacking. Indeed, in the experiments, periods on the order of 1 min are frequently observed among the different cell types; in contrast, in the models, periods larger than 10 s have not been obtained with realistic values for the kinetic constants of the $InsP_3$ receptor (Sneyd et al., 1995).

On the other hand, in populations of permeabilized cells, $InsP_3$-induced Ca^{2+} release has been shown to occur in a quantal manner (Muallem et al., 1989; Meyer and Stryer, 1990; Taylor and Potter, 1990; Combettes et al., 1993a,b; Bootman et al., 1992, 1994). A submaximum $InsP_3$ concentration is indeed unable to release the total Ca^{2+} content of the pools. Moreover, it has been shown in some cases that the residual Ca^{2+} can be released when a slightly higher $InsP_3$ concentration is added to the medium. This property has been referred to as incremental detection (Meyer and Stryer, 1990). Various hypotheses have been proposed to explain this partial discharge: differences in sensitivities for $InsP_3$ of the various Ca^{2+} pools (Kindman and Meyer, 1993; Bootman and Berridge, 1995), dependence of the $InsP_3$ receptor/Ca^{2+} channel activity on luminal Ca^{2+} (Irvine, 1990), rapid activation of the Ca^{2+} pump (Steenbergen and Fay, 1996), or the existence of a so-called memory molecule (Swillens, 1992). It has also been shown that quantal Ca^{2+} release can be readily accounted for by the known properties of the $InsP_3$ receptor/Ca^{2+} channel, if it is assumed that the channel's activity is regulated by the Ca^{2+}

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level in a particular intermediate domain, located downstream from the gate (Swillens et al., 1994). The latter model indeed considers that the $InsP_3$-induced Ca^{2+} efflux generates a transient increase in Ca^{2+} concentration in the vicinity of the channel mouth. When a submaximum increase in $InsP_3$ is simulated, the model does lead to channel activation, leading to a rapid Ca^{2+} release from the stores. But the amount of released Ca^{2+} is limited by a channel shutdown, due to the desensitization induced by the increased Ca^{2+} level in the domain; thus the channel closes before complete emptying of the stores. This property of the model is at the basis of its "incremental detection-like" behavior.

Although closely related, Ca^{2+} oscillations and quantal Ca^{2+} release have generally been treated as distinct phenomena that both require their own approaches. In the present study we perform further analysis of the model previously proposed by Swillens et al. (1994) for transient $InsP_3$-induced Ca^{2+} release, trying to reconcile quantal release and oscillations. Bearing in mind the postulate that both oscillatory and quantal behaviors rely on the intrinsic properties of the $InsP_3$ receptor, the same model should be capable of accounting for both phenomena, in appropriate conditions. Moreover, because CICR is an essential ingredient of the model of Swillens et al. (1994), it is expected a priori that this model can also generate Ca^{2+} oscillations. Experimentally, quantal Ca^{2+} release or incremental detection have been most clearly observed in populations of permeabilized cells or vesicle preparations, whereas sustained Ca^{2+} oscillations have always been observed in intact isolated cells. The main difference between these situations thus lies with the "cytosolic" volume. In permeabilized cells, the medium containing the vesicles acts as the cytosol; because this volume is much larger than the vesicles themselves, variations in cytosolic Ca^{2+} concentration are small and can be neglected, as in the original study (Swillens et al., 1994). On the contrary, such an approximation does not hold in intact cells. Here we extend the initial model by taking into account variations in the cytosolic Ca^{2+} level as well as Ca^{2+} pumping into the stores. As a result, quantal Ca^{2+} release, incremental detection, and Ca^{2+} oscillations can all be simulated with the same model.

We then study in more detail the characteristics of the Ca^{2+} oscillations obtained with the model, assuming the existence of a Ca^{2+} intermediate domain. We focus on the possible role of the domain in altering the kinetics of oscillations and show that a marked lengthening of the period of Ca^{2+} oscillations can be obtained when Ca^{2+} transfer from the domain into the cytosol is fast enough. The importance of cooperativity in the mechanism of Ca^{2+} oscillations is then investigated. The model predicts in this respect that positive and negative regulations of the $InsP_3$ receptor/Ca^{2+} channel activity by Ca^{2+} are probably characterized by a high degree of positive cooperativity.

OVERVIEW OF THE MODEL PREVIOUSLY PROPOSED FOR TRANSIENT $InsP_3$-INDUCED Ca^{2+} RELEASE

Our study is based on the model of a $InsP_3$-sensitive Ca^{2+} channel described previously (Swillens et al., 1994) and schematized in Fig. 1. For simplicity, intracellular Ca^{2+} is supposed to be distributed between three homogeneous compartments: the lumen ($InsP_3$-sensitive store), the intermediate domain (downstream from the channel gate), and the cytosol. Channel activity is stimulated by $InsP_3$ and regulated in a biphasic manner by the Ca^{2+} level in the domain. Ca^{2+}-induced desensitization develops slowly, whereas $InsP_3$- and Ca^{2+}-mediated activations are assumed to be instantaneous. Both activation and inhibition induced by Ca^{2+} are positively cooperative processes. Ca^{2+} released

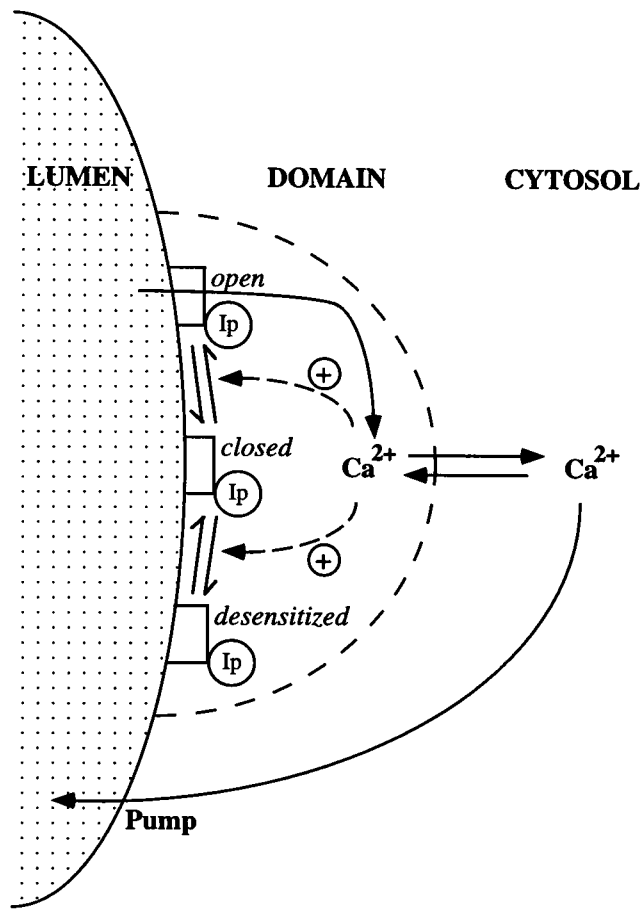

FIGURE 1 Schematic representation of the model developed by Swillens et al. (1994) to account for transient $InsP_3$-induced Ca^{2+} release. The $InsP_3$ (Ip) receptor, which can exist in three different states (open, closed, and desensitized), is biphasically regulated by the Ca^{2+} level prevailing in an intermediate domain located downstream from the gate (see text, and Swillens et al., 1994, for details). For simplicity, only the closed form of the receptor is supposed to desensitize; simulation results would remain unchanged if the open state could also directly desensitize. In the present study, the model has been extended to simulate Ca^{2+} oscillations by considering Ca^{2+} replenishment of the stores through the Ca^{2+} ATPases (*arrow* marked Pump).

from the stores passes through the domain before entering the cytosol. Efflux from the stores is regulated by the fraction of nondesensitized receptors with only activating Ca^{2+} and $InsP_3$ bound to their respective sites, and is proportional to the Ca^{2+} gradient between the lumen and the domain; a small $InsP_3$-independent efflux is also considered ($k_1 b$ in the equations below). The Ca^{2+} flux from the domain to the cytosol is proportional to the Ca^{2+} gradient between these two compartments.

To simulate quantal release and incremental detection, it was assumed that cytosolic Ca^{2+} (*Ccyto*) is maintained at a constant concentration and is not pumped back into the stores by Ca^{2+} ATPases. These constraints reproduce the conditions of stopped-flow experiments. On such bases, the evolution of the fraction of desensitized receptors (*Rdes*) and of Ca^{2+} in the lumen (*Clum*) and in the domain (*Cdom*) is given by

$$\frac{dRdes}{dt} = k_+ Cdom^{n_i} \frac{1 - Rdes}{1 + \left(\frac{Cdom}{K_{act}}\right)^{n_a}} - k_- Rdes \quad (1)$$

$$\frac{dCdom}{dt} = \frac{\alpha}{\beta} k_1 (b + Ira) \times (Clum - Cdom) - k_2 (Cdom - Ccyto) \quad (2)$$

$$\frac{dClum}{dt} = -k_1 (b + Ira)(Clum - Cdom), \quad (3)$$

with

$$Irable = (1 - Rdes) \frac{Ip}{1 + Ip} \quad (4)$$

$$Ira = Irable \frac{1}{1 + \left(\frac{K_{act}}{Cdom}\right)^{n_a}}. \quad (5)$$

(see Swillens et al., 1994, for more details). The differences in the volumes of the lumen and the domain are taken into account through the ratio α/β, with α and β equal, respectively, to the following ratios of volumes: $V_{lumen}/V_{cytosol}$ and $V_{domain}/V_{cytosol}$. Thus, only the ratio V_{lumen}/V_{domain} is relevant to simulating incremental detection. In fact, in Eqs. 1–3, $V_{cytosol}$ is supposed to tend to infinity, so that cytosolic Ca^{2+} concentration can be assumed to remain constant; this assumption significantly enlarges the region of the parameter space in which incremental detection occurs in the numerical simulations. The present definitions for the volume ratios, however, allow us to keep the same symbols when simulating incremental detection and oscillations. Ip refers to the normalized $InsP_3$ level (i.e., the $InsP_3$ concentration divided by its equilibrium dissociation constant). Other parameters are defined as in Swillens et al. (1994). The slow Ca^{2+}-induced desensitization of the channel is governed by the values of the kinetic constants of Ca^{2+} association to (k_+) and dissociation from (k_-) the inhibiting site. K_{act} and K_{inh} (with $K_{inh}^{ni} = k_-/k_+$) stand for the dissociation constants of Ca^{2+} to the activating and inhibiting sites, respectively. The latter processes are characterized by Hill coefficients, noted n_a and n_i. The Ca^{2+} transfer between the lumen and the domain is characterized by the kinetic constant k_1, and k_2 governs the flux between the domain and the cytosol. Finally, $k_1 b$ accounts for a basal efflux from the lumen in the absence of $InsP_3$.

As previously shown (Swillens et al., 1994), numerical integration of the system defined by Eqs. 1–5 leads to the typical incremental detection behavior. Fig. 2 *A* shows that the system responds to successive $InsP_3$ stimuli above a certain thresold, by transient and rapid decreases of Ca^{2+} in the lumen. The activation of the channel by Ca^{2+} in the

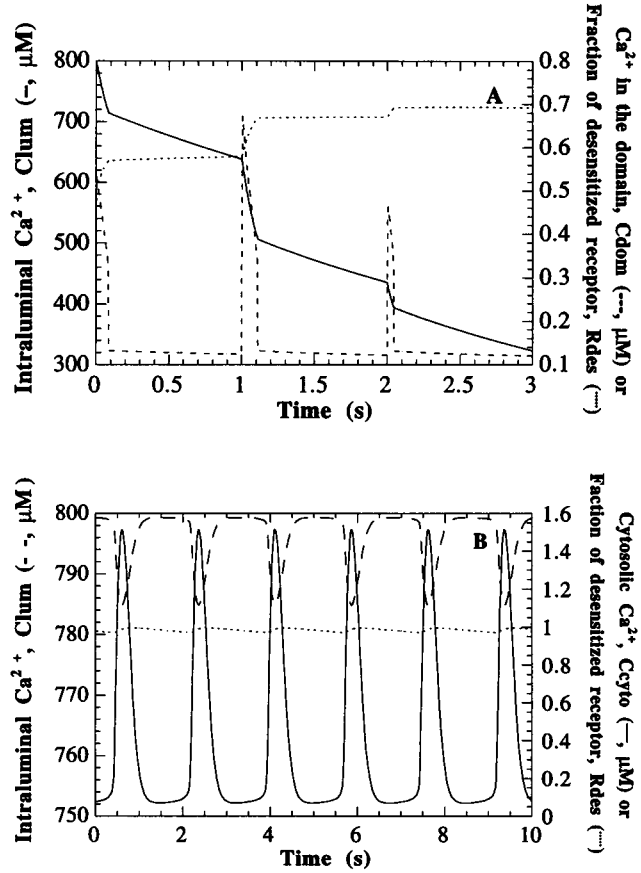

FIGURE 2 Incremental detection and Ca^{2+} oscillations in the model initially developed for transient $InsP_3$-induced Ca^{2+} release, for the same set of parameter values. (*A*) Incremental detection behavior obtained by numerical integration of Eqs. 1–5, with *Ccyto* = 0.1 μM, *Clum* initial = 800 μM, K_{act} = 0.3 μM, n_a = 3, K_{inh} = 0.1 μM, n_i = 4, k_- = 0.02 s^{-1}, $\rho = 7 \times 10^6$, $k_2 = 3 \times 10^8$ s^{-1}, $b = 10^{-4}$, α = 0.1, $\beta = 10^{-6}$. In this case, there is no Ca^{2+} pumping activity back from the cytosol to the lumen. The three successive decreases in Ca^{2+} in the lumen correspond to the following Ip levels, respectively: 0.1, 0.2, and 0.3. (*B*) Sustained Ca^{2+} oscillations obtained when Ca^{2+} pumping is included in the model, by numerical integration of Eqs. 1 and 4–8 for the same set of parameter values as in *A*, with, moreover, $V_{MP} = 10$ μM s^{-1}, K_P = 0.3 μM, n_P = 2, *Ip* = 0.3, and Catot = 80 μM. At the scale of the figure, the evolution of the Ca^{2+} concentration in the domain is undistinguishable from the evolution of the Ca^{2+} concentration in the cytosol.

domain and thus the onset of Ca^{2+} release from the store are instantaneous. However, each step of Ca^{2+} release leads to delayed desensitization of a fraction of receptors. Consequently, the threshold for activation by $InsP_3$ is increased. A further activation thus requires either an $InsP_3$ increase or a decrease of the threshold, which could occur through Ca^{2+} reaccumulation in the lumen (Swillens et al., 1994). Because luminal Ca^{2+} concentration is only decreasing in the situation considered in Fig. 2 *A*, the threshold remains high: the system may thus reactivate only if a sufficiently high $InsP_3$ increment is detected by the receptors.

SUSTAINED Ca^{2+} OSCILLATIONS IN THE MODEL FOR QUANTAL Ca^{2+} RELEASE

As both Ca^{2+} oscillations and quantal Ca^{2+} release occur through Ca^{2+} channel activity of the $InsP_3$ receptor, any realistic model for quantal release must also account for oscillations. Thus we investigated if the model of Swillens et al. (1994), based on the kinetic properties of the $InsP_3$ receptor and on the existence of a Ca^{2+} domain located downstream from the gate, can generate Ca^{2+} oscillations. A major difference between quantal Ca^{2+} release and Ca^{2+} oscillations pertains to the fact that variations in the level of cytosolic Ca^{2+} and pumping through the ATPases must be considered, as Ca^{2+} oscillations occur through periodic exchanges of Ca^{2+} between the cytosol and the internal stores (see Fig. 1). The evolution of the concentrations of luminal and cytosolic Ca^{2+} are given by the following equations (which thus replace Eqs. 3 and 2):

$$\frac{dClum}{dt} = -k_l(b + Ira)(Clum - Cdom) + \frac{V_{MP}}{\alpha}\frac{Ccyto^{n_p}}{Ccyto^{n_p} + K_P^{n_p}} \quad (6)$$

$$\frac{dCcyto}{dt} = \beta k_2(Cdom - Ccyto) - V_{MP}\frac{Ccyto^{n_p}}{Ccyto^{n_p} + K_P^{n_p}}, \quad (7)$$

with the constraint

$$Catot = \alpha Clum + \beta Cdom + Ccyto, \quad (8)$$

where Ca_{tot} represents the total intracellular Ca^{2+} concentration, defined with respect to the cytosolic volume. Ca^{2+} ATPase activity is controlled by cytosolic Ca^{2+} according to a Hill expression; the characteristic parameters of the pump, V_{MP}, K_P, and n_p, refer to the maximum velocity, the constant for half-maximal activity, and the Hill coefficient, respectively. The values ascribed to these parameters in the simulations shown below agree with experimental observations (Carafoli, 1987; Lytton et al., 1992; Camello et al., 1996).

As shown in Fig. 2 *B*, sustained Ca^{2+} oscillations can be obtained with the model initially developed to account for transient $InsP_3$-induced Ca^{2+} release. Note that all values for common parameters have been kept the same as in Fig. 2 *A*. It must also be noted that the oscillatory behavior is crucially dependent on the values of the pumping parameters; if, for instance, significantly higher V_{MP} and K_P values were used, oscillations would not appear with the set of parameter values used in Fig. 2 *A*.

Parts *A* and *B* of Fig. 2 first differ by the fact that cytosolic Ca^{2+} varies in the course of oscillations only in *B*, because of the much smaller "cytosolic" volume and possible luminal Ca^{2+} reaccumulation. In fact, the ratio α/β has been kept unchanged, but when oscillations are considered, the absolute values of α and β also become important. In this respect, it should be noted that the system defined by Eqs. 1 and 4–8 reduces to the system 1–5 if the volume of the cytosol tends to infinity (i.e., α and β tend to zero, as does V_{MP}, given that the latter parameter is defined with respect to the cytosolic volume).

From a mechanistic point of view, the major difference between Fig. 2 *A* and Fig. 2 *B* lies in the variable governing the temporal behavior of the system. In the case of quantal release and incremental detection, the response is induced by successive changes in $InsP_3$ concentration; the channel indeed adapts to any $InsP_3$ change through the Ca^{2+} transients in the domain, and through the increasing fraction of desensitized channels. On the other hand, for oscillations, variations in $InsP_3$ concentration are not considered. The state of the receptor only varies in response to Ca^{2+} changes, which thus govern the whole dynamics of the system. As examplified in Fig. 2 *B*, these changes in the fraction of desensitized receptor can, for some parameter values, be very small.

One of the basic properties of Ca^{2+} oscillations is that their frequency increases with the level of stimulus. Fig. 3 shows how the variables of the system defined by Eqs. 1 and 4–8 evolve when the stimulus is increased. Oscillations occur from Ip = 0.12 to Ip = 0.47. Not shown on Fig. 3 is the fact that the period varies from 17.0 s (Ip = 0.12) down to 3.15 s (Ip = 0.47). For all three panels, the central line indicates the value of the steady state as a function of the $InsP_3$ level; the latter steady-state can be stable (plain line) or unstable (dashed line), in which case oscillations develop. In the oscillatory domain, the plain lines locate maxima and minima reached by the different variables in the course of the oscillations. Thus it can be seen that the amplitude of the spikes in cytosolic Ca^{2+} does not vary much with the stimulus level, in agreement with experimental observations. The central panel emphasizes that, in the model, the Ca^{2+} concentration in the lumen remains nearly constant during the course of oscillations. In the situation considered in Fig. 3, the pool never loses more than 25% of its content. In fact, oscillations appear to be nearly unchanged if the Ca^{2+} level in the lumen is assumed to remain constant. Finally, the bottom panel shows that variations in the fraction of desensitized receptors decrease with the

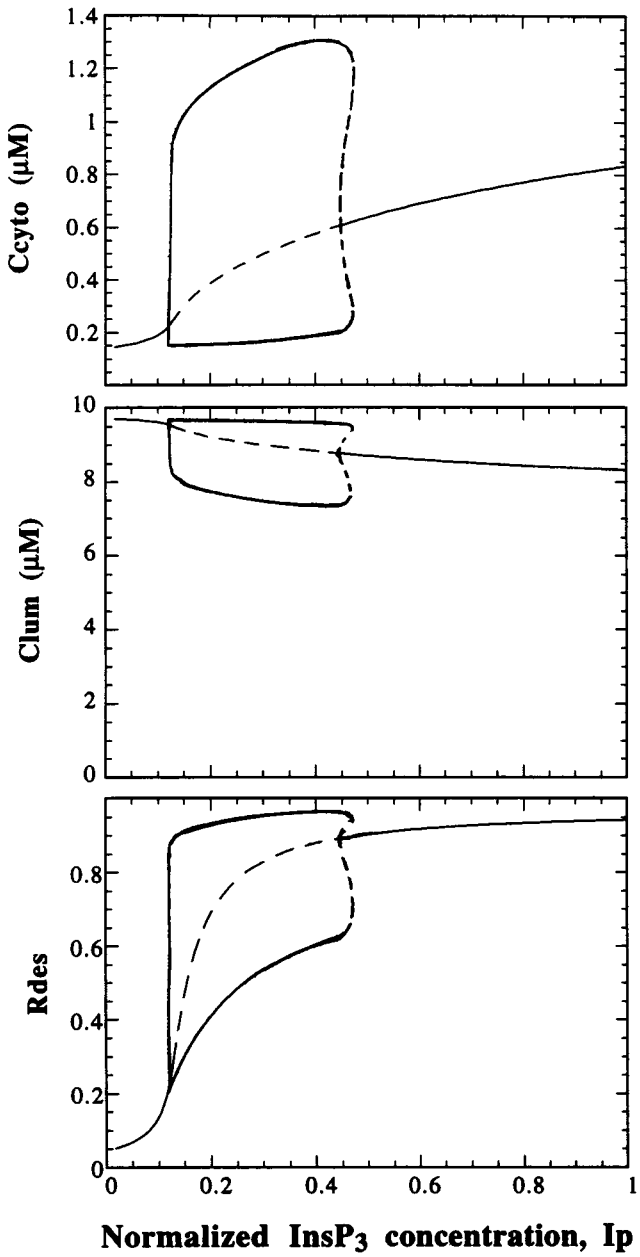

FIGURE 3 Bifurcation diagram as a function of the stimulus level (Ip) of the extended model for transient $InsP_3$-induced Ca^{2+} release defined by Eqs. 1 and 4–8. The central line indicates the steady-state values (unstable if dashed), and the two other lines show the maxima and minima reached during oscillations. The period varies from 17.0 s to 3.5 s in the oscillatory domain (not shown). These results have been obtained with the bifurcation program AUTO, with the following set of parameter values: $K_{act} = 0.6$ μM, $n_a = 3$, $K_{inh} = 0.3$ μM, $n_i = 4$, $k_- = 0.2\ s^{-1}$, $\rho = 85$, $k_2 = 2000\ s^{-1}$, $b = 4 \times 10^{-3}$, $\alpha = 1$, $\beta = 1$, $V_{MP} = 5$ μM s^{-1}, $K_P = 0.3$ μM, $n_P = 2$, and Catot = 10 μM. The volumic differences of the various compartments have been ignored, because otherwise the system could not be solved by using the program AUTO. The results would, however, remain qualitatively unchanged if α and β were smaller than 1. The oscillatory domain is limited by two Hopf bifurcation points, the right one corresponding to a subcritical bifurcation, which explains the small elbow near the largest Ip values leading to oscillations (Nicolis, 1995). The maxima and minima of the unstable limit cycle (near the right bifurcation point) are also indicated by a dashed line.

$InsP_3$ concentration, because the receptors have less time to resensitize between two successive spikes. In fact, the decrease in period with the level of $InsP_3$ is the consequence of an increased efflux from the lumen into the domain, allowing the Ca^{2+} level in the latter compartment to reach the activation threshold earlier.

Another factor that markedly affects the period of the Ca^{2+} oscillations generated by the model is the rate at which the $InsP_3$ receptor/Ca^{2+} channel resensitizes (parameter k_-). As shown in Fig. 4, the period decreases when the rate of resensitization increases. In contrast to the situation described in Fig. 3, the amplitude is significantly affected; in fact, for very small values of k_- (i.e., slow resensitization) Ca^{2+} oscillations do not disappear, but the amplitude of the simulated Ca^{2+} spikes becomes too large from a physiological point of view. Realistic estimates of the rate constant of $InsP_3$ receptor/Ca^{2+} channel reactivation lie between 0.1 and 2 s^{-1} (Finch et al., 1991; Combettes et al., 1994). Thus it seems unlikely that the kinetics of the resensitization process—the characteristic time of which is less than 10 s—may explain experimentally observed oscillation periods as long as several tens of seconds.

EFFECT OF THE Ca^{2+} INTERMEDIATE DOMAIN ON Ca^{2+} OSCILLATIONS

The existence of a Ca^{2+} domain in which the Ca^{2+} concentration may rise very fast and in a transient manner is essential for simulating a Ca^{2+} response similar to the incremental detection process. However, during the course of oscillations shown in Fig. 2 *B*, the Ca^{2+} concentrations in the domain and in the cytosol remain very similar. Thus, one can question the relevance of the domain to the oscillatory behavior. It appears that for the set of parameter values used in Fig. 2 *B*, the oscillations appear nearly unchanged if one neglects the existence of the domain (not shown). This can be done either by assuming that cytosolic

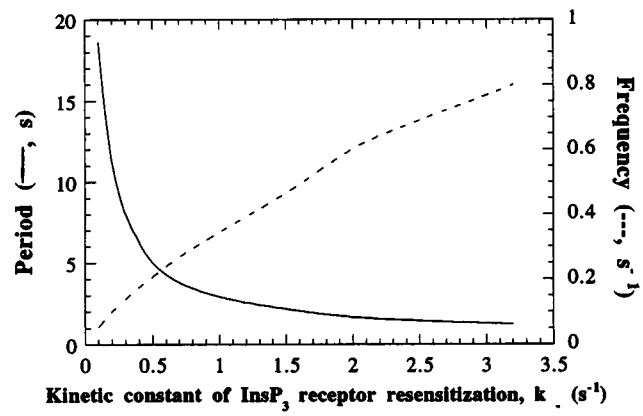

FIGURE 4 Dependence of the period of Ca^{2+} oscillations on the rate constant k_- of $InsP_3$ receptor/Ca^{2+} channel resensitization. Results have been obtained by numerical integration of Eqs. 1, 4–8, with the same set of parameter values as in Fig. 2 *B*.

Ca^{2+} (*Ccyto*) directly regulates channel activity, or by making a rapid equilibrium approximation on the Ca^{2+} concentration in the domain (i.e., by assuming that *Cdom* always equals its stationary value, given by the solution to d*Cdom*/dt = 0). In both cases, oscillations remain nearly unchanged with respect to the ones obtained with the full model. Whereas the first choice indicates that during the course of oscillations *Ccyto* essentially equals *Cdom*, the second choice means that *Cdom* is always in near equilibrium with the other variables of the system. Thus, in the case of Fig. 2 *B*, the Ca^{2+} domain plays no role in the generation of Ca^{2+} oscillations.

One could imagine, however, that in some cases, the domain plays a significant role in the dynamics of the system defined by Eqs. 1 and 4–8. In particular, if Ca^{2+} accumulation in the domain becomes a limiting kinetic factor, one could imagine some effect of this additional variable. This is indeed the case for the parameter values used in Fig. 5 *A*, where the period is as large as 53.2 s, which is on the same order as experimentally observed periods (Berridge, 1993). This period, obtained with realistic values for the parameters governing the dynamics of the receptor, is obviously not imposed by the kinetics of the receptor resensitization for which the characteristic time is on the order of a few seconds. Moreover, it can be clearly seen in Fig. 5 *A* that the receptor (dashed line) is fully resensitized well before the onset of a new Ca^{2+} spike. In contrast, the accumulation of Ca^{2+} plays a major role, and oscillations disappear under the conditions of rapid equilibrium approximation. The kinetic constants characterizing the Ca^{2+} exchanges (k_1 and k_2) are indeed such that Ca^{2+} entering from the lumen in the domain is very rapidly extruded into the cytosol. Only when the gradient between the Ca^{2+} levels in the domain and in the cytosol has become small enough can the domain fill up again to activate CICR. In other conditions, i.e., when ρ (= k_2/k_1) decreases, the period also rapidly decreases (Fig. 6). From a physiological point of view, for large values of ρ, the cytosol can be regarded as a "Ca^{2+} sponge" that very rapidly absorbs any localized Ca^{2+} increase. The simulated relationship between parameter ρ and the period is, however, very stiff, a point that is most probably linked to the fact that the Ca^{2+} fluxes in the domain are modeled in an oversimplified manner.

The period can further increase if one assumes that the Ca^{2+} pump acts at the level of the domain and not the cytosol. From a physiological point of view, this hypothesis implies a close colocalization of the $InsP_3$ receptors and the Ca^{2+} ATPases. Fig. 5 *B* demonstrates that the Ca^{2+} accumulation in the domain needed to reach the CICR threshold is considerably slowed down in this case; with realistic

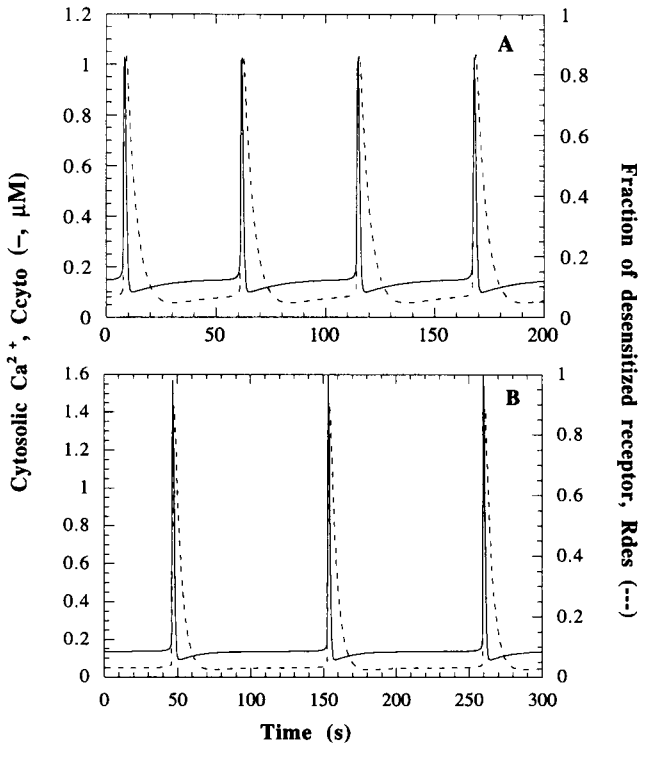

FIGURE 5 Effect of the existence of a Ca^{2+} intermediate domain on the computed period of Ca^{2+} oscillations. (*A*) Effect of the kinetics of Ca^{2+} exchange between the domain and the two other compartments. Results have been obtained by numerical integration of the model defined by Eqs. 1 and 4–8 with the following set of parameters values: K_{act} = 0.5 μM, n_a = 3, K_{inh} = 0.32 μM, n_i = 4, k_- = 0.217 s^{-1}, ρ = 4.736 × 10^7, k_2 = 1.48 × 10^7 s^{-1}, b = 4.10^{-3}, α = 0.1, β = 10^{-6}, V_{MP} = 1.55 μM s^{-1}, K_P = 0.3 μM, n_P = 2, Ip = 0.2, and Ca_{tot} = 100 μM. (*B*) Effect of the existence of Ca^{2+} ATPases pumping Ca^{2+} into the lumen from the intermediate domain, and not from the cytosol. Results have been obtained as in *A*, except that the pump is now acting on C_{dom}, and Eqs. 6 and 7 have been modified adequately. Parameter values are the same as in *A*, except for Ip = 0.3, K_{act} = 0.45 μM, ρ = 4.9012 × 10^7, V_{MP} = 1.77 × 10^6 μM s^{-1}. The fact that the value of V_{MP} has been increased by a factor of 10^6 in comparison with the simulation shown in *A* comes from the fact that this maximum velocity must now be defined with respect to the volume of the domain.

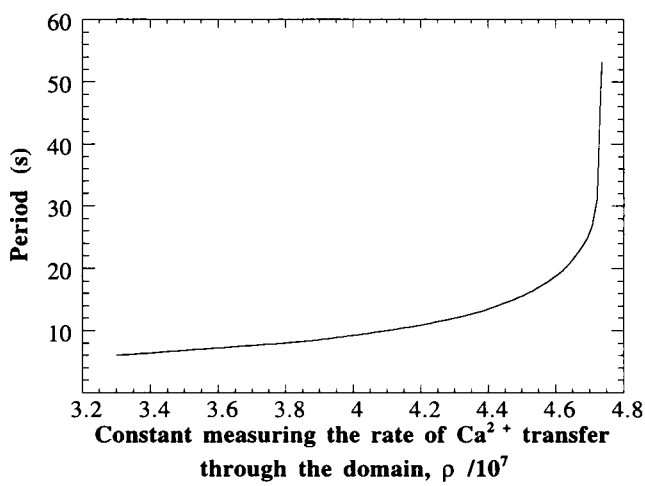

FIGURE 6 Dependence of the period of Ca^{2+} oscillations on the ratio ρ (= k_2/k_1) of rate constants governing the velocities of influx into and efflux out of the domain. Results have been obtained by numerical integration of the model defined by Eqs. 1 and 4–8 with the same parameter values as in Fig. 5 *A*.

values for the parameters governing the dynamics of the receptor, the period can become as large as 100 s.

IMPORTANCE OF COOPERATIVE PROCESSES

From a theoretical point of view, the existence and properties of the Ca^{2+} spikes largely rely on the cooperative nature of several steps involved in their generation (Kuba and Takeshita, 1981; Goldbeter et al., 1990; Swillens and Mercan, 1990; De Young and Keizer, 1992; Atri et al., 1993; Stucki and Somogyi, 1994; Tang et al., 1996). It is indeed well known that cooperative processes play a key role in destabilizing the stationary state (Goldbeter and Dupont, 1990; Goldbeter, 1996). On the other hand, the Ca^{2+} ATPase responsible for Ca^{2+} pumping from the cytosol into the stores is known to show positive cooperativity, with a Hill coefficient of about 2 (Carafoli, 1987; Lytton et al., 1992) or 3 (Camello et al., 1996) with respect to cytosolic Ca^{2+}. Moreover, the fact that the $InsP_3$ receptor is a homotetramer suggests that Ca^{2+} activation and inhibition could also be cooperative processes, a hypothesis that has received experimental support (Bezprozvanny et al., 1991; Schrenzel et al., 1995). Numerical investigation of the present model suggests that experimental-like oscillations cannot occur with realistic parameter values in the absence of cooperativity at the level of the control by Ca^{2+} of the activity of both the $InsP_3$ receptor/Ca^{2+} channel (n_a and n_i) and of the Ca^{2+} ATPases (n_p). In particular, linear stability analysis shows that instability is greatly favored by an increase in the Hill coefficients for receptor regulation by Ca^{2+}.

In addition, simulation results exhibit a mutual dependence on the Hill coefficients for channel activation (n_a) and pumping (n_p): oscillations only occur for an adequate combination of these two parameters (grey shaded region in Fig. 7). For experimental-like values of the Hill coefficients characterizing pumping ($1 < n_P < 3$), sustained oscillations mostly occur for higher values of the Hill coefficient for receptor activation ($n_a > n_p$). The intuitive explanation is that low levels of extraluminal Ca^{2+} activate the receptor with more efficiency than they activate the Ca^{2+} pump; this favors the destabilization of the steady state, leading to the development of oscillations. On the basis of experimental reports indicating that the Ca^{2+} pump cooperatively depends on Ca^{2+}, the present results support the hypothesis that activation of the $InsP_3$ receptor is cooperatively dependent on extraluminal Ca^{2+}, in agreement with the recent analysis of Schrenzel et al. (1995). The present results concerning the relative values of the Hill coefficients have been obtained with the full model defined by Eqs. 1 and 4–8, but would remain qualitatively unchanged if the existence of the domain were not considered.

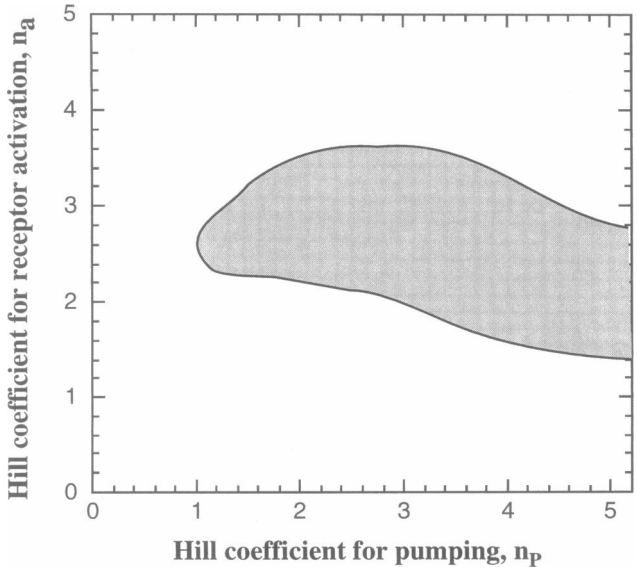

FIGURE 7 Relationship between the values of the Hill coefficients characterizing pumping and Ca^{2+}-induced activation of the receptor, which can be used in the model to get oscillations. The gray region of the parameter space (n_P, n_a) represents the region of instability, in which sustained oscillations occur. Results have been obtained by numerical integration of the model defined by Eqs. 1 and 4–8, with the same parameter values as in Fig. 3, except that Ip = 0.2, $V_{MP} = 4\ \mu M\ s^{-1}$, and $n_i = n_a + 1$. If n_i is not increased in parallel with n_a, parameter values no longer lead to sustained oscillations.

DISCUSSION

In the present study we have shown for the first time that quantal release, incremental detection, and Ca^{2+} oscillations can be accounted for by the same theoretical model. A previously developed model (Swillens et al., 1994), based on the regulatory properties of Ca^{2+} on the $InsP_3$ receptor and on the existence of a Ca^{2+} intermediate domain located around the mouth of the channel, can indeed account for the three phenomena in different conditions. As shown previously, quantal release and incremental detection can be obtained when the cytosolic volume is assumed to be so large that variations in the level of cytosolic Ca^{2+} and pumping into the stores can be neglected. This situation merely accounts for stopped-flow experiments, or, to a lesser extent, for experiments in which permeabilized cells are directly stimulated by $InsP_3$. On the other hand, numerical simulations of the same model, using the same set of parameter values, exhibit periodic Ca^{2+} oscillations if cytosolic Ca^{2+} is allowed to vary and if pumping is taken into account, i.e., in the conditions of an intact cell. One straightforward prediction of this study is that quantal release behavior observed in response to $InsP_3$ stimulations on permeabilized cells would progressively disappear and transform into damped oscillations if the volume of the bath were diminished. However, this theoretical prediction could hold only if permeabilization does not affect the kinetic characteristics of the key components, namely the $InsP_3$ receptor and the Ca^{2+} ATPase.

The present study does not account for quantal release in intact cells, as observed in experiments by Muallem et al. (1989) and Bootman et al. (1992, 1994). First, the results

shown in this paper do not apply to cell populations, as considered by Muallem et al. (1989). Second, we could not reproduce the situation encountered in the HeLa cells used by Bootman et al. (1992, 1994) to investigate quantal release in intact cells; in these studies, oscillations are eliminated by reducing extracellular Ca^{2+}. As in the present model, Ca^{2+} influx is not required to generate oscillations—as is the case in other cell types like hepatocytes or *Xenopus* oocytes, Ca^{2+} oscillations in response to $InsP_3$ always arise in a simulated intact cell. However, some analogy between the model and the latter experimental situation can be envisaged because a marked reduction in extracellular Ca^{2+} prevents replenishment of the intracellular stores (a point that resembles the model hypothesis of neglecting the pumps to simulate quantal release and incremental detection) and because the amplitude of the first spike increases with the level of stimulus both in the present model (data not shown) and in the experiments of Bootman et al. (1992, 1994).

In the model (Swillens et al., 1994), the existence of a Ca^{2+} intermediate domain located downstream from the gate is crucial to incremental detection. It allows rapid and transient Ca^{2+} accumulation near the channel, inducing receptor desensitization before emptying of the stores. If the domain were not considered and the receptor were in contact with the constant—or nearly constant—cytosolic concentration, the channel activity would not return to a basal level between successive IP_3 additions. In this respect, it should be noted that recent experimental studies suggest that luminal Ca^{2+} regulates channel activity by acting at the cytosolic sites, a hypothesis resembling the assumption of the existence of a Ca^{2+} intermediate domain (Horne and Meyer, 1995; Tripathy and Meissner, 1996).

Numerical simulations of the same enlarged model to investigate Ca^{2+} oscillations allowed us to uncover another potential role of this domain. In appropriate conditions, the latter compartment can markedly increase the period of oscillations, a property that is of primary importance given the fact that in the absence of a domain, experimental-like periods cannot be obtained by conventional models when realistic values for the kinetic constants governing resensitization of the $InsP_3$ receptor are used. The domain indeed allows us to consider that the Ca^{2+} released through the $InsP_3$ receptor/Ca^{2+} channel does not induce an instantaneous and homogeneous increase of the Ca^{2+} level in the cytosol. On the contrary, Ca^{2+} released in the domain is first sucked up into the entirety of the cytosol until homogeneity is achieved. Then Ca^{2+} can start to increase near the channel until a point where it reaches the CICR threshold, leading to the onset of a new spike. Definite evidence in favor of the physiological significance of such an effect of the domain on the kinetics of Ca^{2+} oscillations would necessitate very fine numerical simulations of the Ca^{2+} dynamics around a channel, based on the knowledge of the characteristics of the receptors and of the buffers affecting Ca^{2+} diffusion in the different cell types. Work in this field has been recently published by Smith et al. (1996) and is currently under investigation. In the present simulations, the increase in period due to the existence of the domain is further amplified if one assumes that the Ca^{2+} pumps are located so close to the $InsP_3$ receptor that they also act on the level of Ca^{2+} in the domain located downstream from the gate of the channel. It is conceivable that the extent of colocalization between the Ca^{2+} pumps and the release channels varies from one cell type to another (Rossier et al., 1991; Favre et al., 1996), thus leading to a variety of periods. Differences in the values of other parameters would also affect the frequency of spiking.

We also investigated theoretically the role of cooperativity in the regulation of the $InsP_3$ receptor by Ca^{2+} for generating oscillations. Our results provide some indirect evidence in favor of the existence of a high degree of positive cooperativity (Hill coefficient larger than 2): the model predicts that the existence of oscillations is much favored when cooperativity in the regulation of the $InsP_3$ receptor by Ca^{2+} is at least as large as cooperativity at the level of the regulation of the Ca^{2+} ATPase by cytosolic Ca^{2+}. This prediction does not depend on the possible existence of a Ca^{2+} intermediate domain but simply arises from the fact that CICR is considered as the central regulation leading to oscillations.

Given the primary role played by CICR in generating oscillations in the present model, it is most probable that its detailed oscillatory behavior would closely resemble that of a previous, more phenomenological model (Goldbeter et al., 1990). In particular, propagation of Ca^{2+} waves would certainly be obtained when the diffusion of cytosolic Ca^{2+} is considered (Dupont and Goldbeter, 1992, 1994). Along the same lines, other models for quantal release that incorporate CICR, particularly the ones based on different sensitivities of the distinct $InsP_3$-sensitive Ca^{2+} pools (Kindman and Meyer, 1993; Bootman and Berridge, 1995), could probably also give rise to Ca^{2+} oscillations. In any case, a general approach incorporating these two different and complementary aspects of Ca^{2+} signaling is certainly required to gain a more precise understanding of intracellular Ca^{2+} organization.

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